The Handbook of Environmental Chemistry 119
Series Editors: Damià Barceló · Andrey G. Kostianoy

Yolanda Picó
Julian Campo *Editors*

Mass Spectrometry in Food and Environmental Chemistry

Springer

The Handbook of Environmental Chemistry

Volume 119

Founding Editor: Otto Hutzinger

Series Editors: Damià Barceló • Andrey G. Kostianoy

In over four decades, *The Handbook of Environmental Chemistry* has established itself as the premier reference source, providing sound and solid knowledge about environmental topics from a chemical perspective. Written by leading experts with practical experience in the field, the series continues to be essential reading for environmental scientists as well as for environmental managers and decision-makers in industry, government, agencies and public-interest groups.

Two distinguished Series Editors, internationally renowned volume editors as well as a prestigious Editorial Board safeguard publication of volumes according to high scientific standards.

Presenting a wide spectrum of viewpoints and approaches in topical volumes, the scope of the series covers topics such as

- local and global changes of natural environment and climate
- anthropogenic impact on the environment
- water, air and soil pollution
- remediation and waste characterization
- environmental contaminants
- biogeochemistry and geoecology
- chemical reactions and processes
- chemical and biological transformations as well as physical transport of chemicals in the environment
- environmental modeling

A particular focus of the series lies on methodological advances in environmental analytical chemistry.

The Handbook of Environmental Chemistry is available both in print and online via https://link.springer.com/bookseries/698. Articles are published online as soon as they have been reviewed and approved for publication.

Meeting the needs of the scientific community, publication of volumes in subseries has been discontinued to achieve a broader scope for the series as a whole.

Mass Spectrometry in Food and Environmental Chemistry

Volume Editors: Yolanda Picó · Julian Campo

With contributions by

D. Barceló· W. Buchberger · J. Campo · B. Chen · C. D. Chouinard · Z. Cui · M. Farré · Y. Feng · Y.-L. Feng · M. He · M. Himmelsbach · B. Hu · C. W. Klampfl · J. Ma · A. S. Maia · K. A. Maloof · A. Mpupa · S. P. Neal · P. N. Nomngongo · A. Nqombolo · Y. Picó · A. R. L. Ribeiro · C. Ribeiro · Á. Ríos · Y. Sapozhnikova · M. E. Tiritan · K. R. Tucker · C. Xu · Q. Zhang · M. Zougagh

Springer

Editors
Yolanda Picó
Food and Environmental Safety Research Group (SAMA-UV)
Desertification Research Centre - CIDE (CSIC-UV-GV)
Moncada, Valencia, Spain

Julian Campo
Food and Environmental Safety Research Group (SAMA-UV)
Desertification Research Centre - CIDE (CSIC-UV-GV)
Moncada, Valencia, Spain

ISSN 1867-979X ISSN 1616-864X (electronic)
The Handbook of Environmental Chemistry
ISBN 978-3-031-19092-6 ISBN 978-3-031-19093-3 (eBook)
https://doi.org/10.1007/978-3-031-19093-3

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Series Editors

Editorial Board Members

Series Preface

With remarkable vision, Prof. Otto Hutzinger initiated *The Handbook of Environmental Chemistry* in 1980 and became the founding Editor-in-Chief. At that time, environmental chemistry was an emerging field, aiming at a complete description of the Earth's environment, encompassing the physical, chemical, biological, and geological transformations of chemical substances occurring on a local as well as a global scale. Environmental chemistry was intended to provide an account of the impact of man's activities on the natural environment by describing observed changes.

While a considerable amount of knowledge has been accumulated over the last four decades, as reflected in the more than 150 volumes of *The Handbook of Environmental Chemistry*, there are still many scientific and policy challenges ahead due to the complexity and interdisciplinary nature of the field. The series will therefore continue to provide compilations of current knowledge. Contributions are written by leading experts with practical experience in their fields. *The Handbook of Environmental Chemistry* grows with the increases in our scientific understanding, and provides a valuable source not only for scientists but also for environmental managers and decision-makers. Today, the series covers a broad range of environmental topics from a chemical perspective, including methodological advances in environmental analytical chemistry.

In recent years, there has been a growing tendency to include subject matter of societal relevance in the broad view of environmental chemistry. Topics include life cycle analysis, environmental management, sustainable development, and socio-economic, legal and even political problems, among others. While these topics are of great importance for the development and acceptance of *The Handbook of Environmental Chemistry*, the publisher and Editors-in-Chief have decided to keep the handbook essentially a source of information on "hard sciences" with a particular emphasis on chemistry, but also covering biology, geology, hydrology and engineering as applied to environmental sciences.

The volumes of the series are written at an advanced level, addressing the needs of both researchers and graduate students, as well as of people outside the field of

"pure" chemistry, including those in industry, business, government, research establishments, and public interest groups. It would be very satisfying to see these volumes used as a basis for graduate courses in environmental chemistry. With its high standards of scientific quality and clarity, *The Handbook of Environmental Chemistry* provides a solid basis from which scientists can share their knowledge on the different aspects of environmental problems, presenting a wide spectrum of viewpoints and approaches.

The Handbook of Environmental Chemistry is available both in print and online via https://link.springer.com/bookseries/698. Articles are published online as soon as they have been approved for publication. Authors, Volume Editors and Editors-in-Chief are rewarded by the broad acceptance of *The Handbook of Environmental Chemistry* by the scientific community, from whom suggestions for new topics to the Editors-in-Chief are always very welcome.

Damià Barceló
Andrey G. Kostianoy
Series Editors

Preface

The science of Environmental Chemistry includes a vast array of disciplines aiming at a complete description of the Earth's environment. It encompasses geological, chemical, physical, and biological transformations of chemical substances occurring on the natural environment, at different scales, providing an overview of the impact of human activities on it. In this regard, the UN have launched the 2030 Agenda for Sustainable Development, which aims to provide a shared blueprint for peace and prosperity for people and the planet, now and in the future. This agenda proposes 17 Sustainable Development Goals that are an urgent call to guarantee food security and promote sustainable use of terrestrial ecosystems. Environmental chemistry is directly related to these SDGs, helping to study and understand the effects of the increasing use of resources to fulfill the consumer's demand for goods and services.

In recent times, great progress has been made in environmental analysis techniques with special attention to mass spectrometry (MS), omics approaches, miniaturization, automatization, nano- and biosensors, and green chemistry. In this book, recent advances in techniques and innovative uses of MS in food safety and environmental quality are summarized and discussed. The latest developments in sample preparation together with improvements in MS platforms have allowed the study of different omics together with the screening of emerging contaminants (ECs) and nanomaterials. *Mass Spectrometry in Food and Environmental Chemistry* offers useful and relevant information on the most recent analytical techniques as well as all major topics of food safety and environmental quality analyzable by them.

The book contains thirteen chapters written by worldwide experts discussing themes such as elemental and isotopic mass spectrometry as well as chromatographic-high resolution and chromatographic–mass spectrometry-based screening methods in food and environmental chemistry analyses. Other approaches such as omics, thermal desorption, and pyrolysis together with chiral mass spectrometry, ambient ionization techniques, ion mobility, and imaging mass spectrometry have also been discussed not forgetting last developments such as chip-based separation

devices coupled to mass spectrometry and their role in food and environmental chemistry. This book is aimed at anyone seeking to learn about new techniques and developments including a broad audience of researchers, from environmental and food chemists, biologists, and food technologists to the food industry and official food and environment control laboratories, together with other professionals responsible for resources management and decision-makers. Important tools for problem-solving in chemical and biological analyses are discussed in detail. The different advances and promising applications described here are state of the art and will be of paramount importance for ensuring food safety and environmental health for current and future generations.

Finally, we would like to thank all the chapters' authors for their continuous and devoted effort and patience. All of them deserve our deepest thanks because without them this book would have been impossible. I also thank the anonymous reviewers of the project for their suggestions. We would also acknowledge with great gratitude Prof. Damià Barceló (Series Editor) who gave us the opportunity of editing this book and the assistance of the Editorial Office of Springer and especially of Dr. Sofia Costa (Editor) and Ms. Ramya Venkitachalam (Project Coordinator for Springer Nature). We hope that this volume lives up to the expectations of the readers starting or with a long experience in food and environmental quality analysis. This book is intended for all of you.

Valencia, Spain

Yolanda Picó
Julian Campo

Contents

An Overview of the State-of-the-Art: Mass Spectrometry in Food and Environment

Yolanda Picó and Julian Campo

Contents

Abstract Mass spectrometry (MS) has achieved great renown as a specific, selective, sensitive, and rapid technique for the analysis and evaluation of a wide range of food products and environmental matrices. The state of the art of MS in food and environmental safety and quality is presented to show the potential of this technique in the qualification and quantification of chemical characteristics of food and environmental samples, in the evaluation of the quality of meat, fish, fruits, vegetables, and other food products, as well as in the classification of environmental samples, and in the determination of contaminants in all their compartments. The features of each mass spectrometry advance for each category were summarized in the aspects of the investigated quality and safety attributes, and the used systems (low and high resolution mass spectrometry). With its success in different applications of food and environmental quality, and safety analysis and assessment, it is evident that MS can facilitate a variety of tasks. Continued development of methodology and instrumentation will enable more sensitive and timely detection in the coming years.

Keywords Adulteration, Authenticity, Chemometrics, Environmental assessment, Food quality, Food safety, High resolution mass spectrometry, Mass spectrometry

Y. Picó (✉) and J. Campo
Environmental and Food Safety Research Group-University of Valencia (SAMA-UV), Desertification Research Centre – CIDE (CSIC-UV-GV), Valencia, Spain
e-mail: Yolanda.pico@uv.es; Julian.campo@uv.es

Yolanda Picó and Julian Campo (eds.), *Mass Spectrometry in Food and Environmental Chemistry*, Hdb Env Chem (2023) 119: 1–24, DOI 10.1007/698_2022_908,

Published online: 18 October 2022

1 Introduction

The environment and food are fundamental to ensuring human well-being and to preserve the world for the next generation [1–4]. There are many environmental problems that need to be addressed in the coming years if the planet earth is to remain habitable for humans in the next future [5, 6]. These problems are well-known, climate change, water scarcity and its effects on human health, biodiversity preservation, resilience, waste and wastewater management, and so on [7–10]. There are many challenges ahead that require an important research investment. There are also several food safety and quality issues to consider in the near future. Food safety and quality are two concepts highly related. Food quality can be defined "as a total of traits and criteria, which characterize food for a consumers' health" [11]. According to the definition of the Food and Agriculture Organization (FAO): "Food security exits when all the people have through physical and economic access to sufficient, safe and nutritious food to meet their food needs" [12]. Thus, food security involves food free of hazards and with appropriate nutritional value. Both can be considered as part of the quality that in addition involves food authentication and adulteration, food characteristics that consist of food nutrients (proteins, lipids, carbohydrates, vitamins and minerals), food additives, contaminants, substance forming during food processing but also values such as sensory characteristics (e.g. taste of food, smell, etc.) and convenience (e.g. easy in preparation, etc.). There is also a strong relation between environment and food quality and safety since intensive food production damages the environment by depleting the soil and drastically reducing marine ecosystems and their biodiversity [10, 11, 13]. In addition, overexploitation of natural resources has put food security and the availability of clean water at risk. Moreover, harmful environmental effects involving climate change and pollution are affecting food safety and quality, as increased temperatures attract greater use of chemicals and change the pattern of biological contaminants in areas and can alter the nutrient composition of food [1, 5, 14].

The third decade of the twenty-first century has started recently, and the challenges related to food and environment that lie ahead, as set out in the UN's 2030 Agenda for Sustainable Development, are many [6]. Figure 1 shows the 17 Sustainable Development Goals (SDGs). The environmental aspect of development of the SDGs cover natural resource management, climate change, water-related issues, marine issues, biodiversity and ecosystems, circular economy, environmentally sound management of chemicals and waste, and many other topics [15–17]. Three of these goals are primarily food safety and quality related: SDG 3 "Good health and well-being" focuses on health (only achievable through a proper nutrition), while SDG 2 "Zero Hunger" involves the access of the whole world to sufficient food and encompasses eradication of malnutrition and nutrition associated disease and SDG 6 "Clean water and sanitation" is a pre-requisite to favor the achievement of the other two [18–20]. This global action plan approved in 2015 sets out specific measures to achieve a fairer, more prosperous, and environmentally friendly world within 10 years. In this sense, the UN itself is concerned of whether we are or not behind

Fig. 1 Summary of the Sustainable Development Goals (SDGs) as reported by the United Nations (UNs)

schedule, and the question now is whether there is still time to ensure food safety and quality saving the planet [6].

In the environmental and food fields, mass spectrometry (MS) provides many of the data that underlie policy decisions directly influencing the health of the people and ecosystems [21]. Recent developments and improvements in MS enable it to provide a faster and more sensitive, accurate, and precise analysis of contaminants and components in food and environmental samples [22–25]. Methods combining MS with different techniques, such as inductively couple plasma (ICP), gas and liquid chromatography (GC and LC), pyrolysis (Py), elemental analyzers (EA), alone or several of them altogether, have proven to be very suitable for the analysis of food and environmental samples [26–35].

With the improved performance of techniques and instrumentation within MS, emerging strategies [such as chiral analysis, ion mobility, compound-specific isotopic analysis (SCIA)] and omics applications (such as lipidomics, metabolomics, and proteomics) have been developed [28, 30, 32, 36]. Contemporary MS progresses also include ambient-ionization MS and imaging (both could reach direct food analysis) and the transformation of bench-top mass spectrometry in lab-on-chip [37–40]. MS has also made an effort to be involved in what is coined green analytical chemistry (GAC) [41, 42]. Although this technique cannot be considered as a green technique in the "strict sense" because it has a high consumption of energy, toxic reagents (especially if hyphenated to LC), gases, and time, it has a high identification power and high-analyte throughput and then, can reduce the use of additional techniques for confirmation [43]. The most classical instrumental formats (ICP-MS, GC-MS, LC-MS, IRMS) are at the present time built using less material

(then, they are smaller and lighter) with more energy efficient and fewer consumption of gases and hazardous chemicals to complete the analysis generating less waste without loss of efficiency that is also important [44, 45]. Recent developments such as ambient-ionization MS and imaging are solvent free and reduced importantly the hazardous wastes, and lab-on-chip is clearly included within one of the most relevant principles of GAC that indicates the need to use automated and miniaturized methods [42, 43, 46]. With all these progresses, MS-based techniques are in the state-of-the-art technique and have become essential in both food and environmental analysis, (1) for determining chemicals in environmental and biological compartments and to determine these chemicals as well as endogenous compounds in food analysis [4, 47], (2) for identifying unknown chemicals using high-resolution mass spectrometry and scan-dependent and -independent MS/MS techniques [48, 49], and (3) for helping to fill the knowledge gaps regarding the molecular mechanisms of toxicity and apical effects caused by a certain chemical [11, 47]. Thus, MS plays an important role in both fundamental and applied research in food and environmental analysis, supporting environmental risk assessment and management as well as food safety.

This chapter presents the history and applications of different types of mass detectors, and how these detectors have been combined or hybridized with other separation techniques to achieve a wider range of applications. This history highlights new advances in recently developed mass spectrometry (MS) methods, such as high-resolution mass spectrometry (HRMS), ambient ionization mass spectrometry (AIMS) for direct food analysis, mass spectrometry imaging (MSI), and lab-on-a-chip technologies. It also discusses and compares the advantages and limitations of different MS techniques in their applications to the environment and to food safety and quality (these applications include metals, contaminants, food components, omics, chiral separation, macromolecules, etc.). Finally, this chapter discusses the future perspectives of the different techniques (many of which will be discussed in depth in the various chapters of this book) and the evolution of applications.

2 Developments in Mass Spectrometry and Current Applications in Environmental and Food Analysis

Mass spectrometry is an important tool for ensuring environmental and food safety [4, 39, 49–58]. Thanks to this technology, hazards ranging from pesticides to heavy metals and toxins can be identified, even if they are only present in minute quantities [54, 55, 58]. Advances in MS have enhanced the potential for analysis and the important role it has played and will continue to play in determining sample characteristics and ensuring food and environmental safety [49–51, 56, 58]. There have been different improvements in MS along history that have marked advances in the analysis of food and environmental samples [21, 26, 59–61]. Table 1 summarizes these achievements. The enormous structural diversity and huge dynamic range of

Table 1 Advances in mass spectrometry have been a milestone in environmental and food analysis

Year	Advancement
1987	Determination of mass of the electron by J.J. Thomson
1912	First mass spectrometer (J.J. Thomson) (Faraday cup)
1918	"Directional focusing" mass spectrograph developed
1934	First double-focusing magnetic analyzer
1946	Discovery of the principle of analysis by measuring the time-of-flight of ions
	Martin and Synge described increasing powerful methods to separate amino acids and other organic compounds
1948	Martin and James developed gas chromatography
1949	Herzog and Viehb established the fundamentals of secondary ion mass spectrometry (SIMS)
1951	Gas chromatography starts to be used in the analytical laboratories
1953	Off-line pyrolysis GC reported
	Integrated pyrolysis mass spectrometry developed
	Patented quadrupole and ion-trap analyzers
1955	Introduction of the electron multiplier
1956	First commercial time-of-flight (TOF) mass spectrometry
1959	First coupling of gas chromatography-mass spectrometry (GC-MS)
1961	Liebe and Herzog developed the first SIMS device
	Pyrolysis gas chromatography off-line mass spectrometry reported
1966	First library of mass spectra published
	Horváth and Lipsky described the fundamentals and first application of ionic exchange chromatography
	Chemical ionization discovery
	First integrated pyrolysis-GC-MS reported
1967	Description of the high performance liquid chromatography (HPLC)
1968	First electrospray (ESI) interface for mass spectrometry
1971	First routine GC-MS with magnetic sector
1972	Introduction of the HPLC commercial devices connected on-line to a detector
1973	Tandem mass spectrometry (MS/MS) described
	Horning and Coll reported atmospheric pressure chemical ionization (APCI)
	Advent of reflection time-of-flight mass spectrometers to avoid energy spread
1976	Moving belt interface – First commercial LC-MS interface.
	Introduction of fused silica capillary columns in GC
	A combustion chamber between GC and MS incorporate.
1978	First NBS (NIH) database published
	First commercial MS/MS instrument
1983	First commercial ICP-MS
1984	Vestal developed the first LC-MS with thermospray interface
1985	Coupled laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)
	Hillenkamp and Karas reported first matrix-assisted laser desorption/ionization (MALDI)
1986	First single particle (SP)-ICP-optical emission spectrometry (OES) reported
1987	First GC-triple quadrupole (MS/MS) reported
1988	Tanaka at Shimadzu introduced the first commercial MALDI-instrument

(continued)

Table 1 (continued)

Year	Advancement
	First commercial GC-C-IRMS was released
1989	First LC-MS commercial instrument with electrospray (designed by Fenn)
1990	Coupling of GC with new low- and high-resolution time-of-flight (TOF)
1991	Frysinger and Gaines first reported GC x GC
1993	SP implemented in ICP-MS
1995	First commercial LC-orthogonal accelerated (oa)-TOF-MS
1997	Micromass introduced the first commercial LC-QqTOF-MS
	Caprioli and Vanderbilt published the first papers on MALDI-imaging
1998	Kinghorn and Marriott proposed the first cryogenic modulator for GC x GC
2001	First GC-oaTOF-MS reported
2002	Application of ion mobility mass spectrometry (IMMS)
2003	First GC with atmospheric pressure photoionization (APPI) reported
2004	Cooks introduced desorption electrospray ionization (DESI)
	Commercialization of liquid chromatography-IRMS (LC-IRMS)
	Application of electron transfer dissociation (ETD)
2005	Introduction of orbitrap
2005	Commercialization of direct analysis in real-time (DART) interface
2006	Cooks' team developed DESI-MS imaging
2010	First coupling of GC-MS with quadrupole time-of-flight (QqTOF) reported
2012	Petersen and Coll coupled GC-MS with an orbitrap

food and environmental matrices provided analytical challenges for analysis of chemicals present on them [30, 62–64]. The reported achievements in MS technology greatly expand the capability of MS-based approaches for food analysis [21, 50, 65].

The interest in knowing the chemical composition of food is well before the interest in environmental problems [12, 66–68]. The first developments in the field of food analysis date back to the late 1800s and include numerous methods based on solvent extraction, acid digestion, and weight difference determination [21, 68]. From 1900 to 1940 food science was immersed in the chemical characterization of the nutrients –carbohydrates, lipids, proteins, vitamins, minerals – as well as in the discovery of the functions that these components had in foods [67, 68]. From this arose the need to develop analytical methods that could verify the nutritional quality of foods. However, the real revolution in food analysis began in the 1950s with the application of the first instrumental techniques such as chromatography [69]. These in combination with mass spectrometry marked a clear milestone in food analysis [52, 70] enabling to extend food analysis to other, not necessarily nutritional, food components [53]. In addition, chromatography-mass spectrometry allowed the development of methods to ensure food safety, prevent fraud, control the presence of undesirable substances, and ultimately guarantee quality and safety.

Comparatively, interest in environmental preservation and the study of environmental problems is later began in the early 1970s [54]. In fact, the beginning of environmental concern is associated with the publication in 1962 of Rachel Carson's book "Silent Spring," which brought to the forefront the problem of the extensive use of pesticides [71]. A few years later, the Environmental Protection Agency (EPA) was created in the USA and in the early 1970s the first organochlorine synthesis pesticides were banned [72].

Despite this time difference between the two disciplines, the advances in both fields are linked to the development of organic mass spectrometry [47]. Gas chromatograph combined with single quadrupole mass spectrometry (GC-MS) was the first technique that marked a revolution in both fields [32, 33]. Until the early 1990s, GC-MS (commercialized since 1968) was the only technique capable of reaching the required detection limits with the necessary selectivity and specificity to be able to detect minority compounds in very complex matrices, such as environmental and food samples [73, 74]. GC-MS worked especially well in the detection of volatile and thermostable compounds such as many organic molecules [36, 41, 75, 76]. In addition, as there were no robust alternatives, numerous derivatization reactions were developed to transform non-volatile molecules into volatile ones, thus greatly extending the range of determinable molecules [36]. For small organic molecules, the combination of liquid chromatography and mass spectrometry (LC-MS) in the late 1980s was a breakthrough [30, 31, 77]. Overall, the 1980s produced major advances in interface and ionization techniques that greatly helped to improve the applications of mass spectrometry [78–80]. In this respect, it is only necessary to recall that in 2002, Fenn and Tanaka received the Nobel Prize for the development of the electrospray source in the 80s that made it possible to combine liquid chromatography and ionization techniques [58]. Both combinations, LC-MS and GC-MS have allowed the separation of organic molecules, providing an upstream separation that incorporates from the use of chromatographic phases that allow the separation of chiral compounds to the new multidimensional separation techniques such as GC x GC and LC x LC that always facilitates the determination of compounds in complex mixtures [76, 81, 82].

During the 80s decade, the combination of the isotope ratio mass spectrometry (IRMS) to an elemental analyzer (EA) was also developed [74]. The term "continuous-flow isotope ratio mass spectrometry" (CF-IRMS) was introduced in 1988 when Preston and McMillan coupled an elemental analyzer to an isotope ratio mass spectrometer via a variable leak [83]. The gas chromatography-combustion-IRMS (GC-C-IRMS) was developed even before but commercialized in 1988 [84]. In this case, since its commercialization, the systems are well established [85]. However, there are major advances in applications, especially in food analysis (geographical origin, authenticity, etc.) and environmental analysis [85]. Although to a lesser extent, there are also advances in instrumentation such as the combination of IRMS and LC [83].

The ICP source coupled to a quadrupole-based mass analyzer was introduced commercially in 1983 [27]. However, it was not until 2001 that this instrument was equipped with collision/reaction cells that increased the capability of the technique

to process samples with a high matrix content. In the following years, ICPMS has become one of the techniques capable of detecting more than 70% of the elements in the periodic table [86]. Thanks to these instruments, the analysis of metals and metalloids is practically solved except for few cases. One of these cases where analysis is not fully resolved yet is the speciation of the different forms of inorganic species. In this field, ICP is also useful but became an on-line detection tool for common separation techniques, such as GC, LC, and CE. There is also recent instrumental advance within this technique, such as the combination of laser ablation (LA) and ICPMS, which is a particularly useful technique for mapping [13] and the single particle (SP)-ICP-MS [86–88].

The decade of the 90s was marked by the appearance of tandem mass spectrometry, especially triple quadrupole (QqQ)-MS/MS, which increased the specificity, selectivity, and sensitivity of the determination [29, 30]. This has led to an increase in the capacity of these techniques to improve the analysis as they are able to simultaneously determine many compounds based on the use of generic extraction methods and a very specific determination by mass spectrometry coupled or not with a previous separation technique [31, 57, 77].

Since the 2000s, food and environmental applications have experienced a movement from low to high-resolution mass spectrometry [89, 90]. This has allowed to shift workflows from target to non-target analysis and to exponentially increase the number of determinable compounds. The ability of HRMS to provide information on the most probable empirical formula as well as on characteristic fragments of the structure helps not only to increase the number of determinable compounds but also to identify unknown compounds [79]. This high throughput option has also opened the field of "omics" within food and environmental analysis [58]. The various mass analyzers, which were originally designed to be combined with liquid chromatography, have been incorporated into other separation systems such as GC-MS or ICP-MS [78]. In this context, chemometrics plays a key role in resolving some issues related to food authentication or traceability, being a useful tool for food scientists to classify unknown samples with similar characteristics for the determination of the authenticity and to associate a food product to the place of origin in the context of traceability [91].

Also in the 2000s, the development of environmental ionization occurred. This includes ionization techniques capable of achieving desorption and ionization of analytes directly from the surface of an untreated sample [35]. The first desorption source was electrospray ionization (DESI), this was closely followed by direct analysis in real time (DART), a plasma-based environmental ionization technique. Since then, a number of desorption sources with different principles have been developed [54]. AIMS allows direct and rapid analysis with no or minimal sample preparation and is particularly applicable to in situ analysis, enables direct analysis of intact substances, and has been developed through portable instruments.

The determination of macromolecules has also brought other advances to mass spectrometry, including the incorporation of pyrolysis prior to separation and determination, the use of AIMS, ESI, or MALDI sources and finally leading to MSI, which has allowed the evaluation of the distribution of both macro and small

molecules in the matrix. These methods have opened new opportunities for the MS of macromolecules by greatly extending the molecular size and type of samples amenable to MS [2, 92].

3 Applications of Mass Spectrometry in Environmental and Food Analysis: Current Status and Future Prospects

The applications of mass spectrometry in environment and food have grown exponentially since its introduction. Table 2 shows the different applications that can be found depending on the type of technique.

The determination of the elements of the periodic table is covered by two techniques: ICP-MS and the IRMS– with all their different advances and combination with other chromatographic techniques. ICP-MS is an inorganic elemental and isotopic analysis technique capable of determining and quantifying most of the elements of the periodic table in a linear dynamic range of six orders of magnitude (ng/L – mg/L) in addition to being able to perform elemental determination in a multi-elemental analysis that provides the composition of the analyzed sample. It can also perform isotopic composition quantification and trace isotope stability studies and most importantly, speciation studies where the technique is able to determine the different species of the metal and to analyze nanomaterials. IRMS is devoted to determining the isotopic analysis of stable isotopes of different molecules. Both cover a wide range of applications, the former to determine the concentration of metals in food and environment and the latter to study the isotope ratio of stable C, O, N, and H. These elemental species techniques are more well-established than those related to organic molecules. However, there are still several pending issues that are not well resolved yet. One of these pending issues is the speciation analysis of the elements present in food and the environment by combining ICP-MS with efficient separation techniques, such as LC, GC, or on-line coupling of flow field-flow fractionation (F^4) [93, 104–107]. The relation between safety and Human Health risk and the element form is well-known but still a concern for the scientific community due to the lack of knowledge about some aspects as the relation between food and environment. Interestingly, one of these efforts to contribute new knowledge described the biogeochemical role of Hg in the soil–plant system and associated health risks, demonstrating the need and the interest of these studies [105]. Other is the release of engineered elemental metal nanoparticles (EMNPs) into the environment. EMNPs pose potential risks to living organisms, including human beings. Among the various techniques reported for the characterization of EMNPs, on-line coupling of F^4-ICPMS – based on size separation – has been well established for the simultaneous separation, identification, and quantification of EMNPs, especially in the complex matrices of environmental samples [56]. However, the real leading technique for the determination of EMNPs in food and environment is single particle (SP)-ICP-MS. In SP-ICPMS each recorded pulse is

Table 2 Applications of mass spectrometry within food and environmental analysis

	Technique	Application	Reference
Elemental analysis	ICP-MS	• Determination of elements of the periodical table in gas, solid, or liquid matrices • Speciation of the different metal's forms • Analysis of EMNPs in food and environment • IRMS of non-conventional stable isotopes	[26, 86–88, 93]
	IRMS	• Determination of the ratio of stable isotopes (C, H, O, S) in bulk samples • CSIA in food and environmental samples' specific compounds (e.g. lipids, proteins, organic matter, water, etc.)	[74, 83–85, 94]
Small organic molecules	GC-MS	• Volatile and thermostable compounds [e.g. volatile organic compounds (VOC), contaminants, lipids, etc.] • Non-volatile compounds transformable in volatile by chemical reaction → derivatization • Separation of chiral compounds • "Omic": metabolomic and lipidomics	[24, 32, 36, 41, 75, 76, 81]
	LC-MS	• Determination of any type of organic molecules (nutrients, endogenous compounds, contaminants, toxins, etc.) • Separation of chiral compounds and ionic species • "Omic": metabolomic and lipidomics	[23, 30, 31, 57, 58, 77–80, 89]
	IM-MS	Additional separation step for mobile ions. For compounds that elute together and need an additional separation	[59, 92, 95–99]
	AIMS	• Determination of organic molecules (nutrients, endogenous compounds, contaminants, toxins, etc.) • "Omic": metabolomic and lipidomics • Analysis of sample surface (no extraction) • On-site analysis	[35, 37, 38, 54]
	Imaging	• Visualization of the molecule in its environment • "Omic": metabolomic and lipidomics • Organic molecules	[92]
Macromolecules	LC-ESI-MS	• Bottom-up and top-down approaches • Omics: proteomics	[90]
	MALDI-TOF	• Omics: proteomics • Food allergens, bacteria, etc.	[100, 101]
	Py GC-MS	• Pyrolysis transforms macromolecules in their units • Organic matter, microplastics	[102, 103, 139]

(continued)

Table 2 (continued)

	Technique	Application	Reference
	AIMS	• Analysis of sample surface (no extraction) • On-site analysis	[35, 37, 38, 54]
	Imaging	• Visualization of the molecule in its environment • "Omic": proteomics	[55, 92]

a single NP. The frequency of the pulses is directly related to the NP concentration (number of nanoparticles in solution) and the intensity of each pulse to the mass of the element, in fact to the number of atoms, in each detected NP (size) [64, 86]. Finally, another aspect of interest is the determination of the ratio of stable isotopes of non-conventional elements (Li, B, Mg, Ca, Fe, Mo, etc.). Among the different techniques, thermal ionization mass spectrometry (TIMS), secondary ion mass spectrometry (SIMS), and multiple collector (MC)-ICP-MS are mainly used for isotope ratio measurements. The determination of the ratio of stable isotopes (C, H, O, and N) is performed by IRMS. The most novel aspects involve compound-specific isotope analysis (SCIA), which is mostly performed by GC-C-IRMS. This instrument achieves first separation of the specific compounds and then, determination of the compound's isotope ratio. CSIA can be applied to food and environmental samples generating data on key components such as aroma compounds, sugars, amino acids, carbon dioxide (in carbonated beverages), organic matter (in soil) as well as on contaminants, such as pesticides. The enormous versatility of GC-C-IRMS is evidenced by the numerous applications that are increasing exponentially with the growing versatility of these techniques and it can be expected that it will become a pivotal technique in the detection of fraud, either alone or in combination with other analytical methods [74].

Small organic molecules are determined mostly by GC-MS [75] and LC-MS [69] but also can be done by AIMS [75, 108] or MSI [40]. The technique will depend on the purpose of the analysis because the two latter were designed to provide spatial information on the location of the compound. Advances in MS instruments have provided a substantial enhancement in sensitivity, scan speed, and quantitative accuracy, making it a useful platform for analysis of complex food composition, including nutrients and other endogenous compounds (e.g. amino acids, polyphenols, lipids, vitamins, carotenoids), contaminants (e.g. pesticides, heterocyclic aromatic amines, polycyclic aromatic hydrocarbons, plastic migrants), and toxins (e.g. alkaloids, phycotoxins, phytotoxins, and mycotoxins) [50–53, 70, 80, 89, 109]. In environmental analysis, these techniques have been focused on the determination of micropollutants [3, 4, 47]. LC- and GC-/MS(MS) instruments today are continuously improving to increase the range of contaminant classes and physicochemical properties that can be determined with a single analytical method providing a broad scope of analysis. As a consequence, there is a transition in both, GC and LC, from the triple quadrupole instruments that have been most widely used in analytical laboratories in the last decade, to high-resolution MS systems to avoid the inherent

limitations of QqQ instruments in terms of restricted number of simultaneous transitions due to the scanning speed and inability to detect compounds not selected "a priori" [110]. Analysis with modern HRMS instruments is able to detect and quantify virtually unlimited amount of analytes based on the full scan data, plus retrospective analysis [34]. Recent improvements in sensitivity of HRMS instruments put them on par with modern QqQ-MS/MS but with much better opportunities to detect a wider range of compounds, as to the traditional "target analysis" they add the possibilities of broad screening (against a database that may contain thousands of compounds) and the identification of unknown compounds (much more laborious). In this sense, the different modes of information acquisition: data-dependent and data-independent (DDA and DIA), as well as the versatility of schemas, have greatly increased the amount of information acquired [111]. These increased capabilities to explore the sample composition beyond the traditional analysis along with the explosion of methods based on chromatography hyphenated to HRMS, which provides enough sensitivity and selectivity to cover a wide range of metabolites and other compounds in complex food and environmental samples, are responsible of the boom of metabolomics [25, 112]. In this sense "omics" techniques represent a bridge between small molecule and macromolecule analysis. The introduction of metabolomics in the case of small molecules (molecular weight $<$1,500 Da) has allowed to work with profiling (target) and fingerprinting (non-target) techniques [25]. Metabolomics identifies differences in small molecules (biomarkers) between a control group and the group of interest (subject to an exposure, a treatment, or any type of difference), in terms of any other endogenous and/or exogenous small molecule whose presence/absence or altered concentration between the two groups may make a difference [112, 113]. The similarities and differences among the samples are established by unsupervised pattern recognition algorithms [principal component analysis (PCA), hierarchical cluster analysis (HCA), etc.] and supervised pattern recognition algorithms [linear discriminant analysis (LDA), partial least squares discriminant analysis (PLS-DA), soft independent modeling of class analogy (SIMCA), the Random Forests (RF), machine learning algorithm, etc.] [112]. The successful implementation of these methods is also related to software improvements that simplify to deal with an enormous amount of generated data. Metabolomics tools are now being used to study volatiles (via GC methods) as a new tool for understanding aroma and flavor chemistry and the mechanisms involved in the formation of these flavor compounds in order to understand better the beneficial and less beneficial aspects of fermentation and other food processing methods [33, 63, 114]. Metabolomics research using GC/MS in food also includes the analysis of non-volatile compounds such as sugars, amino acids, and organic acids that achieved discrimination of food samples based on cultivars and authentication of food samples to prevent food fraud, characterizing the profile of food samples to provide a general overview of the sample, evaluating stress-response, optimizing postharvest processes based on metabolic changes, monitoring changes during growth and food processing, evaluating and predicting food quality, and evaluating food shelf-life [32]. However, untargeted metabolomics by LC-MS is increasingly used to discover metabolic markers for food authentication in routine

control and the assessment of biological identity, geographical origin, agricultural production, processing technology, freshness, cause of animals' death, and so on, through three main steps, data acquisition, biomarker discovery, and biomarker validation [30, 113]. Within environmental analysis, metabolomics is mostly devoted to the identification of different biomarkers of the anthropic influence in the environment. The applications in wastewater-based epidemiology are one of the most relevant examples because these biomarkers can give particular fingerprinting for populations [115]. Difficulties of the implementation of non-target metabolomics in other environmental samples are related to the low concentrations of contaminants present that prevent full development of the technique [10].

The other techniques that can be applied to the analysis of small molecules and macromolecules are AIMS and MSI. These techniques were designed to provide spatial information, that is to offer information on the position of the molecules of interest within the matrix. AIMS techniques were developed to detect the analytes in the surface of the sample and have shown substantial potential in both environmental and food analysis. However, now their spatial dimension is not exploited in most applications and AIMS techniques are just considered as highly suitable for the analysis of large sample sets – especially for qualitative screening, sometimes providing additional (at most) semi-quantitative information due to the increasing implementation of a previous sample preparation (clean-up and/or preconcentration). Food and environmental applications have been mostly focused on the determination of contaminants, such as pesticides, pharmaceuticals, brominated flame retardants, etc. Furthermore, within food quality, these interfaces have been used to study dried fruits used in traditional Chinese herbal medicine, for the authentication of fruit juices, to determine the pungency of peppers, a characteristic that can be caused by capsaicinoids, amino acids in food, etc. [35, 38, 116]. This sample preparation step has become almost mandatory as it decreases sample complexity and concentrates the analyte to meet the sensitivity requirements for analysis but most of the times eliminates the spatial information. Sample preparation is already implemented in most of the applications to food analysis and is even more pronounced in environmental analysis, where (ultra-)trace analysis is the norm. The analysis of the data by chemometric tools that helps to understand and classify the complex information obtained has increased the number of applications related to metabolomics [37]. Although all the limitations reported, AIMS techniques could play an important role in "omics" related analytical chemistry – mainly due to their ease of use and the possibility to process a large number of samples within a short time [117]. AIMS techniques are also well suited to meet the challenges related to on-site analysis. Ideally all parts of the equipment (i.e., the ion-source as well as the MS instrument with all its peripheral devices) should be portable. This would become a clear advantage of these devices for mass spectrometry.

Unlike AIMS, imaging mass spectrometry's (IMS) ability to image molecules in space is its main attraction. MSI is a prevailing tool that enables untargeted investigations into the spatial distribution of molecular species in a variety of samples [118]. Thousands of molecules, such as metabolites, lipids, peptides, proteins, and glycans, can be recorded as imaging in a single experiment without labeling. The

combination of information gained from mass spectrometry (MS) and visualization of spatial distributions in thin sample sections makes this a valuable chemical analysis tool for food and environmental specimen characterization. MSI can study almost anything owing to the wide variety of ionization choices and sample preparation strategies that can be paired depending upon your analyte(s) of interest. From pesticides to peptides and microplastics to metabolites, the future of MSI promises to extend the answerable questions in environmental and food science beyond those that were previously limited to temporal and quantitative data sets [40, 55]. Implementing MSI in environmental and food science research space means that spatial data will become part of routine analysis [92].

The determination of organic macromolecules is another field where MS-based techniques have a growing role. Within this field, omic technologies provide a holistic view of the molecules that make up a cell, tissue, or organism. Omic technologies in the case of macromolecules aimed primarily at the universal detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics), in a specific sample in a non-targeted and non-biased manner. These techniques are named "foodomics" when integrated into food and nutrition and effectively provide analytic information about the effects of food on human health and help to improve food processing, quality control, traceability and authentication. In the field of environmental sciences, omic technologies will provide a better understanding of the impact of complex mixtures of contaminants integrated with other environmental stressors. Nevertheless, "omic" strategies still provide many challenges, such as the bioinformatic tools that are still evolving. There is a lack of standardized methodologies, and it is necessary to establish carefully designed experiments, accompanied by appropriate analytical techniques and statistical analyses.

Within food analysis, LC-ESI-MS techniques with both high-resolution and low-resolution tandem mass analyzers are applied to the identification and detection of qualitative and quantitative information about the proteome, in order to extract new integrative information from the complex multivariable space of omics [2]. The two principal workflows to recognizing and typifying proteins using MS are the "bottom-up," which analyze peptides after proteolytic digestion, and "top-down," which analyze intact proteins. Bottom-up indicates that information about the constituent proteins is reconstructed from individually identified fragment peptides. This new information is necessary to achieve a higher level of understanding of processes in food science and technology; consequently, in new functions of food and improved markers of food quality and safety to completely transform the concept of food safety. As an interesting example, Stachniuk et al. [90] presented sets of myofibrillar and sarcoplasmic proteins, which turned out to be the source of 105 heat-stable peptides, detectable in processed meat using LC-MS/MS. A list of heat-stable species-specific peptides was compiled for 11 types of white and red meat including chicken, duck, goose, turkey, pork, beef, lamb, rabbit, buffalo, deer, and horse meat, which can be used as markers for meat authentication. Great efforts are ongoing to clarify the allergenic mechanisms of the diverse allergenic proteins of food origin, aimed to both designing suitable therapies and for a timely and precise diagnosis of the allergic condition [48, 119]. Among the other omics sciences, mass spectrometry

(MS)-based proteomics is gaining a steadily increasing interest by the whole scientific community because of its high versatility. Peptide marker identification is one of the most important steps in the development of a mass spectrometry (MS)-based method for allergen detection, since the robustness and sensitivity of the overall analytical method will strictly depend on the reliability of the proteotypic peptides tracing for each allergen. Pilolli et al. [120] described the results of a comprehensive evaluation of the current literature on MS-based allergen detection aiming at collecting all available information about proteins and peptide markers validated in independent studies for milk, egg, peanut, soybean, hazelnut, and almond. Within the field of environmental analysis, proteomics has been applied to the identification of macromolecules in wastewater analysis including also the proteins that determine the presence of residues of some animal species [115].

The other technique highly used in proteomic and also in the identification of other macromolecules is matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) that has become an indispensable tool in the analysis of macromolecules, such as proteins and peptides. Some biomolecules are too large and can break down when heated, and traditional techniques fragment or destroy the macromolecules. In the case of MALDI-MS, macromolecules of interest are embedded in a matrix of small molecules. Then, MALDI is a soft ionization in which a laser is incident on the matrix that contains analytes so that these macromolecules pass into the gas phase without fragmenting or decomposing them. MALDI-MS is commonly combined with time-of-flight (TOF)-MS and is appropriate to provide the peptide mass fingerprinting (PMF), as well as the selected fragmentation of various precursors using collisional-induced dissociation (CID) or post-source decay (PSD). Recognition of food adulterations, description of food allergens, identification of food spoilers and food-borne pathogens, safety assessment of genetically modified foods and investigation of protein structural modifications induced by various industrial processes that could be an issue in terms of food quality and safety are some of the last key applications of these techniques in food analysis [33, 101]. Furthermore, MALDI-MSI has found within the environmental research various applications in different fields, e.g., animal and plant biology, entomology as well as microbiology. In the latter, despite a simpler cell structure, MALDI-MSI has been applied to unravel the complex microbial communication network and the correlation between the localization of certain molecules and their biological function [55].

The other technique applied to characterize macromolecules is Py-GC-MS, recognized as a valuable technique in food and environmental chemistry [121]. Macromolecules are broken down by pyrolysis into simpler molecules that can be separated by GC and detected by MS [102]. The versatility of Py-GC-MS offers ample possibilities to be exploited in multiple applications related to food industry, from food authentication to the analysis of composition, quality, and additives, including contaminants as well [121]. In environmental analysis, Py-GC-MS has been applied to the chemical characterization of organic matter compounds in soil and sediment but also to assess the possible modifications that disturbances as forest fires or land use changes can cause in such compounds. Evaluation of the contamination by micro and nanoplastics of environmental samples has also become a hot

topic addressed by the application of this technique [102]. Most of the reported studies are focused on the robustness of the pyrolyzer and therefore, latest innovations in GC-MS have not been yet introduced but it is expected that Py-GC/MS takes advantage of the great separation power of GCxGC, the speed of rapid GC, and the identification capability that HRMS and HRMS in tandem can provide [103]. The large number of results obtained and the difficulty in interpreting them are constraints that also need to be considered [102].

There are two global trends related to the advances in the techniques that deserve to be highlighted. One is ion mobility spectrometry (IM-MS) that has grown dramatically over the last decade [95]. Ion mobility spectrometry (IMS) consists of separating ions in an electric field in the presence of a collision gas that separate them according to their shape (three-dimensional arrangement) and electrons [122]. Since both, IMS and MS are performed on ions in the gas phase, they can be easily combined into a single instrument – an ion mobility-mass spectrometer (IM-MS). IM-MS has been implemented in almost all the techniques related to mass spectrometry (e.g., LC-IM-MS, GC-IM-MS, IM-AIMS and IM-MSI, MALDI-IM-MS) [92, 96–99]. This can give an idea of its usefulness. Currently, the food industry utilizes IMS in nutritional analysis, food safety, food fingerprinting, and process control and quality assurance/quality control (QA/QC). Some of these categories are dominated by GC/LC-IM-MS instrument design and development, while others are focused on portable, inexpensive, and fast standalone IMS instrumentation. IM-MS enables the separation of molecules that have an isobaric mass, and in which chromatographic separation fails, becoming indispensable in these cases. Applications of IM-MS within the field of food chemistry involved metabolomics, proteomics, lipidomics including determination of lipopeptides as well as phenolic compounds, terpenes, volatile compounds, biogenic amines, etc. This has provided insights into the study on food control, food processing, authentication, and fraud [123]. In environmental analysis, it is a promising and powerful tool for the (non-) target and suspect analysis of small organic molecules in complex matrices.

The other current trend in mass spectrometry stems from the need to reduce pollution from chemical laboratories and to develop greener methods [41]. Chip-based separation devices coupled to mass spectrometry are a promising platform for food and environmental analysis, which will particularly replace traditional separation systems (LC-MS, GC-MS, and CE-MS) [42]. Compared to traditional systems, they offer several advantages: (1) they can provide quantitative and qualitative information of analytes at molecular level, (2) they have low cost and consume small volumes of samples and reagent, and (3) permit multi-step operations, since sample preparation, separation, and signal analysis can be simultaneously integrated into a single chip-based separation device [46]. Although important technology innovations aiming the fully-integration of chip-based separation devices and mass analyzers have been accomplished, their use for routine analyses is still under evaluation. Applications are still very scarce, but the prospects for the future of this reduction make research and improvement in this field necessary.

4 Conclusions

The full possibilities of MS within food and environmental analysis are far away to be reached. Short-term future trends of MS include the wider implementation of (ultra)high-resolution tandem mass analyzers providing high-mass accuracy, IM-MS (considering it as a second MS dimension), AIMS, and MSI among other widely applied techniques within this field. However, in the long term, it is expected that miniaturization, transportable systems that can measure at the sampling site and finally the implementation of the lab-on-chip will make the technique greener. It is expected that in the near future, the number of applications of these techniques will grow exponentially and that the advances in the mass spectrometers used will also develop rapidly.

Within the most classical, LC or GC combined with MS, challenges to analyze more contaminants faster, more efficiently, with low detection limits and to reduce false positive/false negative rates in complex samples place high demands on future improvements in instrumentation and techniques. Improvements in MS detectors' sensitivity, speed, selectivity, specificity, and wider dynamic linear range are expected. Features aimed to reduce instrumental downtime needed for maintenance, and new and improved ionization sources and interfaces, plus combinations of existing ion sources used simultaneously are expected.

Mass spectrometry is ideally suited for use in most areas of environmental and food analysis, including the determination of organic and inorganic contaminants, with special emphasis on emerging ones, characterization of environmental samples, analysis of food components, food processing, food quality/authenticity assessment, and analysis of food additives and contaminants. The actual and potential contributions of mass spectrometry to these areas are unlimited. Recent advances in mass spectrometry have provided new options to ensure food quality and safety, or to determine environmental contaminants, and to establish their fate in the environment as well as the characterization of different components of environmental compartments based mainly on profiling/fingerprinting, and metabolomics approaches. As recent progress demonstrates, chemometrics can be used in parallel with data collected from food samples to test food associations, to explore biological mechanisms, and to determine origin and authenticity. This will be also an area of future research within MS.

Acknowledgments This work has been supported by Grant RTI2018-097158-B-C31 funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe" and the grant of the Generalitat Valenciana Prometeo Programme CIPROM/2021/032.

References

1. Hossain A et al (2022) Next-generation climate-resilient agricultural technology in traditional farming for food and nutritional safety in the modern era of climate change. In: Plant abiotic stress physiology volume 1: responses and adaptations, pp 225–291
2. Andjelković U, Josić D (2018) Mass spectrometry based proteomics as foodomics tool in research and assurance of food quality and safety. Trends Food Sci Technol 77:100–119
3. Magi E, Di Carro M (2018) Marine environment pollution: the contribution of mass spectrometry to the study of seawater. Mass Spectrom Rev 37(4):492–512
4. Muter O, Bartkevics V (2020) Advanced analytical techniques based on high-resolution mass spectrometry for the detection of micropollutants and their toxicity in aquatic environments. Curr Opin Environ Sci Health 18:1–6
5. Talari G et al (2021) State of the art review of big data and web-based decision support systems (DSS) for food safety risk assessment with respect to climate change. Trends Food Sci Technol
6. Report GSD (2019) The future is now. Science for achieving sustainable development
7. Viitasalo M, Bonsdorff E (2022) Global climate change and the Baltic Sea ecosystem: direct and indirect effects on species, communities and ecosystem functioning. Earth Syst Dynam 13(2):711–747
8. Sabater S et al (2016) Shared effects of organic microcontaminants and environmental stressors on biofilms and invertebrates in impaired rivers. Environ Pollut 210:303–314
9. Navarro-Ortega A et al (2012) Assessing and forecasting the impacts of global change on Mediterranean rivers. The SCARCE consolider project on Iberian basins. Environ Sci Pollut Res 19(4):918–933
10. Hospido A, Membré JM (2022) Evaluation of climate change effects on food sustainability, safety and quality. Trends Food Sci Technol 126:205–206
11. Feliciano RJ et al (2022) Strategies to mitigate food safety risk while minimizing environmental impacts in the era of climate change. Trends Food Sci Technol
12. Summit WF (1996) Rome declaration on world food security
13. Duchenne-Moutien RA, Neetoo H (2021) Climate change and emerging food safety issues: a review. J Food Prot 84(11):1884–1897
14. Tripathi J, Variyar PS (2021) Food safety and methods to ensure food security in the face of climate change. CAB Rev Perspect Agric Vet Sci Nutr Nat Resour 16(15)
15. Proskuryakova LN, Loginova I (2021) Energy and environment: sustainable development goals and global policy landscape. In: Advanced sciences and technologies for security applications, pp 355–374
16. Scharlemann JPW et al (2020) Towards understanding interactions between sustainable development goals: the role of environment–human linkages. Sustain Sci 15(6):1573–1584
17. Racioppi F et al (2020) Reaching the sustainable development goals through healthy environments: are we on track? Eur J Public Health 30:I14–I18
18. Echendu AJ (2022) Flooding, food security and the sustainable development goals in Nigeria: an assemblage and systems thinking approach. Soc Sci 11(2)
19. Viana CM et al (2022) Agricultural land systems importance for supporting food security and sustainable development goals: a systematic review. Sci Total Environ 806
20. Rashidi Chegini K et al (2021) Is there a linkage between household welfare and income inequality, and food security to achieve sustainable development goals? J Clean Prod 326
21. Griffiths J (2008) A brief history of mass spectrometry. Anal Chem 80(15):5678–5683
22. Giuffrida D, Zoccali M, Mondello L (2020) Recent developments in the carotenoid and carotenoid derivatives chromatography-mass spectrometry analysis in food matrices. Trends Anal Chem 132
23. Xiao Y et al (2022) Determination of Antibiotic residues in aquaculture products by liquid chromatography tandem mass spectrometry: recent trends and developments from 2010 to 2020. Separations 9(2)

24. Balaram V (2021) Strategies to overcome interferences in elemental and isotopic geochemical analysis by quadrupole inductively coupled plasma mass spectrometry: a critical evaluation of the recent developments. Rapid Commun Mass Spectrom 35(10)
25. Collins SL et al (2021) Current challenges and recent developments in mass spectrometry-based metabolomics. In: Annual review of analytical chemistry, pp 467–487
26. Sylvester PJ, Jackson SE (2016) A brief history of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Elements 12(5):307–310
27. Ohls K, Bogdain B (2016) History of inductively coupled plasma atomic emission spectral analysis: from the beginning up to its coupling with mass spectrometry. J Anal At Spectrom 31(1):22–31
28. Chien HJ et al (2022) Determination of adulteration, geographical origins, and species of food by mass spectrometry. Mass Spectrom Rev
29. El-Shazly M et al (2016) Use, history, and liquid chromatography/mass spectrometry chemical analysis of aconitum. J Food Drug Anal 24(1):29–45
30. Zhong P et al (2022) Untargeted metabolomics by liquid chromatography-mass spectrometry for food authentication: a review. Compr Rev Food Sci Food Saf 21(3):2455–2488
31. Fisher CM, Croley TR, Knolhoff AM (2021) Data processing strategies for non-targeted analysis of foods using liquid chromatography/high-resolution mass spectrometry. Trends Anal Chem 136
32. Putri SP et al (2022) Application of gas chromatography-mass spectrometry-based metabolomics in food science and technology. J Biosci Bioeng 133(5):425–435
33. Adebo OA et al (2021) Application of gas chromatography–mass spectrometry (GC-MS)-based metabolomics for the study of fermented cereal and legume foods: a review. Int J Food Sci Technol 56(4):1514–1534
34. Suman M et al (2021) Fighting food frauds exploiting chromatography-mass spectrometry technologies: scenario comparison between solutions in scientific literature and real approaches in place in industrial facilities. Trends Anal Chem 142
35. Lu H et al (2018) Ambient mass spectrometry for food science and industry. Trends Anal Chem 107:99–115
36. Stilo F et al (2021) Untargeted approaches in food-omics: the potential of comprehensive two-dimensional gas chromatography/mass spectrometry. Trends Anal Chem 135
37. Filho JFA et al (2020) Ambient ionization mass spectrometry in food metabolomics. In: Comprehensive foodomics, pp 54–76
38. Arrizabalaga-Larrañaga A et al (2020) Ambient ionization mass spectrometry in food analysis. In: Food toxicology and forensics, pp 271–312
39. Yoshimura Y, Zaima N (2020) Application of mass spectrometry imaging for visualizing food components. Foods:**9**(5)
40. Morisasa M et al (2019) Application of matrix-assisted laser desorption/ionization mass spectrometry imaging for food analysis. Foods 8(12)
41. Owens JE et al (2016) Analysis of whiskey by dispersive liquid-liquid microextraction coupled with gas chromatography/mass spectrometry: an upper division analytical chemistry experiment guided by green chemistry. J Chem Educ 93(1):186–192
42. Oedit A et al (2015) Lab-on-a-chip hyphenation with mass spectrometry: strategies for bioanalytical applications. Curr Opin Biotechnol 31:79–85
43. Molina-Díaz A et al (2019) Ambient mass spectrometry from the point of view of green analytical chemistry. Curr Opin Green Sustain Chem 19:50–60
44. Picó Y, Barceló D (2021) Analysis of microplastics and nanoplastics: how green are the methodologies used? Curr Opin Green Sustain Chem 31
45. Picó Y, Barceló D (2022) Micro(Nano)plastic analysis: a green and sustainable perspective. J Hazard Mater Adv 6:100058
46. Balogh MP (2009) The reality of lab-on-a-chip technology for the mass spectrometry laboratory. LCGC N Am 27(10):900–915

47. Carmona E, Picó Y (2018) The use of chromatographic methods coupled to mass spectrometry for the study of emerging pollutants in the environment. Crit Rev Anal Chem 48(4):305–316
48. Marzano V et al (2020) Perusal of food allergens analysis by mass spectrometry-based proteomics. J Proteomics 215
49. Domínguez I, Frenich AG, Romero-González R (2020) Mass spectrometry approaches to ensure food safety. Anal Methods 12(9):1148–1162
50. Cao G et al (2020) Mass spectrometry for analysis of changes during food storage and processing. J Agric Food Chem 68(26):6956–6966
51. Giorgi G (2020) Mass spectrometry methods for food safety/detection of toxins in food. In: NATO science for peace and security series A: chemistry and biology, pp 47–60
52. Pang GF (2018) Analytical methods for food safety by mass spectrometry: volume II veterinary drugs. 1-656
53. Pang GF (2018) Analytical methods for food safety by mass spectrometry. 1:1–880
54. Das S, Bhatia R (2022) Liquid extraction surface analysis-mass spectrometry: an advanced and environment-friendly analytical tool in modern analysis. J Sep Sci
55. Sgobba E, Daguerre Y, Giampà M (2021) Unravel the local complexity of biological environments by MALDI mass spectrometry imaging. Int J Mol Sci 22(22)
56. Bai Q et al (2021) Flow field-flow fractionation hyphenated with inductively coupled plasma mass spectrometry: a robust technique for characterization of engineered elemental metal nanoparticles in the environment. Appl Spectrosc Rev
57. Díaz-Cruz MS, Barceló D (2006) Determination of antimicrobial residues and metabolites in the aquatic environment by liquid chromatography tandem mass spectrometry. Anal Bioanal Chem 386(4):973–985
58. Lopez De Alda MJ et al (2003) Liquid chromatography-(tandem) mass spectrometry of selected emerging pollutants (steroid sex hormones, drugs and alkylphenolic surfactants) in the aquatic environment. J Chromatogr A 1000(1–2):503–526
59. Schneider BB et al (2016) Differential mobility spectrometry/mass spectrometry history, theory, design optimization, simulations, and applications. Mass Spectrom Rev 35(6):687–737
60. Zhang X, Ren X, Chingin K (2021) Applications of direct analysis in real time mass spectrometry in food analysis: a review. Rapid Commun Mass Spectrom 35(6)
61. Giesen C et al (2012) History of inductively coupled plasma mass spectrometry-based immunoassays. Spectrochim Acta B At Spectrosc 76:27–39
62. Valletta M et al (2021) Mass spectrometry-based protein and peptide profiling for food frauds, traceability and authenticity assessment. Food Chem:365
63. Park MK, Kim YS (2021) Mass spectrometry based metabolomics approach on the elucidation of volatile metabolites formation in fermented foods: a mini review. Food Sci Biotechnol 30(7):881–890
64. Vidmar J (2021) Detection and characterization of metal-based nanoparticles in environmental, biological and food samples by single particle inductively coupled plasma mass spectrometry. In: Comprehensive analytical chemistry, pp 345–380
65. Dou X et al (2022) Mass spectrometry in food authentication and origin traceability. Mass Spectrom Rev
66. McGorrin RJ (2009) One hundred years of progress in food analysis. J Agric Food Chem 57(18):8076–8088
67. Honda, M. and H. Nishi. Household nutrition analysis and food recommendation U sing purchase history. 2021
68. Wu F, Rodricks JV (2020) Forty years of food safety risk assessment: a history and analysis. Risk Anal 40:2218–2230
69. Zhang X (2019) Mass spectrometry for food quality and safety evaluation. In: Evaluation technologies for food quality, pp 405–438
70. Martínez-Bueno MJ et al (2019) An overview of non-targeted screening strategies based on high resolution accurate mass spectrometry for the identification of migrants coming from plastic food packaging materials. Trends Anal Chem 110:191–203

71. Carson R et al (2002) Silent spring. Houghton Mifflin
72. Unger RW (2010) Introduction: Hoffmann in the historiography of environmental history. In: Brill's series in the history of the environment, pp 1–21
73. Pico Y, Alfarhan AH, Barcelo D (2020) How recent innovations in gas chromatography-mass spectrometry have improved pesticide residue determination: an alternative technique to be in your radar. Trends Anal Chem:122
74. van Leeuwen KA et al (2014) Gas chromatography-combustion-isotope ratio mass spectrometry for traceability and authenticity in foods and beverages. Compr Rev Food Sci Food Saf 13(5):814–837
75. Janssen HG, Cicourel AG, Tranchida PQ (2020) Chapter 4: conventional gas chromatography: mass spectrometry hyphenation and applications in food analysis. In: Food chemistry, function and analysis, pp 131–165
76. Dymerski T (2018) Two-dimensional gas chromatography coupled with mass spectrometry in food analysis. Crit Rev Anal Chem 48(4):252–278
77. Khan MR, Deng R, Öz F (2021) Current developments and trends in the liquid chromatography-mass spectrometry study of food integrity and authenticity. In: Chromatographic and related separation techniques in food integrity and authenticity, pp 25–41
78. Creydt M, Fischer M (2020) Food phenotyping: recording and processing of non-targeted liquid chromatography mass spectrometry data for verifying food authenticity. Molecules 25(17)
79. Guo Z et al (2020) Non-targeted screening of pesticides for food analysis using liquid chromatography high-resolution mass spectrometry – a review. Food Addit Contam A Chem Anal Control Expo Risk Assess 37(7):1180–1201
80. Antunes M et al (2020) Liquid chromatography-mass spectrometry as a tool to identify adulteration in different food industries. In: Food toxicology and forensics, pp 123–180
81. Rasmussen HT, Huang K (2012) 8.7 Chromatographic separations and analysis: chromatographic separations and analysis of enantiomers. In: Comprehensive chirality, pp 96–114
82. Ilisz I, Pataj Z, Péter A (2010) Macrocyclic glycopeptide-based chiral stationary phases in high performance liquid chromatographic analysis of amino acid enantiomers and related analogs. In: Macrocyclic chemistry: new research developments, pp 129–157
83. Manaj S, Kim ST (2021) Techniques for measuring carbon and oxygen isotope compositions of atmospheric CO2 via isotope ratio mass spectrometry. Rapid Commun Mass Spectrom 35(4)
84. Mohr W et al (2014) Protein stable isotope fingerprinting: multidimensional protein chromatography coupled to stable isotope-ratio mass spectrometry. Anal Chem 86(17):8514–8520
85. Zhang Y et al (2012) Calibration and data processing in gas chromatography combustion isotope ratio mass spectrometry. Drug Test Anal 4(12):912–922
86. Chang PP et al (2022) ICP-MS-based methodology in metallomics: towards single particle analysis, single cell analysis, and spatial Metallomics. At Spectrosc 43(3):255–265
87. Flores K et al (2021) Environmental applications and recent innovations in single particle inductively coupled plasma mass spectrometry (SP-ICP-MS). Appl Spectrosc Rev 56(1):1–26
88. Montaño MD et al (2016) Single particle ICP-MS: advances toward routine analysis of nanomaterials. Anal Bioanal Chem 408(19):5053–5074
89. Silva C et al (2020) Forensic attribution profiling of food using liquid chromatography-mass spectrometry. In: Food toxicology and forensics, pp 97–121
90. Stachniuk A et al (2019) Liquid chromatography–mass spectrometry bottom-up proteomic methods in animal species analysis of processed meat for food authentication and the detection of adulterations. Mass Spectrom Rev
91. Bianchi F, Careri M (2020) Mass spectrometry in food authenticity and traceability. In: Food authentication and traceability, pp 101–130
92. McCann A et al (2021) Rapid visualization of lipopeptides and potential bioactive groups of compounds by combining ion mobility and MALDI imaging mass spectrometry. Drug Discov Today Technol 39:81–88

93. Lorenc W et al (2022) LC/ICP-MS and complementary techniques in bespoke and nontargeted speciation analysis of elements in food samples. Mass Spectrom Rev 41(1):32–50
94. Perini M, Bontempo L (2022) Liquid chromatography coupled to isotope ratio mass spectrometry (LC-IRMS): a review. Trends Anal Chem 147
95. Paglia G, Smith AJ, Astarita G (2022) Ion mobility mass spectrometry in the omics era: challenges and opportunities for metabolomics and lipidomics. Mass Spectrom Rev 41(5): 722–765
96. Delvaux A, Rathahao-Paris E, Alves S (2022) Different ion mobility-mass spectrometry coupling techniques to promote metabolomics. Mass Spectrom Rev 41(5):695–721
97. Moran-Garrido M et al (2022) Recent developments in data acquisition, treatment and analysis with ion mobility-mass spectrometry for lipidomics. Proteomics
98. Kohoutek KM, Harrington PDB (2021) Electrospray ionization ion mobility mass spectrometry. Crit Rev Anal Chem
99. Charles L, Chendo C, Poyer S (2020) Ion mobility spectrometry – Mass spectrometry coupling for synthetic polymers. Rapid Commun Mass Spectrom 34(S2)
100. Zambonin C (2021) Maldi-tof mass spectrometry applications for food fraud detection. Appl Sci 11(8)
101. Calvano CD et al (2021) Proteomic analysisof food allergens by MALDI TOF/TOF mass spectrometry. In: Methods in molecular biology, pp 357–376
102. Picó Y, Barceló D (2020) Pyrolysis gas chromatography-mass spectrometry in environmental analysis: focus on organic matter and microplastics. Trends Anal Chem 130
103. Sobeih KL, Baron M, Gonzalez-Rodriguez J (2008) Recent trends and developments in pyrolysis-gas chromatography. J Chromatogr A 1186(1-2):51–66
104. Patriarca M et al (2021) Atomic spectrometry update: review of advances in the analysis of clinical and biological materials, foods and beverages. J Anal At Spectrom 36(3):452–511
105. Natasha et al (2020) A critical review of mercury speciation, bioavailability, toxicity and detoxification in soil-plant environment: Ecotoxicology and health risk assessment. Sci Total Environ:711
106. Wang F et al (2019) How closely do mercury trends in fish and other aquatic wildlife track those in the atmosphere? – implications for evaluating the effectiveness of the Minamata convention. Sci Total Environ 674:58–70
107. Butler OT et al (2018) Atomic spectrometry update-a review of advances in environmental analysis. J Anal At Spectrom 33(1):8–56
108. Gentili A, Fanali S, Mainero Rocca L (2019) Desorption electrospray ionization mass spectrometry for food analysis. Trends Anal Chem 115:162–173
109. Sindona G, Di Donna L (2020) Evaluation of quality and safety of foods by Tandem mass spectrometry. In: NATO science for peace and security series A: chemistry and biology, pp 1–10
110. Yan XT et al (2022) Technical overview of orbitrap high resolution mass spectrometry and its application to the detection of small molecules in food (update since 2012). Crit Rev Anal Chem 52(3):593–626
111. Gavage M, Delahaut P, Gillard N (2021) Suitability of high-resolution mass spectrometry for routine analysis of small molecules in food, feed and water for safety and authenticity purposes: a review. Foods 10(3)
112. Reisdorph N et al (2020) Metabolomics mass spectrometry data processing: applications in food analysis. In: Comprehensive foodomics, pp 339–352
113. Lacalle-Bergeron L et al (2021) Chromatography hyphenated to high resolution mass spectrometry in untargeted metabolomics for investigation of food (bio)markers. Trends Anal Chem 135
114. Diez-Simon C, Mumm R, Hall RD (2019) Mass spectrometry-based metabolomics of volatiles as a new tool for understanding aroma and flavour chemistry in processed food products. Metabolomics 15(3)

115. Picó Y, Barceló D (2021) Mass spectrometry in wastewater-based epidemiology for the determination of small and large molecules as biomarkers of exposure: toward a global view of environment and human health under the COVID-19 outbreak. ACS Omega 6(46): 30865–30872
116. Beneito-Cambra M et al (2020) Ambient (desorption/ionization) mass spectrometry methods for pesticide testing in food: a review. Anal Methods 12(40):4831–4852
117. Klampfl CW (2018) Ambient mass spectrometry in foodomics studies. Curr Opin Food Sci 22: 137–144
118. Ueki R, Fukusaki E, Shimma S (2022) History of hair analysis by mass spectrometry imaging. J Biosci Bioeng 133(2):89–97
119. Bräcker J, Brockmeyer J (2018) Characterization and detection of food allergens using high-resolution mass spectrometry: current status and future perspective. J Agric Food Chem 66(34):8935–8940
120. Pilolli R et al (2020) Critical review on proteotypic peptide marker tracing for six allergenic ingredients in incurred foods by mass spectrometry. Food Res Int 128
121. Ma S et al (2018) Soil organic matter chemistry: based on pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS). Mini-Rev Org Chem 15(5):389–403
122. Gabelica V (2022) Chapter 1 ion mobility–mass spectrometry: an overview. In: Ion mobility-mass spectrometry: fundamentals and applications. The Royal Society of Chemistry, pp 1–25
123. Hernández-Mesa M et al (2019) Ion mobility spectrometry in food analysis: principles, current applications and future trends. Molecules 24(15)

Food and Environmental Samples Handling and Preparation for Mass Spectrometry

Philiswa Nosizo Nomngongo, Azile Nqombolo, and Anele Mpupa

Contents

Abstract In current food safety and environmental monitoring, mass spectrometry methodologies are widely used for identification and quantification of various substances. However, studies have shown that various compounds in food and environment samples are found at ultra-trace concentration levels and the complexity of the sample matrix might limit the performance of selective and sensitive analytical method. Therefore, selection of a proper sampling strategy and sample preparation methods plays a critical role in achieving accurate and reliable results. Thus, this chapter is mainly focused on the recent trends in sample handling and preparation of food and environmental samples prior to MS analysis. Methodologies regarding mass spectrometry coupled with chromatographic separation have been reviewed to highlight the improvement in the analytical determinations of various compounds in food and environmental samples.

Keywords Environmental monitoring, Food samples, Sample handling, Solid phase extraction, Stir-bar-sorptive extraction

P. N. Nomngongo (✉), A. Nqombolo, and A. Mpupa
Department of Chemical Sciences, University of Johannesburg, Johannesburg, South Africa

Department of Science and Innovation-National Research Foundation South African Research Chair Initiative (DSI-NRF SARChI): Nanotechnology for Water, University of Johannesburg, Doornfontein, South Africa
e-mail: pnnomngongo@uj.ac.za

Yolanda Picó and Julian Campo (eds.), *Mass Spectrometry in Food and Environmental Chemistry*, Hdb Env Chem (2023) 119: 25–52, DOI 10.1007/698_2022_891,

Published online: 7 October 2022

1 Introduction

The developments in food and environmental sample analysis have become more challenging over the past decades [1, 2]. This is due to complexity of the sample matrices as well as presence of mixtures of substances, which are continuously changing. Food samples usually encompass wide range of physical types including biological matrices (such as meat), liquids (beverages, milk) dry powders and solids, among others [3]. Environmental samples, on the other hand, include water, sediments, soil, air, biological materials (plants), and wastes (liquids, solids, or sludges) [4]. The variability of the nature of these sample matrices thus demands rapid, reliable, accurate, and sensible analytical methods. Recently, major trends in food and environmental analysis are focused on the development of rapid and efficient methods for trace analysis of organic compounds (both targeted and nontargeted) [5]. Despite the recent advancements in analytical instrumentation (especially mass spectrometry, MS), sample handing and preparation prior to MS detection are still mandatory.

MS is one of the most powerful analytical techniques that are widely used for qualitative and quantitative analysis of a wide range of analytes in food and environmental matrices [6–8]. This is due to its attractive features such as selectivity, high detectability, and rapidity [6–8]. However, to achieve quality results from an MS instrument, classical analytical workflow including sampling, sample handling, sample preparation (extraction, pre-concentration, clean-up) as well as chromatographic separation is required [8, 9]. It is important to note that every step of the analytical workflow is critical for analytical method performance but bottleneck steps such as sample preparation and separation become the rate determining steps of the overall analysis process [10, 11]. In cases where rapid or urgent decisions need to be made (especially in the area of food safety), the use of fast, sensitive, efficient, and reliable analytical methods is critical [8, 12, 13]. Therefore, there is a call for development of alternative methods that minimize the lengthy analytical workflows. This is achieved by developing improved and efficient sample preparation steps while skipping the chromatographic separation, thus resulting to direct analysis of pretreated samples using MS [12, 14, 15].

Sample preparation prior to the determination of analytes in food and environmental matrices using MS involves critical steps, chiefly because of the complexity of the sample composition as well as low concentrations of many analytes [16, 17]. Therefore, the isolation of target analytes from food and environmental samples requires careful selection of a suitable extraction and pre-concentration procedure [18]. The selection of the sample preparation method is subject to complexity of the sample matrix, chemical structure, and characteristics of the analytes of interests [16, 17]. This chapter gives a summary of recent progress in regard to the development of sample handling and preparation methods in the areas of food and environmental analysis.

2 Sample Handling and Preparation

2.1 Sample Handling and Collection

Chemicals in food and environmental samples exhibit different physical and chemical properties. For instance, diffusion of different pollutants from various sources into water systems is strongly influenced by their characteristics such as partition coefficient, polarity, volatility as well as the properties of the interacting media [19, 20]. As the results, more hydrophilic substances are mostly found in water samples while hydrophobic compounds (those with log $K_{ow} > 4$) are retained in sediments and suspended matter [19]. Therefore, food and environment sample handling/collection becomes one of the most significant stages because improper collection, processing, and storage could alter the quality of the samples as well as chemical or molecular structures of the target analytes [21, 22]. Among the steps taking place prior to analyte extraction, detection, and quantification, sample collection plays a vital role because a sketchy sampling can lead to incorrect results regardless of flawlessness of the remaining analytical steps [23]. Therefore, the sampling step should be carried out in such way that any aliquot of food and environmental sample taken for analysis must be a good representative of the original bulk sample [23]. For example, it is recommended that for solid food and environmental samples an appropriate crushing must be used to attain smaller representative sample sizes [23, 24]. The amount of sample to be used for further analysis depends on the type of analysis required. For trace analysis in food and environmental samples, larger sample portions are normally required to achieve low detection limits. However, for the analysis of sample composition smaller portions of samples are often used [22, 25].

2.1.1 Sampling Strategies for Environmental and Food Samples

Sampling of environmental samples depends on the nature of the sample. As such, several sampling strategies have been reported in the literature. These include passive sampling, grab or discrete sampling at a specific time, time-integrated sampling, and event-based sampling [19, 26]. Grab samples are used to provide a snapshot of the water body or solid food and environmental samples at the specific collecting time and location. Grab sampling method is simple and cost effective but it does not fully represent the status of the water body [19, 26–28]. Once the samples are collected by grab sampling technique, they are usually kept in pre-cleaned bottles (glass amber bottles for organic substances and polypropylene bottles for trace metals analysis) and stored at 4°C until pretreatment. Time-integrated sampling techniques are individual time- or flow-proportional samples collected in individual containers [19, 26]. The collected time-integrated samples are combined to form a composite sample before they are subjected to a specific sample preparation method [19, 26]. Event-based sampling is often conducted at times when high concentration

of pollutants is expected [19, 26]. These events include heavy chemical spills, heavy rainfalls, oil spill, improper industrial and sewage discharges. Event-based sampling can include grab or in-situ time-integrated samples. Passive sampling utilizes samplers that are deployed in water over a set of time to accumulate pollutants in a time-weighted average or equilibrium concentrations [19, 28]. Passive sampling uses one device per selected study area as compared with liters of grab water samples [19, 28]. Passive sampling devices comprise of an adsorbent that is placed between two membranes which allows the analytes to pass through the sorbent and be extracted from the water [19, 28, 29]. Several review articles have extensively discussed the principles of different passive samplers as well as their application in environmental analysis [28–31].

2.2 Sample Preparations

Sample preparation is the major bottleneck in food and environmental analysis because of the complexity of the sample matrices and ultra-trace concentration of target analytes [32]. The selection of a suitable sample preparation method depends on various factors such as chemical properties of the analytes, sample matrix, and the analytical detection method [23, 33]. Recently, attempts have been made toward the development of various sample preparation methods from extraction and enrichment of numerous analytes with different chemical properties in food and environmental samples. These methods include solid and liquid phase-based extraction methods.

2.2.1 Solid Phase Extraction-Based Methods

Solid phase extraction (SPE) is one of the most used sample preparation techniques [34–36]. SPE is based on the separation and distribution of analyte(s) between a solid adsorbent and a liquid mobile phase [37]. One of the main advantages of using SPE-based methods is the diverse choice of sorbents and the ease of modification [38]. The main steps of SPE include activation of sorbent, analyte retention, removal of interferences, and elution of analytes [35]. These steps can be varied/modified depending on the nature of the sorbent used [39].

Traditional SPE has drawbacks such as solvent and time consumption [35, 40] and this has led to the development of modified SPE methods [42]. These include techniques such as (1) solid phase microextraction, (2) stir bar-sorptive extraction, (3) matrix solid phase dispersion, and (4) in-tube solid phase microextraction. These sample preparation techniques have been extensively reviewed in recent years [23, 34, 35, 38, 41–44]. The selection of a suitable analysis method depends on the sample matrix nature as well as the type of analytes. For example, in the head space solid phase microextraction (HS-SPME) there is no direct contact between the fiber and the sample [23, 45]. In contrast, in the direct immersion SPME (DI-SPME),

the extracting phase is directly immersed on the sample for direct analyte transfer [46].

Application of Solid Phase-Based Extraction Methods in Food Samples

Currently, a simple "solid phase extraction" search on web of science returns 15,781 results. The same search modified to show studies from the last 5 years (2017–2021) gives 5,967 while modifying the search with "mass spectrometry (MS)" returns 3,305 results. This shows the vast usage of SPE-based techniques combined with MS for residue analysis. In the past years, solid phase-based extraction methods combined with MS methodologies have received growing interest in food analysis which include food contaminants and components (Table 1). These MS based methodologies include liquid chromatography mass spectrometry (LC-MS) [47], gas chromatography mass spectrometry (GC-MS) [48], high-resolution mass spectrometry (HRMS) [49–51], matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) [52], LC coupled to tandem mass spectrometry-electrospray ionization or atmospheric pressure chemical ionization (LC-ESI-MS/MS or LC-APCI-MS/MS) [53], MALDI-time-of-flight tandem mass spectrometry (MALDI-TOF-MS) [54], LC-quadrupole-TOF (LC-Q-TOF-MS/MS) [55, 56], and GC-MS/MS [57, 58]. Table 1 suggests that a large number of MS-based method for analysis of food samples mostly utilizes SPE and other related procedures as preliminary extraction, clean-up and pre-concentration step. In these methods various adsorbent materials are selected based on the nature of the analytes of interest.

A study reported by Zhao et al. developed a magnetic solid phase extraction (MSPE) using polydopamine functionalized magnetic core-shell mesoporous silica nanoparticles (Fe_3O_4@nSiO_2@mSiO_2-PDA) as an adsorbent. The developed MSPE method was coupled with ultra-high-performance liquid chromatography coupled with Q-Exactive high-resolution mass spectrometry (UHPLC-Q-Exactive HRMS) for extraction, pre-concentration, and determination of fipronil and its metabolites in egg samples. The method showed limits of quantification (LOQs) 0.006–0.010 μg/kg and limits of detection (LODs) 0.002–0.004 μg/kg. Repeatability 4.4–10.7% inter-day and 2.2–6.7% intra-day precision were obtained with recoveries in the range of 80.6–95.3% [59]. LC-HRMS coupled with dispersive micro-SPE was used for the determination of carcinogenic 4-methylimidazole (4-MEI) in food from roasting process and cooking. The analyte was extracted from vinegar, tea, coffee, and soy sauce [60]. The determination of Bisphenol A (BPA) carried out by LC-ESI-MS using a microwave assisted extraction showed relative standard deviation (RSD %) of 7–10% with low LODs in the ng/g level. The authors reported that concentrations of BPA in canned food were in the range of 7.3 and 42.3 ng/g [61]. Liu et al. reported the use of novel core-shell mesoporous silica (CSMS) microspheres coated by titanium dioxide (CSMS@TiO2) as a sorbent for extraction of phosphorus-containing amino-acid-like herbicides from baby food. They developed a dispersive SPE coupled with capillary electrophoresis-mass spectrometry (CE-MS). LOQs were in the range of 0.3–1.6 ng/mL with excellent repeatability (intra/inter-day,

Table 1 Selected applications of solid phase-based extraction methods in food samples

Techniques	Samples	Analytes	Performance	References
SPE UPLC-MS/MS	Food	Bisphenol A	LOD:0.01 μg/L LOQ: 0.04 μg/L RSD <1% ($n = 5$) <3% ($n = 5$)	[66]
SPE-UHPLC-ESI-MS/MS	Milk, animal tissues and eggs	Gamithromycin	LOQ:0.80–1.0 μg/kg LOD:0.30–0.40 μg/kg	[67]
SPE-UHPLC-MS/MS	Beef, eggs, milk, pork, and lamb	Diuretics	LOQs: 0.53–2.43 μg/kg	[68]
MIP-PE HPLC-MS/MS	Pork	Sulfonamides (SAs)	Concentrations: 3.79–3.57 μg/kg	[69]
d-SPE-UHPLC-MS/MS	Chicken, sheep tissue, bovine & swine	11 macrolides	LOQs:5.0 μg/kg LODs: 0.1–2.0 μg/kg	[70]
d-micro SPE LC-HRMS	Swine body fluids	Fluoroquinolones	LOQs: 0.06–0.1 μg/L LODs: 0.02–0.03 μg/L	[71]
DSPE-LC-APCI-HRMS/MS	Coconut milk	Pesticide residues	LODs: 0.5–5 ng/g LOQs: 1–10 ng/g Concentrations: 5.54–206.99 ng/g.	[72]
DSPE-GC-APCI Q-TOF	Orange, chili pepper, and brown rice	Pesticide multiresidues	LODs: 0.11–0.6 μg/mL LOQs: 0.34–1.9 μg/mL Recoveries: 70–120%	[73]
SPME-GC-MS/MS	Beverages	Parabens, phenolic antioxidants, sulfonamide plasticizer, and flame retardant	LODs: 0.005–0.2 μg/L %RSD: 0.8–5.4% Recoveries: 98–109% Concentrations: 4.75–2220.99 μg/L	[58]
d-micro SPE-LC-HRMS	Milk and honey samples	Sulfonamides	LODs: 0.003–0.2 μg/L; 0.01–1 μg/kg	[74]

(continued)

Table 1 (continued)

Techniques	Samples	Analytes	Performance	References
			%RSD: 1.3–10.4% Recoveries: 68.8–115.8% Concentrations: 0.3–9.7 μg/L; (3.9 μg/kg	
μ-SPE-GC-orbitrap-MS	Cereals	Pesticide residues	LODs: 0.005–0.01 mg/kg %RSD: <20% Recoveries: 70–120%	[75]
MIP-μ-SPE/LC-MS/MS	Non-dairy beverages	Aflatoxins (AFB1 and AFB2)	LODs: 0.085–0.207 μg/L %RSD: <18% Recoveries: 91–104% Concentrations: 0.230–0.632 μg/L	[76]
MIP-μ-SPE/HPLC-MS/MS	Cultured fish	Aflatoxins	LODs: 0.29–0.61 μg/kg %RSD: <20% Recoveries: 80–100%	[77]
D-μ-SPE/GC-MS	Honey samples	Polycyclic aromatic hydrocarbons	LODs: 0.14–0.37 ng/g %RSD: <7.5% Recoveries: 72–81%.	[78]
MSPE/HPLC-MS/MS	Milk, pork meat, and fish meat	Sulfonamide antibiotics	LODs: 0.40–1.5 ng/g %RSD: <10% Recoveries: 0.40–1.5 ng/g	[79]
MSPE/HPLC-MS/MS	Milk and meat samples	Fluoroquinolones	LODs: 0.7–6.0 ng/L %RSD: >10% Recoveries: 75.3–104.7%. Concentrations: 0–8.60 μg/kg	[80]
SPE/UPLC-MS/MS	Chicken meat; eggs	Quinolone antibiotics	LODs: 0.1–0.16 μg/kg Recoveries: 66.9–99.0% Concentrations: 6.22–44.4 μg/kg	[81]

(continued)

Table 1 (continued)

Techniques	Samples	Analytes	Performance	References
MSPE–LC–MS/MS	Milk and pork samples.	β-agonists and fluoroquinolones	LODs: 0.1–0.2 ng/g %RSD: <6.8% Recoveries: 94.5–105.8% Concentrations: 0.12–0.42 ng/g	[82]
MI-SPE/LC-MS/MS	Various food samples	Patulin	LOD: 0.05–0.2 ng/g %RSD: <4.5% Recovery: 81.3–106.3%	[83]

SPE solid phase extraction, *UPLC-MS/MS* ultra-performance liquid chromatography coupled to tandem mass spectrometry, *SPME–GC–MS/MS* solid phase microextraction – coupled to a gas chromatography–ion trap tandem mass spectrometry, *GC-APCI-Q-TOF* gas chromatography-atmospheric pressure chemical ionization quadrupole time-of-flight, *DSPE/d-SPE* dispersive solid phase extraction, *UHPLC-ESI-MS/MS* ultra-high performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry, *LC-APCI-HRMS/MS* liquid chromatography-atmospheric pressure chemical ionization high-resolution tandem mass spectrometry, *HPLC-MS/MS* high-performance liquid chromatography with tandem mass spectrometry, *LC-HRMS* liquid chromatography-high-resolution mass spectrometry, *MIP-SPE* molecularly imprinted polymer based solid phase extraction, *d-micro SPE* dispersive micro solid phase extraction, *μ-SPE* microsolid-phase extraction system, *GC-Orbitrap-MS* gas chromatography-orbitrap mass spectrometry

RSDs of 2.1–8.3%, $n = 5$) [62]. Sun et al. reported a novel sample preparation method based on pipette-tip microsolid phase extraction (PT-μSPE) coupled corona discharge ionization-ion mobility spectrometry (CD-IMS) for the determination of benzodiazepines (BZDs) in dietary supplements. The PT-μSPE was fabricated using poly (styrene-co-divinylbenzene) (St-co-DVB) monoliths via an in-situ polycondensation reaction [63]. The LODs of the developed method ranged from 5 to 15 ng mL^{-1}. Furthermore, the developed PT-μSPE-IMS was applied in spiked samples and acceptable recoveries ranging from 84.2 to 112.1%.

Combination of MS based techniques with suitable solid SPE/SPME procedures allows the detection of food adulteration. For instance, Peng et al. conducted a study on determination of maltol, ethyl maltol, vanillin, and ethyl vanillin in foods using isotope dilution headspace SPME coupled with GC-MS. Under optimum conditions, the LODs of the SPME-GC-MS were 0.005–0.50 μg/g and concentration of ethyl maltol and vanillin in milk tea were up to 833 μg/g and 1,044 μg/g, respectively. Moreover, concentrations ranging from 0.286 to 1.65 μg/g for maltol, ethyl maltol, and vanillin were detected in blended sesame oil [64]. In another study, Peng et al. investigated the possibility of adulteration of rice using synthetic aromatic flavorings. The synthetic aromatic flavors are used to deceitfully mimic commercially valuable fragrant rice varieties [65]. In their study, the authors applied HS-SPME coupled with GC-MS using selected ion monitoring (GC–MS–SIM) for determination of 2-acetylpyrazine (2-APZ), 2-acetylthiazole (2-ATZ), 2-acetylpyrrole (2-APR), and 2-acetylpyridine (2-APD). The HS-SPME/GC–MS-SIM method had

LODs ranging from 0.5 to 10 ng/mL, thus allowing the analysis of volatiles in rice with minimal pretreatment. The commercially available fragrant rice samples were found to contain 2-ATZ, 2-APD, and 2-APR 2 at the concentrations ranging from 0 0.018 to 0.035 mg/kg, respectively. Other applications of solid phase-based techniques shown in Table 1 revealed high recoveries, low LOQs and LODs in most of the studies, allowing quantitative analysis of various analytes.

Application of Solid Phase-Based Extraction Methods in Environment Samples

A web of science search for "solid phase extraction, environment" for the last 5 years returns 7,834 results, similarly showing the extensive use of SPE in environmental monitoring studies. This is because SPE and related procedures have the capability to simultaneously extract large number of compounds in a sample thus warranting them to be simple, rapid, and somehow generic sample preparation methods. By extracting large number of analytes at the same time, the cost, time, and labor are significantly reduced. Table 2 summarizes some of the studies conducted in environmental analysis using SPE-based methods. Similar to food samples, solid phase-based methods allow the analysis of wide range of complex environmental matrices. The application of these methods leads to remarkable analytical performance which achieves better separation by the chromatographic technique and more effective quantification/identification of analytes. Coupling MS methodologies with suitable sample preparation method allows accurate identification of contamination sources. For example, it is suspected that plastic bottles containing bisphenol A (BPA) may release this compound into the drinking water they contain. A study using Bond-Elut-C18 SPE combined with UPLC-MS/MS method was developed and applied in extraction of BPA from bottled water (glass and plastic). The analytical characteristics of the developed method in terms of LOQ, LOD, and intra/inter-day precision RSD were 0.04 μg/L, 0.01 μg/L, and $<31\%$ ($n = 5$), respectively. Bond Elut C18 which is a hydrophobic bonded silica sorbent showed better extraction efficiency compared to Oasis® Max (mixed-mode polymeric) and Oasis® HLB (polymeric reversed phase) sorbents. The results showed that glass bottled samples contain higher concentration (4.34–41.19 μg/L) compared to PET bottled samples (0.29–24.88 μg/L) [66]. A HS-SPME method using metal-organic framework CIM-80(Al) (CIM-80(Al) MOF) as extraction phase in combination with GC-MS was used to determine seven musk fragrances and six methyl siloxanes from water samples. Low LODs in the range of 0.2 and 0.5 μg/L were observed, and RSD lower than 17% and 22%, respectively, [84]. Stir bar sorptive-dispersive microextraction (SBSDME) method using polydopamine-coated cobalt ferrite magnetic nanoparticles ($CoFe_2O_4$@PDA) was employed as an extraction material prior to thermal desorption GC-MS (TD-GC-MS) for the determination of nitro musks from water samples. The method showed low LOQs and LODs (ng/L) and repeatability (inter/intra-day) with RSD $<15\%$ [85].

Gao and co-workers [86] reported a method for the determination of androgens and progestogens in environmental water samples with linearity, LODs, and

Table 2 Selected applications of solid phase-based extraction methods in environment samples

Techniques	Samples	Analytes	Performance	References
Porous thin film-GC-APCI-MS/MS	Seawater and river water	Polycyclic aromatic hydrocarbons	LODs: 1–100 pg/mL Recoveries: 79–115%	[89]
SPE- LC-MS/MS	Surface water and wastewater	Sterols and steroid hormones	LODs: 0.7–15.0 ng/L Recoveries: 70–114% Concentrations: 176–5,162 ng/L)	[90]
SPE-LC-MS/MS	Surface water, groundwater, and drinking water	Per- and polyfluoroalkyl substances (PFASs)	LOQs: 0.6–26 ng/L Recoveries: 83–107%. RSDs: 0.7–15% Concentration: 0.045–17 μg/L	[91]
SPE-LC–MS/MS	Drinking, surface, and effluent wastewaters	Pesticides, hormones, or pharmaceuticals	LODs: 0.1–1.4 ng/L LOQs: 0.3–4.8 ng/L Concentrations: 3–68 ng/L	[92]
SPE-UHPLC-MS/MS	Surface and groundwater	Anthelmintic compounds	LODs:0.05–1.0 ng/L LOQs:0.5–40 ng/L	[93]
SPE-UPLC-MS/MS	Sediments	Carbazole and polyhalogenated carbazoles (PHCs)	LODs: 0.003–0.22 ng/g LOQs: 0.01–0.75 ng/g Recoveries: 63–124.7%	[94]
d-SPE HPLC-MS	Water samples	Bisphenol A, AF, B & tetrabromobisphenol A	LODs: 0.02 μg/L Linear range (0.1–1,000 μg/L)	[95]
SPE-LC-MS	Drinking water	Drugs of abuse and pharmaceuticals	LODs: 0.01–1.09 ng/L %RSD: <15.0% Recoveries: 62:107% Concentrations: 0.14–2.81 ng/L	[64]
d-SPE/LC-MS	Water samples			[96]

(continued)

Table 2 (continued)

Techniques	Samples	Analytes	Performance	References
		Phenoxycarboxylic acids	LODs: 0.55–3.84 /L %RSD: 2.0–9.0% Recoveries: 86.9–101.3% Concentrations: 0.14–2.81 ng/L	
SPE LC-MS/MS	Urban wastewater	Pharmaceuticals	LODs: 0.1–1.5 ng/L %RSD: 2.2–8.6% Recoveries: 84.7–97.2% Concentrations: 11–145,250 ng/L	[97]
MSPE/LC–MS/MS	Water, soil, river, and sediment	Chiral pesticides	LODs: 0.0–0.62 ng/L; 0.02–0.17 ng/g %RSD: 2.1–13.4% Recoveries: 80.3–106.3% Concentrations: 0–45.9 ng/L; 0–79.4 ng/g	[98]

d-SPE dispersive solid phase extraction, *SPE* solid phase extraction, *GC-APCI-MS/MS* gas chromatography coupled to triple quadrupole mass spectrometry, *LC-MS/MS* liquid chromatography-tandem mass spectrometry, *HLPC-MS* high-performance liquid chromatography-tandem mass spectrometry, *LC-MS* liquid chromatography-tandem mass spectrometry, *UPLC-MS/MS* ultra-performance liquid coupled to tandem mass spectroscopy, *UHPLC-MS/MS* ultra-high performance liquid chromatography-tandem mass spectrometry

recoveries ranging 8–2,032 ng/L, 2.0–10.0 ng/L and 80.5–102.4%, respectively, using UiO-66(Zr) MOF as the adsorbent of μ-SPE device combined with LC-MS/MS. The μ-SPE-LC-MS/MS method had intra-day and inter-day analysis in terms of RSDs of 1.8–6.3% and 2.8–6.5%, respectively. Combining the μ-SPE with LC-MS/MS enabled the detection of trace concentrations of progesterone ranging between 11.3 and 72.2 ng/L. Barreca et al. [87] developed an online SPE method coupled with LC-MS/MS for pre-concentration and determination of perfluoroalkyl acids (PFASs) in natural water. The SPE- LC-MS/MS method had remarkable LODs (0.2–5 ng/L) and linearity (0.2–250 ng/L) as well as acceptable accuracy (80–120%), and precision (RSD $<15\%$).

González-Sálamo et al. [88] reported the use of Basolite® F300 MOF based DSPE for extraction of phthalic acid esters from water samples prior to LC-MS determination. The matrix-matched calibration, trueness, and LODs of the developed method were 5–500 μg/L and 70–118%, 0.022–0.069 μg/L, respectively. The analytes were present in all investigated samples at concentrations ranging from 0.21

to 4.04 μg/L. Wang et al. [15] prepared graphene/Fe_3O_4 (G-Fe_3O_4) nanocomposites as an adsorbent for MSPE of ketoconazole, econazole, miconazole, butoconazole, sertaconazole, fenticonazole, and isoconazole from environmental water samples. The imidazole antifungals were quantified using LC-MS/MS. The electrostatic, cation-π, hydrophobic and π-π stacking interactions allowed efficient extraction of the analytes. Satisfactory recoveries (70–92%), acceptable intra-day/inter-day precisions (%RSD 3.0–12.0%), and LOD (0.11–0.32 ng/L) were obtained.

2.2.2 Liquid Phase-Based Extraction Methods

Liquid–liquid extraction (LLE) uses organic solvents such as ethyl acetate, hexane, and isooctane among others to partition target analytes from a sample matrix to the organic extractant [99]. LLE is one of the oldest extraction techniques [34, 100]. Due to its ease of application, LLE has been widely applied. Traditional LLE has been modified by the development of miniaturized forms of LLE such as single drop microextraction (SDME), dispersive liquid–liquid microextraction (DLLME), hollow fiber liquid phase microextraction (HF-LPME), and cloud point extraction (CPE) [101, 102]. Various review articles have covered the principles of various liquid phase-based extraction methods [102, 103], and in this chapter their application in food and environmental samples prior to various MS methodologies is highlighted.

Application of Liquid Phase-Based Extraction Methods in Food Samples

Advances in food analysis has evolved from conventional analysis of the major components of food to more complex and broader aspects. This is because various chemical substances present in food play a major role its production and distribution [104, 105]. Notable the improvement in livestock production and crop yields requires the use of protection, phytosanitary products, and veterinary drugs. In addition, the improvement in food quality requires the use of substances such as colorants, flavors, enzymes, and other additives [106]. Packaging materials (such as paper, polyethylene terephthalate (PET) plastics and paper, among others), on the other hand, are utilized for preservation, presentation improvement, storage, ease of transportation, and maintaining hygienic conditions of food [107]. Even though the above-mentioned efforts increase food production and security, the chemical substances used may lead to their presence in food, thus presenting a potential contamination risk [108]. Figure 1 illustrates that food analysis does not only focus on contaminants, but it also involves analysis of foodstuff nutritional values (such as proteins, amino acids, essential fatty acids, fibers, vitamins, carbohydrates, lipids, minerals, etc.) [109]. Furthermore, food analysis has included the determination of natural contaminants such as mycotoxins and algal biotoxins as well as food authentication, adulteration, and fraud detection [110–113].

As discussed, food analysis encompasses the determination of numerous chemical compounds with different chemical nature. Therefore, sensitive and accurate

Fig. 1 Typical application of MS based methods in analysis of various chemical substances in food. Adapted from Ref. [104] with Elsevier permission

analytical techniques (GC and LC coupled with MS) combined with various modes of liquid phase extraction procedures have been extensively used in monitoring/quantification of various substances in food samples. Nemati et al. [114] developed a liquid microextraction method based on solidification of floating organic droplet-liquid-liquid microextraction (SFOD-LLME) coupled with HPLC-MS/MS for extraction and determination of four antibiotics in honey. In their findings, they reported low LOQs (1.9–2.6 ng/g) and LODs (0.55–0.79 ng/g) with $\leq$6.9% RSDs [114]. Previous study reported the development of a micro-LLE coupled with UPLC-MS/MS for the extraction and quantification of herbicides from agricultural products. Method quantification limit (MQL) 0.6–1.0 ng/g and recoveries ranging from 73.7 to 91.4% with RSD of less than 10% were obtained [115].

Zheng et al. have developed an LLE method coupled with LC-MS for quantification of salicylic acid (SA) and acetylsalicylic acid (ASA). These two analytes (SA and ASA) were extracted from flatfish, milk, egg, eel, and porcine muscle. The obtained LOQs were 7 and 10 μg/kg for SA and ASA, respectively, with RSDs of $\leq$9.0% (inter-day and intra-day) [116]. Salim et al. [117] developed and validated DLLME combined with LC-MS/MS for analysis of multi-mycotoxin (that is aflatoxins (AFB1, AFB2, AFG1, and AFG2), ochratoxin A (OTA), deoxynivalenol (DON), fumonisins (FB1 and FB2), sterigmatocystin (STG), T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS) and zearalenone (ZEA)) in rice bran. The developed method had good linearity (0.5–150 ng/g), LOD (0.5–50 ng/g) and acceptable recoveries (70.2–99.4%) with a %RSD below 2%. The concentrations of mycotoxin in rice bran samples were between 0 and 157 ng/g. Alsharif et al. [118] developed a modified HF-DLLME of multi-aflatoxins and ochratoxin A in commercial apple, grape, orange, and pomegranate juice prior to HPLC-MS/MS detection. The linearity, LOD, recoveries, and %RSD were 0.1–30 μg/L, 0.04–0.13 μg/L, 70–120% and $<$11%, respectively. Table 3 shows that different types of analytes with varying

Table 3 Selected applications of liquid phase-based extraction methods in food analysis

Techniques	Samples	Analytes	Performance	References
HLLE/LC-orbitrap HRMS	Pork, fish, egg, and milk	19 quinolones	LOQs: 0.028–0.192 μg/kg	[119]
SPE salting-out assisted LLE	Aqueous food simulants	Carcinogenic primary aromatic amines (PAAs)	LOD: 0.05 μg/L LOQ: 0.15 μg/L	[120]
LDDES-DLLME-GC-MS/MS	Soft drinks	PAHs	LOQ: 0.2 μg/L LOD: 0.01 μg/L RSD: <16–87%	[121]
SALLE-UHPLC–MS/MS	Infant foods	Tetracycline	LOD: 0.05–0.14 μg/kg LOQ: 0.16–0.48 μg/kg %RSD: <7.3%	[122]
Inkjet-based DLLME-UHPLC–MS/MS	Wheat	Aflatoxins	LODs: 0.06–6 μg/kg %RSD: <4.6% Recoveries: 83.2–93.0% Concentrations:0–2.88 μg/kg	[123]
LLE-LC-MS/MS	Meat-, fish-, seafood-based products, various dairy ingredients, infant formulae, and fats	Tetracyclines	LOD: 10 μg/kg	[124]
VA-DLLME/LC-MS/MS	Tomato fruits	Fipronil and its metabolite fipronil sulfone	LOD: 0.075 μg/kg %RSD: <12% Recoveries: 89.8–96.3%	[125]
IL-based DLLME/LC–MS/MS	Vegetables and fruits	Multi-pesticide residues	LODs: 0.02–0.32 μg/kg %RSDs: 1–16% Recoveries: 70–137%	[126]
DLLME/UPLC-MS/MS	Honey	Pyrrolizidine alkaloids	LODs: 0.009–0.018 μg/kg %RSD: 1–12% Recoveries: 63 to 103% Concentrations: up to 17.5 μg/kg	[127]
DLLME/GC-MS	Milk	Amino acids (alanine, glycine, and glutamic acid)	LODs: 0.31–0.84 μg/mL %RSDs: 7.8–10.3%	[128]

SS-LPE Switchable solvent liquid phase extraction, *HLLE* homogeneous liquid–liquid extraction, *SALLE* salting-out assisted liquid–liquid extraction, *VA-DLLME* vortex assisted dispersive liquid–liquid microextraction, *IL-based DLLME* ionic liquid based dispersive liquid–liquid microextraction

physical and chemical properties from complex food samples can be analyzed using MS methodologies via liquid phase-based extraction methods as preliminary extraction and enrichment step. These analytes include pesticides, pharmaceuticals, and PAHs, among others.

Application of Liquid Phase-Based Extraction Methods in Environmental Samples

Table 4 presents the summary of some of the recent studies describing the application of liquid phase-based extraction methods for enrichment of different analytes in

Table 4 Application of liquid phase-based extraction methods in environmental analysis

Method	Samples	Analyte	Performance	References
LPE-SPE-GC-MS	Water	PAHs	%RSD < 7.5% and recoveries 87–104% LODs: 4–110 ng/kg	[136]
DLLME-GC-MS	Surface water	Pesticides	LOQs: 0.003–3.75 μg/L LODs: 0.001–1.125 μg/L	[137]
SPE-DLLE UHPLC-MS/MS	Water	Pharmaceuticals and personal care products	LOQ: 3 ng/L LOD: 1 ng/L	[138]
DLLME-SFO-SBE/UHPLC-(+) APCI-MS/MS	Water samples	Nitrated-PAHs(oxy-PAHs)	LOQs: 0.15–1.10 ng/mL LODs: 0.02–0.85 ng/mL	[139]
DLLME/GC-MS	Human saliva	Parabens, triclocarban, bisphenols, and benzophenones	LODs: 0.01–0.15 ng/mL LOQs: 0.05–0.40 ng/mL	[140]
LLE/GC-MS	Wastewater	40 analytes in refinery wastewater	LODs: 0.3–1.7 μg/L	[95]
DLLME/GC-MS	Wastewater	Bisphenol A and UV-filters	LOQ:10 ng/L LOD: 2 ng/L	[141]
DLLME /LC-MS/MS	Water	Pesticides and personal care products	LOQs:0.0125–1.25 μg/L	[142]
HF-LPME L/C-MS/MS	Water samples	27 emerging contaminants	LOQs: 2.13–126.50 ng/L LODs:1.09–98.15 ng/L	[143]
SS-LPE/GC-MS	Well, tap, and lake water samples	Chlorthiamid, ethyl parathion, penconazole, and fludioxonil pesticides	LODs: 0.28–0.58 ng/mL LOQs: 0.93–1.9 ng/mL	[144]

SS-LPE switchable solvent liquid phase extraction, *DLLME-SFO-SBE* dispersive liquid–liquid phase microextraction based on the solidification of a floating organic drop coupled with solvent assisted back-extraction, *HF-LPME* Hollow fiber liquid phase microextraction

environmental samples prior to MS analysis. The results obtained by different researchers show that these methods have significant influence on the performance and efficiency of the detection techniques. This supported the differences in the analytical figures of merit (Table 4). For instance, LLE-GC-MS extraction method was developed to extract 9 haloacetic acids from water samples. LOD and LOQ were less than 2.6 and 3.9 μg/L, while the RSDs for inter-day and intra-day precisions ranged from 1.68–19.07 to 0.01–7.31%, respectively. Quantitative recoveries ranging from 79.7 to 113.22% were obtained [129]. These results show that traditional LLE is applicable because it allowed efficient extraction of target analytes prior to GC-MS analysis.

In another study, Erarpat et al. developed a GC-MS method based on liquid phase extraction of estrone, fluoxetine, and pesticides from water. The results obtained demonstrated that the method had relatively low LODs (0.16–8.6 ng/mL) and LOQs (0.5–29 ng/mL) [130]. Another study reported the application of LPME with GC-MS for extraction of hormones and pesticides [131]. The method exhibited high recovery greater than or equal to 110% with RSD $<10\%$. The LODs and LOQs ranged from 0.20–13 ng/mL to 0.90–46 ng/mL, respectively [131]. This study proved that liquid phase extraction method could be used for extraction of analytes that have different chemical properties. Cacho et al. developed an ionic liquid based dispersive liquid–liquid microextraction (IL-DLLME) coupled with GC-MS for the extraction of nine organophosphorus pesticides from water samples. The obtained RSDs were between 4.1 and 9.7% while the LODs were in the range of 5–16 ng/L [132]. Ionic liquid dispersive liquid phase microextraction (IL-DLPME) coupled with GC-MS has been used in pre-concentration of chlorobenzene compounds. The developed method was used in both water and soil samples. The LODs were in the range of 8.4–252 ng/kg for soil and 0.5–7.2 ng/L for water. The concentrations of the analytes in soil and water samples were 1.5–50 ng/L and 2.7–256 ng/L, respectively [133]. A DLLME method coupled with MS for the detection of parathion methyl from water samples was reported elsewhere [134]. The optimized method exhibited high recoveries (up to 99.9%) and acceptable precision 0.32%. A DLLME-GC-MS method was used for the extraction of hormones, pesticides, and endocrine disruptors in sea water, tap water, well water, and municipal wastewater [135]. The method displayed good analytical performance with LODs ranging from 0.3 to 2.0 ng/mL and acceptable precision.

2.3 Separation Techniques

Mass spectrometry-based (MS) techniques exhibit excellent selectivity and high sensitivity, as well as abundant fragments that can be used for structural identification. When coupled with chromatographic techniques, MS makes the separation methods to be suited for both targeted detection and nontargeted [145, 146]. The most commonly used chromatographic separation techniques with MS are gas and liquid chromatography (GC and LC) [42]. GC is used for the separation of non-polar

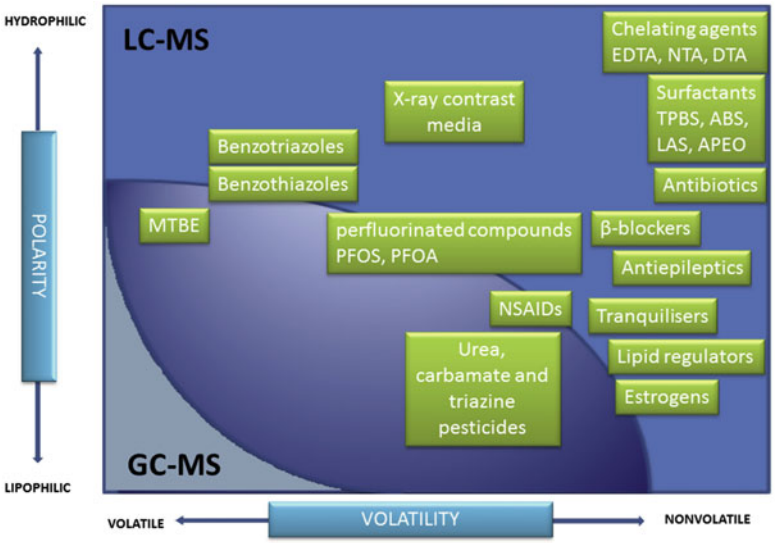


Fig. 2 Application of GC-MS and LC-MS in wide range of organic pollutants. Adapted from Ref. [148] with Elsevier permission

and volatile compounds using helium or nitrogen as a mobile phase (Fig. 2). In contrast, LC can separate a wide range of polar, non-volatile, and ionic compounds (Fig. 2) using mainly reversed phase C18 columns as the stationary phase and mixtures of methanol-water or acetonitrile water with volatile additives as mobile phase [147]. The combination of MS with these separation techniques results in a powerful technique with the ability to identify and confirm the identity of compounds, provided structural information needed for analyte characterization as well as identifying coeluting compounds (Fig. 2 and Tables 1, 2, 3, 4).

3 Conclusions

Recent advances in the sample preparation of food and environmental samples for mass spectrometric analysis have been discussed in this chapter. Furthermore, sample handling procedures such as passive sampling, grab or discrete sampling at a specific time, time-integrated sampling, and event-based sampling have been briefly discussed. With respect to sample preparation, the use of solid phase- and liquid phase-based extraction techniques, such as SPE, SPE, DSPE, DLLME, LLE, and thin films that allow extraction and pre-concentration of analytes as well as sample clean-up prior to MS analysis was also presented. Studies showed that solid phase-based extractions remain the most commonly used sample pretreatment procedures for both food and environmental samples. Additionally, MS based methodologies based on GC and LC/UHPLC for separation of volatile compounds and polar

analytes are still the separation methods of choice prior to MS detection. Furthermore, the interest in use of HRMS, Q-TOF-MS/MS, TOF-MS, and APCI/ESI-MS/MS coupled with GC or LC for food and environmental sample is growing owing to advantages such as high resolution and efficiency. Additionally, the above-mentioned MS approaches provide an improvement in the performance of the analytical methods thus allowing these methodologies to be suitable for control laboratories.

Acknowledgments The authors wish to thank the Department of Chemical Sciences (Faculty of Science, University of Johannesburg, South Africa) for providing necessary facilities. The authors are also grateful for financial support offered by the Department of Science and Innovation/National Research Foundation South African Research Chairs Initiative (DSI/NRF SARChI) (grant no. 91230).

References

1. Farré M, Kantiani L, Petrovic M, Pérez S, Barceló D (2012) Achievements and future trends in the analysis of emerging organic contaminants in environmental samples by mass spectrometry and bioanalytical techniques. J Chromatogr A 1259:86–99
2. Pilařová V, Plachká K, Khalikova MA, Svec F, Nováková L (2019) Recent developments in supercritical fluid chromatography–mass spectrometry: is it a viable option for analysis of complex samples? TrAC Trends Anal Chem 112:212–225
3. Chemat F, Rombaut N, Meullemiestre A, Turk M, Perino S, Fabiano-Tixier A-S et al (2017) Review of green food processing techniques. Preservation, transformation, and extraction. Innov Food Sci Emerg Technol 41:357–377
4. Net S, Delmont A, Sempéré R, Paluselli A, Ouddane B (2015) Reliable quantification of phthalates in environmental matrices (air, water, sludge, sediment and soil): a review. Sci Total Environ 515:162–180
5. Wang S, Chen H, Sun B (2020) Recent progress in food flavor analysis using gas chromatography–ion mobility spectrometry (GC–IMS). Food Chem 315:126158
6. Deng J, Yang Y, Wang X, Luan T (2014) Strategies for coupling solid-phase microextraction with mass spectrometry. TrAC Trends Anal Chem 55:55–67
7. Fang L, Deng J, Yang Y, Wang X, Chen B, Liu H et al (2016) Coupling solid-phase microextraction with ambient mass spectrometry: strategies and applications. TrAC Trends Anal Chem 85:61–72
8. Khaled A, Belinato JR, Pawliszyn J (2020) Rapid and high-throughput screening of multi-residue pharmaceutical drugs in bovine tissue using solid phase microextraction and direct analysis in real time-tandem mass spectrometry (SPME-DART-MS/MS). Talanta 217:121095
9. Alcántara-Durán J, Moreno-González D, Gilbert-López B, Molina-Díaz A, García-Reyes JF (2018) Matrix-effect free multi-residue analysis of veterinary drugs in food samples of animal origin by nanoflow liquid chromatography high resolution mass spectrometry. Food Chem 245:29–38
10. Álvarez-Sánchez B, Priego-Capote F, de Castro MDL (2010) Metabolomics analysis II. Preparation of biological samples prior to detection. TrAC Trends Anal Chem 29(2):120–127
11. Pourchet M, Debrauwer L, Klanova J, Price EJ, Covaci A, Caballero-Casero N et al (2020) Suspect and non-targeted screening of chemicals of emerging concern for human biomonitoring, environmental health studies and support to risk assessment: from promises to challenges and harmonisation issues. Environ Int 139:105545

12. Gentili A, Fanali S, Rocca LM (2019) Desorption electrospray ionization mass spectrometry for food analysis. TrAC Trends Anal Chem 115:162–173
13. Wang X, Wang S, Cai Z (2013) The latest developments and applications of mass spectrometry in food-safety and quality analysis. TrAC Trends Anal Chem 52:170–185
14. Vasiljevic T, Singh V, Pawliszyn J (2019) Miniaturized SPME tips directly coupled to mass spectrometry for targeted determination and untargeted profiling of small samples. Talanta 199:689–697
15. Wang Z, Zhao P, Yu J, Jiang Z, Guo X (2018) Experimental and molecular docking study on graphene/Fe3O4 composites as a sorbent for magnetic solid-phase extraction of seven imidazole antifungals in environmental water samples prior to LC-MS/MS for enantiomeric analysis. Microchem J 140:222–231
16. Mohamed AH, Noorhisham NA, Yahaya N, Mohamad S, Kamaruzzaman S, Osman H et al (2021) Sampling and sample preparation techniques for the analysis of organophosphorus pesticides in soil matrices. Crit Rev Anal Chem:1–22
17. Tankiewicz M, Fenik J, Biziuk M (2010) Determination of organophosphorus and organonitrogen pesticides in water samples. TrAC Trends Anal Chem 29(9):1050–1063
18. Baggiani C, Anfossi L, Giovannoli C (2007) Solid phase extraction of food contaminants using molecular imprinted polymers. Anal Chim Acta 591(1):29–39
19. Bataineh M, Schymanski EL, Gallampois CMJ (2021) Recent analytical methods for risk assessment of emerging contaminants in ecosystems. In: Pollution assessment for sustainable practices in applied sciences and engineering. Elsevier, pp 739–778
20. Farré M, Pérez S, Gajda-Schrantz K, Osorio V, Kantiani L, Ginebreda A et al (2010) First determination of C60 and C70 fullerenes and N-methylfulleropyrrolidine C60 on the suspended material of wastewater effluents by liquid chromatography hybrid quadrupole linear ion trap tandem mass spectrometry. J Hydrol 383(1–2):44–51
21. Dudzik D, Barbas-Bernardos C, García A, Barbas C (2018) Quality assurance procedures for mass spectrometry untargeted metabolomics. A review. J Pharm Biomed Anal 147:149–173
22. Ridgway K, Lalljie SPD, Smith RM (2007) Sample preparation techniques for the determination of trace residues and contaminants in foods. J Chromatogr A 1153(1–2):36–53
23. Pérez-Rodríguez M, Pellerano RG, Pezza L, Pezza HR (2018) An overview of the main foodstuff sample preparation technologies for tetracycline residue determination. Talanta 182: 1–21
24. Saleh TA (2020) Trends in the sample preparation and analysis of nanomaterials as environmental contaminants. Trends Environ. Anal Chem:e00101
25. Moser A, Korstjens I (2018) Series: practical guidance to qualitative research. Part 3: sampling, data collection and analysis. Eur J Gen Pract 24(1):9–18
26. Brack W, Ait-Aissa S, Burgess RM, Busch W, Creusot N, Di Paolo C et al (2016) Effect-directed analysis supporting monitoring of aquatic environments – an in-depth overview. Sci Total Environ 544:1073–1118
27. Panno SV, Krapac IG, Keefer DA (1998) A new device for collecting time-integrated water samples from springs and surface water bodies. Environ Eng Geosci 4(3):375–383
28. Taylor AC, Fones GR, Vrana B, Mills GA (2021) Applications for passive sampling of hydrophobic organic contaminants in water – a review. Crit Rev Anal Chem 51(1):20–54
29. Charriau A, Lissalde S, Poulier G, Mazzella N, Buzier R, Guibaud G (2016) Overview of the Chemcatcher® for the passive sampling of various pollutants in aquatic environments part A: principles, calibration, preparation and analysis of the sampler. Talanta 148:556–571
30. Gong X, Li K, Wu C, Wang L, Sun H (2018) Passive sampling for monitoring polar organic pollutants in water by three typical samplers. Trends Environ Anal Chem 17:23–33
31. Vrana B, Smedes F, Prokeš R, Loos R, Mazzella N, Miege C et al (2016) An interlaboratory study on passive sampling of emerging water pollutants. TrAC Trends Anal Chem 76:153–165

32. Ashley J, Shahbazi M-A, Kant K, Chidambara VA, Wolff A, Bang DD et al (2017) Molecularly imprinted polymers for sample preparation and biosensing in food analysis: progress and perspectives. Biosens Bioelectron 91:606–615
33. Manousi N, Zachariadis GA, Deliyanni EA, Samanidou VF (2018) Applications of metal-organic frameworks in food sample preparation. Molecules 23(11):2896
34. Dimpe KM, Nomngongo PN (2016) Current sample preparation methodologies for analysis of emerging pollutants in different environmental matrices. TrAC Trends Anal Chem 82:199–207
35. Khatibi SA, Hamidi S, Siahi-Shadbad MR (2021) Current trends in sample preparation by solid-phase extraction techniques for the determination of antibiotic residues in foodstuffs: a review. Crit Rev Food Sci Nutr 61(20):3361–3382
36. Samsidar A, Siddiquee S, Shaarani SM (2017) A review of extraction, analytical and advanced methods for determination of pesticides in environment and foodstuffs. Trends Food Sci Technol 2018(71):188–201
37. Buszewski B, Szultka M (2012) Past, present, and future of solid phase extraction: a review. Crit Rev Anal Chem 42(3):198–213
38. Selahle SK, Mpupa A, Nomngongo PN (2021) A review of extraction, analytical, and advanced methods for the determination of neonicotinoid insecticides in environmental water matrices. Rev Anal Chem 40(1):187–203
39. Cunha SC, Fernandes JO (2018) Extraction techniques with deep eutectic solvents. TrAC Trends Anal Chem 105:225–239
40. Picó Y, Fernández M, Ruiz MJ, Font G (2007) Current trends in solid-phase-based extraction techniques for the determination of pesticides in food and environment. J Biochem Biophys Methods 70(2):117–131
41. Alampanos V, Samanidou V, Papadoyannis I (2019) Trends in sample preparation for the hplc determination of penicillins in biofluids. J Appl Bioanal 5(1):9–17
42. Das SK (2014) Recent developments in clean up techniques of pesticide residue analysis for toxicology study: a critical review. Univers J Agric Res 2(6):199–203
43. Płotka-Wasylka J, Szczepańska N, de La Guardia M, Namieśnik J (2015) Miniaturized solid-phase extraction techniques. TrAC Trends Anal Chem 73:19–38
44. Zuloaga O, Navarro P, Bizkarguenaga E, Iparraguirre A, Vallejo A, Olivares M et al (2012) Overview of extraction, clean-up and detection techniques for the determination of organic pollutants in sewage sludge: a review. Anal Chim Acta 736:7–29
45. Tankiewicz M, Fenik J, Biziuk M (2011) Solventless and solvent-minimized sample preparation techniques for determining currently used pesticides in water samples: a review. Talanta 86:8–22
46. Sajid M, Khaled Nazal M, Rutkowska M, Szczepańska N, Namieśnik J, Płotka-Wasylka J (2019) Solid phase microextraction: apparatus, sorbent materials, and application. Crit Rev Anal Chem 49(3):271–288
47. Li X, Li H, Ma W, Guo Z, Li X, Li X et al (2018) Determination of patulin in apple juice by single-drop liquid-liquid-liquid microextraction coupled with liquid chromatography-mass spectrometry. Food Chem 257:1–6
48. Bueno M, Resconi VC, Campo MM, Ferreira V, Escudero A (2019) Development of a robust HS-SPME-GC-MS method for the analysis of solid food samples. Analysis of volatile compounds in fresh raw beef of differing lipid oxidation degrees. Food Chem 281:49–56
49. Bessaire T, Ernest M, Christinat N, Carrères B, Panchaud A, Badoud F (2021) High resolution mass spectrometry workflow for the analysis of food contaminants: application to plant toxins, mycotoxins and phytoestrogens in plant-based ingredients. Food Addit Contam A:1–19
50. Dong H, Xian Y, Li H, Wu Y, Bai W, Zeng X (2020) Analysis of heterocyclic aromatic amine profiles in Chinese traditional bacon and sausage based on ultrahigh-performance liquid chromatography–quadrupole-orbitrap high-resolution mass spectrometry (UHPLC-Q-orbitrap-HRMS). Food Chem 310:125937

51. Wong JW, Wang J, Chow W, Carlson R, Jia Z, Zhang K et al (2018) Perspectives on liquid chromatography–high-resolution mass spectrometry for pesticide screening in foods. J Agric Food Chem 66(37):9573–9581
52. Salla V, Murray KK (2013) Matrix-assisted laser desorption ionization mass spectrometry for identification of shrimp. Anal Chim Acta 794:55–59
53. Du B, Shen M, Pan Z, Zhu C, Luo D, Zeng L (2021) Trace analysis of multiple synthetic phenolic antioxidants in foods by liquid chromatography–tandem mass spectrometry with complementary use of electrospray ionization and atmospheric pressure chemical ionization. Food Chem:131663
54. Kritikou AS, Aalizadeh R, Damalas DE, Barla IV, Baessmann C, Thomaidis NS (2022) MALDI-TOF-MS integrated workflow for food authenticity investigations: an untargeted protein-based approach for rapid detection of PDO feta cheese adulteration. Food Chem 370:131057
55. Jin P, Xu S, Xu W, He X, Kuang Y, Hu X (2020) Screening and quantification of fourteen synthetic antidiabetic adulterants in herbal pharmaceuticals and health foods by HPLC and confirmation by LC-Q-TOF-MS/MS. Food Addit Contam A 37(1):11–18
56. Yang M, Li J, Zhao C, Xiao H, Fang X, Zheng J (2021) LC-Q-TOF-MS/MS detection of food flavonoids: principle, methodology, and applications. Crit Rev Food Sci Nutr 1–21
57. Niu Y, Liu J, Yang R, Zhang J, Shao B (2020) Atmospheric pressure chemical ionization source as an advantageous technique for gas chromatography-tandem mass spectrometry. TrAC Trends Anal Chem:116053
58. Žniderščič L, Mlakar A, Prosen H (2019) Development of a SPME-GC-MS/MS method for the determination of some contaminants from food contact material in beverages. Food Chem Toxicol 134:110829
59. Zhao Y, Bai X-L, Liu Y-M, Liao X (2021) Determination of fipronil and its metabolites in egg samples by UHPLC coupled with Q-exactive high resolution mass spectrometry after magnetic solid-phase extraction. Microchem J 169:106540
60. Chen D, Yan P, Lv B, Zhao Y, Wu Y (2018) Parallel reaction monitoring to improve the detection performance of carcinogenic 4-methylimidazole in food by liquid chromatography-high resolution mass spectrometry coupled with dispersive micro solid-phase extraction. Food Control 88:1–8
61. Maragou NC, Thomaidis NS, Theodoridis GA, Lampi EN, Koupparis MA (2020) Determination of bisphenol A in canned food by microwave assisted extraction, molecularly imprinted polymer-solid phase extraction and liquid chromatography-mass spectrometry. J Chromatogr B 1137:121938
62. Liu J, Feng W, Tian M, Qu Q, Yang L (2021) Titanium dioxide-coated core-shell silica microspheres-based solid-phase extraction combined with sheathless capillary electrophoresis-mass spectrometry for analysis of glyphosate, glufosinate and their metabolites in baby foods. J Chromatogr A:462519
63. Sun T, Ali MM, Wang D, Du Z (2020) On-site rapid screening of benzodiazepines in dietary supplements using pipette-tip micro-solid phase extraction coupled to ion mobility spectrometry. J Chromatogr A 1610:460547
64. Peng J, Wei M, Hu Y, Yang Y, Guo Y, Zhang F (2019) Simultaneous determination of maltol, ethyl maltol, vanillin, and ethyl vanillin in foods by isotope dilution headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry. Food Anal Methods 12(8):1725–1735
65. Peng J, Yang Y, Zhou Y, Hocart CH, Zhao H, Hu Y et al (2020) Headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry with selected ion monitoring for the determination of four food flavoring compounds and its application in identifying artificially scented rice. Food Chem 313:126136
66. AlAmmari AM, Khan MR, Aqel A (2020) Trace identification of endocrine-disrupting bisphenol a in drinking water by solid-phase extraction and ultra-performance liquid chromatography-tandem mass spectrometry. J King Saud Univ 32(2):1634–1640

67. Yan Y, Zhang H, Ai L, Kang W, Lian K, Wang J (2021) Determination of gamithromycin residues in eggs, milk and edible tissue of food-producing animals by solid phase extraction combined with ultrahigh-performance liquid chromatography-tandem mass spectrometry. J Chromatogr B 1171:122637
68. Yan Y, Lian K, Zhang H, An J, Zhang Y, Kang W et al (2021) Doping-control analysis of 14 diuretics in animal-derived foods using ultra-high-performance liquid chromatography-tandem mass spectrometry. Microchem J:106948
69. Zhao X, Wang J, Wang J, Wang S (2018) Development of water-compatible molecularly imprinted solid-phase extraction coupled with high performance liquid chromatography–tandem mass spectrometry for the detection of six sulfonamides in animal-derived foods. J Chromatogr A 1574:9–17
70. Du J, Li X, Tian L, Li J, Wang C, Ye D et al (2021) Determination of macrolides in animal tissues and egg by multi-walled carbon nanotube-based dispersive solid-phase extraction and ultra-high performance liquid chromatography–tandem mass spectrometry. Food Chem 365: 130502
71. Wang R, Li S, Chen D, Zhao Y, Wu Y, Qi K (2021) Selective extraction and enhanced-sensitivity detection of fluoroquinolones in swine body fluids by liquid chromatography–high resolution mass spectrometry: application in long-term monitoring in livestock. Food Chem 341:128269
72. Talari K, Ganji SK, Kommu M, Tiruveedula RR, Upadhyayula V (2021) Quantitative determination of targeted and untargeted pesticide residues in coconut milk by liquid chromatography–atmospheric pressure chemical ionization–high energy collisional dissociation tandem high-resolution mass spectrometry. J Chromatogr A:462649
73. Cha KH, Lee J, Lee J, Kim J-H (2021) Development of a quantitative screening method for pesticide multiresidues in orange, chili pepper, and brown rice using gas chromatography-quadrupole time of flight mass spectrometry with dopant-assisted atmospheric pressure chemical ionization. Food Chem:131626
74. Hu S, Zhao M, Xi Y, Mao Q, Zhou X, Chen D et al (2017) Nontargeted screening and determination of sulfonamides: a dispersive micro solid-phase extraction approach to the analysis of milk and honey samples using liquid chromatography–high-resolution mass spectrometry. J Agric Food Chem 65(9):1984–1991
75. Hakme E, Poulsen ME (2021) Evaluation of the automated micro-solid phase extraction clean-up system for the analysis of pesticide residues in cereals by gas chromatography-orbitrap mass spectrometry. J Chromatogr A 1652:462384
76. Chmangui A, Jayasinghe GDTM, Driss MR, Touil S, Bermejo-Barrera P, Bouabdallah S et al (2021) Assessment of trace levels of aflatoxins AFB1 and AFB2 in non-dairy beverages by molecularly imprinted polymer based micro solid-phase extraction and liquid chromatography-tandem mass spectrometry. Anal Methods 13(30):3433–3443
77. Jayasinghe GDTM, Domínguez-González R, Bermejo-Barrera P, Moreda-Piñeiro A (2020) Miniaturized vortex assisted-dispersive molecularly imprinted polymer micro-solid phase extraction and HPLC-MS/MS for assessing trace aflatoxins in cultured fish. Anal Methods 12(35):4351–4362
78. Afshar Mogaddam MR, Jouyban A, Nemati M, Farajzadeh MA, Marzi KE (2021) Application of curcumin as a green and new sorbent in deep eutectic solvent-based dispersive micro-solid phase extraction of several polycyclic aromatic hydrocarbons from honey samples prior to gas chromatography–mass spectrometry determination. J Sep Sci 44(21):4037–4047
79. Zhao Y, Wu R, Yu H, Li J, Liu L, Wang S et al (2020) Magnetic solid-phase extraction of sulfonamide antibiotics in water and animal-derived food samples using core-shell magnetite and molybdenum disulfide nanocomposite adsorbent. J Chromatogr A 1610:460543
80. Yu H, Jia Y, Wu R, Chen X, Chan T-WD (2019) Determination of fluoroquinolones in food samples by magnetic solid-phase extraction based on a magnetic molecular sieve nanocomposite prior to high-performance liquid chromatography and tandem mass spectrometry. Anal Bioanal Chem 411(13):2817–2826

81. Lu Z, Deng F, He R, Tan L, Luo X, Pan X et al (2019) A pass-through solid-phase extraction clean-up method for the determination of 11 quinolone antibiotics in chicken meat and egg samples using ultra-performance liquid chromatography tandem mass spectrometry. Microchem J 151:104213
82. Hu K, Shi Y, Zhu W, Cai J, Zhao W, Zeng H et al (2021) Facile synthesis of magnetic sulfonated covalent organic framework composites for simultaneous dispersive solid-phase extraction and determination of β-agonists and fluoroquinolones in food samples. Food Chem 339:128079
83. Zhao M, Shao H, He Y, Li H, Yan M, Jiang Z et al (2019) The determination of patulin from food samples using dual-dummy molecularly imprinted solid-phase extraction coupled with LC-MS/MS. J Chromatogr B 1125:121714
84. González-Hernández P, Pacheco-Fernández I, Bernardo F, Homem V, Pasán J, Ayala JH et al (2021) Headspace solid-phase microextraction based on the metal-organic framework CIM-80 (Al) coating to determine volatile methylsiloxanes and musk fragrances in water samples using gas chromatography and mass spectrometry. Talanta 232:122440
85. Grau J, Benedé JL, Chisvert A (2021) Polydopamine-coated magnetic nanoparticles for the determination of nitro musks in environmental water samples by stir bar sorptive-dispersive microextraction. Talanta 231:122375
86. Gao G, Xing Y, Liu T, Wang J, Hou X (2019) UiO-66(Zr) as sorbent for porous membrane protected micro-solid-phase extraction androgens and progestogens in environmental water samples coupled with LC-MS/MS analysis: the application of experimental and molecular simulation method. Microchem J 146:126–133. http://www.sciencedirect.com/science/article/pii/S0026265X18315996
87. Barreca S, Busetto M, Vitelli M, Colzani L, Clerici L, Dellavedova P (2018) Online solid-phase extraction LC-MS/MS: a rapid and valid method for the determination of perfluorinated compounds at sub ng· L− 1 level in natural water. J Chem 2018
88. González-Sálamo J, González-Curbelo MÁ, Hernández-Borges J, Rodríguez-Delgado MÁ (2019) Use of Basolite® F300 metal-organic framework for the dispersive solid-phase extraction of phthalic acid esters from water samples prior to LC-MS determination. Talanta 195: 236–244
89. Shahhoseini F, Azizi A, Egli SN, Bottaro CS (2020) Single-use porous thin film extraction with gas chromatography atmospheric pressure chemical ionization tandem mass spectrometry for high-throughput analysis of 16 PAHs. Talanta 207:120320
90. Jauković ZD, Grujić SD, Bujagić IVM, Laušević MD (2017) Determination of sterols and steroid hormones in surface water and wastewater using liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry. Microchem J 135:39–47
91. Janda J, Nödler K, Brauch H-J, Zwiener C, Lange FT (2019) Robust trace analysis of polar (C 2-C 8) perfluorinated carboxylic acids by liquid chromatography-tandem mass spectrometry: method development and application to surface water, groundwater and drinking water. Environ Sci Pollut Res 26(8):7326–7336
92. Rubirola A, Boleda MR, Galceran MT (2017) Multiresidue analysis of 24 water framework directive priority substances by on-line solid phase extraction-liquid chromatography tandem mass spectrometry in environmental waters. J Chromatogr A 1493:64–75
93. Mooney D, Coxon C, Richards KG, Gill L, Mellander P-E, Danaher M (2019) Development and optimisation of a multiresidue method for the determination of 40 anthelmintic compounds in environmental water samples by solid phase extraction (SPE) with LC-MS/MS detection. Molecules 24(10):1978
94. Zhou W, Huang X, Lin K (2019) Analysis of polyhalogenated carbazoles in sediment using liquid chromatography–tandem mass spectrometry. Ecotoxicol Environ Saf 170:148–155
95. Song XL, Lv H, Wang DD, Liao KC, Wu YY, Li GM, Chen Y (2022) Graphene oxide composite microspheres as a novel dispersive solid-phase extraction adsorbent of bisphenols prior to their quantitation by HPLC–mass spectrometry. Microchem J 172:106920

96. Ma J-Q, Liu L, Wang X, Chen L-Z, Lin J-M, Zhao R-S (2019) Development of dispersive solid-phase extraction with polyphenylene conjugated microporous polymers for sensitive determination of phenoxycarboxylic acids in environmental water samples. J Hazard Mater 371:433–439
97. Semreen MH, Shanableh A, Semerjian L, Alniss H, Mousa M, Bai X et al (2019) Simultaneous determination of pharmaceuticals by solid-phase extraction and liquid chromatography-tandem mass spectrometry: a case study from Sharjah sewage treatment plant. Molecules 24(3):633
98. Zhao P, Wang Z, Li K, Guo X, Zhao L (2018) Multi-residue enantiomeric analysis of 18 chiral pesticides in water, soil and river sediment using magnetic solid-phase extraction based on amino modified multiwalled carbon nanotubes and chiral liquid chromatography coupled with tandem mass spectrometry. J Chromatogr A 1568:8–21
99. Marsousi S, Karimi-Sabet J, Moosavian MA, Amini Y (2019) Liquid-liquid extraction of calcium using ionic liquids in spiral microfluidics. Chem Eng J 356:492–505
100. Hashemi B, Zohrabi P, Kim K-H, Shamsipur M, Deep A, Hong J (2017) Recent advances in liquid-phase microextraction techniques for the analysis of environmental pollutants. TrAC Trends Anal Chem 97:83–95
101. He Y (2012) Liquid-based microextraction techniques for environmental analysis. In: Pawliszyn JL (ed) Comprehensive sampling and sample preparation: analytical techniques for scientists, vol 3. Academic Press, Oxford, pp 835–862
102. Rutkowska M, Płotka-Wasylka J, Sajid M, Andruch V (2019) Liquid–phase microextraction: a review of reviews. Microchem J 149
103. Hashemi SH, Ziyaadini M, Kaykhaii M, Jamali Keikha A, Naruie N (2020) Separation and determination of ciprofloxacin in seawater, human blood plasma and tablet samples using molecularly imprinted polymer pipette-tip solid phase extraction and its optimization by response surface methodology. J Sep Sci 43(2):505–513
104. Nie Q, Nie S (2019) High-performance liquid chromatography for food quality evaluation. In: Evaluation technologies for food quality, Elsevier, p. 267–299
105. Arisseto-Bragotto AP, Feltes MMC, Block JM (2017) Food quality and safety progress in the Brazilian food and beverage industry: chemical hazards. Food Qual Saf 1(2):117–129
106. Moradi M, Razavi R, Omer AK, Farhangfar A, McClements DJ (2022) Interactions between nanoparticle-based food additives and other food ingredients: a review of current knowledge. Trends Food Sci Technol 120:75–87
107. Sanchis Y, Yusà V, Coscollà C (2017) Analytical strategies for organic food packaging contaminants. J Chromatogr A 1490:22–46
108. Jiang H-L, Li N, Cui L, Wang X, Zhao R-S (2019) Recent application of magnetic solid phase extraction for food safety analysis. TrAC Trends Anal Chem 120:115632
109. Donno D, Mellano MG, Gamba G, Riondato I, Beccaro GL (2020) Analytical strategies for fingerprinting of antioxidants, nutritional substances, and bioactive compounds in foodstuffs based on high performance liquid chromatography–mass spectrometry: an overview. Foods 9(12):1734
110. Callao MP, Ruisánchez I (2018) An overview of multivariate qualitative methods for food fraud detection. Food Control 86:283–293
111. Rodionova OY, Pomerantsev AL (2020) Chemometric tools for food fraud detection: the role of target class in non-targeted analysis. Food Chem 317:126448
112. Gupta PK (2020) Poisonous foods and food poisonings. In: Problem solving questions in toxicology, Springer, p. 229–238
113. Gupta R, Raza N, Bhardwaj SK, Vikrant K, Kim K-H, Bhardwaj N (2021) Advances in nanomaterial-based electrochemical biosensors for the detection of microbial toxins, pathogenic bacteria in food matrices. J Hazard Mater 401:123379
114. Nemati M, Mogaddam MRA, Farazajdeh MA, Tuzen M, Khandaghi J (2021) In-situ formation/decomposition of deep eutectic solvent during solidification of floating organic droplet-

liquid-liquid microextraction method for the extraction of some antibiotics from honey prior to high performance liquid chromatography-tandem mass spectrometry. J Chromatogr A:462653
115. Watanabe E, Seike N (2020) Detection of herbicide clopyralid at nanogram per gram level in agricultural products using easy-to-use micro liquid–liquid extraction followed by analysis with ultraperformance liquid chromatography–tandem mass spectrometry. J Chromatogr A 1630:461578
116. Zheng W, Yoo K-H, Abd El-Aty AM, Park D-H, Choi J-M, Kim S-K et al (2019) Quantitative determination of carbasalate calcium derived metabolites, acetylsalicylic acid and salicylic acid, in six animal foods using liquid-liquid extraction method coupled with liquid chromatography-tandem mass spectrometry. Food Chem 278:744–750
117. Salim SA, Sukor R, Ismail MN, Selamat J (2021) Dispersive liquid–liquid microextraction (DLLME) and LC-MS/MS analysis for multi-mycotoxin in rice bran: method development, optimization and validation. Toxins (Basel) 13(4):280
118. Alsharif AMA, Choo Y-M, Tan GH, Abdulra'uf LB. (2019) Determination of mycotoxins using hollow fiber dispersive liquid–liquid–microextraction (HF-DLLME) prior to high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS). Anal Lett 52(12):1976–1990
119. Hu S, Zhao M, Wang Z, Yang J, Chen D, Yan P (2021) Development of a pH-dependent homogeneous liquid-liquid extraction by cold-induced phase separation in acetonitrile/water mixtures for determination of quinolone residues in animal-derived foods. J Chromatogr A 1649:462235
120. Szabo BS, Jakab PP, Hegedűs J, Kirchkeszner C, Petrovics N, Nyiri Z et al (2021) Determination of 24 primary aromatic amines in aqueous food simulants by combining solid phase extraction and salting-out assisted liquid–liquid extraction with liquid chromatography tandem mass spectrometry. Microchem J 164:105927
121. Caldeirão L, Fernandes JO, Gonzalez MH, Godoy HT, Cunha SC (2021) A novel dispersive liquid-liquid microextraction using a low density deep eutectic solvent-gas chromatography tandem mass spectrometry for the determination of polycyclic aromatic hydrocarbons in soft drinks. J Chromatogr A 1635:461736
122. Moreno-González D, García-Campaña AM (2017) Salting-out assisted liquid–liquid extraction coupled to ultra-high performance liquid chromatography–tandem mass spectrometry for the determination of tetracycline residues in infant foods. Food Chem 221:1763–1769
123. Sun M, Xu D, Wang S, Uchiyama K (2019) Inkjet-based dispersive liquid–liquid microextraction method coupled with UHPLC–MS/MS for the determination of aflatoxins in wheat. Anal Chem 91(4):3027–3034
124. Desmarchelier A, Anizan S, Minh Tien M, Savoy M-C, Bion C (2018) Determination of five tetracyclines and their epimers by LC-MS/MS based on a liquid-liquid extraction with low temperature partitioning. Food Addit Contam A 35(4):687–695
125. Abdallah OI, Ahmed NS (2019) Development of a vortex-assisted dispersive liquid-liquid microextraction (VA-DLLME) and LC-MS/MS procedure for simultaneous determination of fipronil and its metabolite fipronil sulfone in tomato fruits. Food Anal Methods 12(10):2314–2325
126. Lawal A, Wong RCS, Tan GH, Abdulra'uf LB, Alsharif AMA. (2018) Multi-pesticide residues determination in samples of fruits and vegetables using chemometrics approach to QuEChERS-dSPE coupled with ionic liquid-based DLLME and LC–MS/MS. Chromatographia 81(5):759–768
127. Celano R, Piccinelli AL, Campone L, Russo M, Rastrelli L (2019) Determination of selected pyrrolizidine alkaloids in honey by dispersive liquid–liquid microextraction and ultrahigh-performance liquid chromatography–tandem mass spectrometry. J Agric Food Chem 67(31):8689–8699
128. Quigley A, Connolly D, Cummins W (2019) Determination of selected amino acids in milk using dispersive liquid–liquid microextraction and GC-MS. Anal Methods 11(28):3538–3545

129. Franco ES, Pádua VL, Rodriguez MDVR, Silva DF, Libanio M, Pereira MC et al (2019) A simple liquid-liquid extraction-gas chromatography-mass spectrometry method for the determination of haloacetic acids in environmental samples: application in water with Microcystis aeruginosa cells. Microchem J 150:104088
130. Erarpat S, Cağlak A, Bodur S, Chormey SD, Engin ÖG, Bakırdere S (2019) Simultaneous determination of fluoxetine, estrone, pesticides, and endocrine disruptors in wastewater by gas chromatography–mass spectrometry (GC–MS) following switchable solvent–liquid phase microextraction (SS–LPME). Anal Lett 52(5):869–878
131. Chormey DS, Bodur S, Baskın D, Fırat M, Bakırdere S (2018) Accurate and sensitive determination of selected hormones, endocrine disruptors, and pesticides by gas chromatography–mass spectrometry after the multivariate optimization of switchable solvent liquid-phase microextraction. J Sep Sci 41(14):2895–2902
132. Cacho JI, Campillo N, Viñas P, Hernández-Córdoba M (2018) In situ ionic liquid dispersive liquid-liquid microextraction coupled to gas chromatography-mass spectrometry for the determination of organophosphorus pesticides. J Chromatogr A 1559:95–101
133. Campillo N, Oller-Ruiz A, Hernandez-Cordoba M, Vinas P (2020) In situ generated ionic liquid and dispersive liquid-phase microextraction to determine chlorobenzenes in environmental samples by gas chromatography-mass spectrometry. Microchem J 159:105515
134. Chormey DS, Er EÖ, Erarpat S, Özzeybek G, Arı B, Bakirdere S (2018) A novel analytical approach for the determination of parathion methyl in water: quadrupole isotope dilution mass spectrometry-dispersive liquid–liquid microextraction using multivariate optimization. Analyst 143(5):1141–1146
135. Bulgurcuoğlu AE, Yılmaz B, Chormey DS, Bakırdere S (2018) Simultaneous determination of estrone and selected pesticides in water medium by GC-MS after multivariate optimization of microextraction strategy. Environ Monit Assess 190(4):1–10
136. Rascón AJ, Azzouz A, Ballesteros E (2018) Multiresidue determination of polycyclic aromatic hydrocarbons in edible oils by liquid-liquid extraction–solid-phase extraction–gas chromatography–mass spectrometry. Food Control 94:268–275
137. Chen B, Jin B, Jiang R, Xie L, Lin Y, Feng W et al (2014) Screening and quantification of 304 pesticides and related organic pollutants in surface water using dispersive liquid–liquid microextraction coupled with gas chromatography-mass spectrometry. Anal Methods 6(6):1743–1752
138. Celano R, Piccinelli AL, Campone L, Rastrelli L (2014) Ultra-preconcentration and determination of selected pharmaceutical and personal care products in different water matrices by solid-phase extraction combined with dispersive liquid–liquid microextraction prior to ultra high pressure liquid chromatography. J Chromatogr A 1355:26–35
139. Guiñez M, Bazan C, Martinez LD, Cerutti S (2018) Determination of nitrated and oxygenated polycyclic aromatic hydrocarbons in water samples by a liquid–liquid phase microextraction procedure based on the solidification of a floating organic drop followed by solvent assisted back-extraction and liquid ch. Microchem J 139:164–173
140. de Oliveira ML, Rocha BA, de Oliveira Souza VC, Barbosa Jr F (2019) Determination of 17 potential endocrine-disrupting chemicals in human saliva by dispersive liquid-liquid microextraction and liquid chromatography-tandem mass spectrometry. Talanta 196:271–276
141. Cunha SC, Pena A, Fernandes JO (2015) Dispersive liquid–liquid microextraction followed by microwave-assisted silylation and gas chromatography-mass spectrometry analysis for simultaneous trace quantification of bisphenol a and 13 ultraviolet filters in wastewaters. J Chromatogr A 1414:10–21
142. Caldas SS, Rombaldi C, de Oliveira Arias JL, Marube LC, Primel EG (2016) Multi-residue method for determination of 58 pesticides, pharmaceuticals and personal care products in water using solvent demulsification dispersive liquid–liquid microextraction combined with liquid chromatography-tandem mass spectrometry. Talanta 146:676–688

143. Salvatierra-stamp V, Muñiz-Valencia R, Jurado JM, Ceballos-Magaña SG (2018) Hollow fiber liquid phase microextraction combined with liquid chromatography-tandem mass spectrometry for the analysis of emerging contaminants in water samples. Microchem J 140:87–95
144. Bulgurcuoğlu AE, Durak BY, Chormey DS, Bakırdere S (2021) Development of a switchable solvent liquid phase extraction method for the determination of chlorthiamid, ethyl parathion, penconazole and fludioxonil pesticides in well, tap and lake water samples by gas chromatography mass spectrometry. Microchem J 168:106381
145. Ren J-L, Zhang A-H, Kong L, Wang X-J (2018) Advances in mass spectrometry-based metabolomics for investigation of metabolites. RSC Adv 8(40):22335–22350
146. Xiang L, Zhu L, Huang Y, Cai Z (2020) Application of derivatization in fatty acids and fatty acyls detection: mass spectrometry-based targeted lipidomics. Small Methods 4(8):2000160
147. De Perre C, Whiting SA, Lydy MJ (2015) A simultaneous extraction method for organophosphate, pyrethroid, and neonicotinoid insecticides in aqueous samples. Arch Environ Contam Toxicol 68(4):745–756
148. Pérez-Fernández V, Rocca LM, Tomai P, Fanali S, Gentili A (2017) Recent advancements and future trends in environmental analysis: sample preparation, liquid chromatography and mass spectrometry. Anal Chim Acta 983:9–41

Elemental Mass Spectrometry in Food and Environmental Chemistry

Bin Hu, Man He, Beibei Chen, Chi Xu, Qiulin Zhang, Junrong Ma, Yiling Feng, and Zewei Cui

Contents

B. Hu (✉), M. He, B. Chen, C. Xu, Q. Zhang, J. Ma, Y. Feng, and Z. Cui
Department of Chemistry, Wuhan University, Wuhan, China
e-mail: binhu@whu.edu.cn

Yolanda Picó and Julian Campo (eds.), *Mass Spectrometry in Food and Environmental Chemistry*, Hdb Env Chem (2023) 119: 53–98, DOI 10.1007/698_2022_890,

Published online: 18 October 2022

Abstract Analytical challenges of elemental mass spectrometry are discussed, introducing the fundamental principles of inductively coupled plasma mass spectrometry (ICP-MS) and its variants such as chromatographic techniques-ICP-MS, multiple-collector-ICP-MS, laser ablation-ICP-MS, and field flow fractionation-ICP-MS. Applications are discussed and commented. Some examples illustrate the analytical approaches, which are used to address specific issues in various areas of both food and nutrition and environmental research, such as food authentication, metallomics, speciation, toxic and nutritive elements, nanomaterials, and migration studies.

Keywords Chromatographic separation, Field flow, Inductively coupled plasma mass spectrometry, Laser ablation, Multiple collector, Solid/liquid phase (micro)extraction

1 Introduction

Elemental Mass Spectrometry refers to the analytical techniques for qualitative and quantitative analysis of elements based on mass-to-charge ratio, including inductively coupled plasma mass spectrometry (ICP-MS), thermal ionization mass spectrometry (TIMS), and secondary ion mass spectrometry (SIMS). The latter two techniques are mainly used for isotope ratio measurements, along with multiple collector (MC)-ICP-MS [1]. Comparatively, ICP-MS merits much application potential in trace elemental quantification and speciation analysis. Traditional ICP-MS is limited to the quantification of trace elements in aqueous solution. By combining with efficient separation techniques, the information of specific elemental species can be obtained by ICP-MS. Moreover, single particle (SP) ICP-MS, based on the generation of discrete pulses of ions arising from SPs sequentially introduced into ICP, has been proposed for the measurement of metal-containing nanoparticles, providing the information of particle size and concentration [2]. Likewise, direct elemental analysis for solid samples with ICP-MS can be carried out replacing conventional pneumatic nebulization with laser ablation as the sample introduction system, enabling mapping of elemental distribution with lateral resolution at μm in bioimaging [3].

This chapter is focused on the development of variants of ICP-MS and their applications in food and environmental chemistry.

2 Solid Phase (Micro)Extraction/Liquid Phase (Micro)Extraction-ICP-MS

The quantification of trace elements by ICP-MS is often affected by the complexity of the sample, and sometimes the instrumental quantification limit does not reach the sensitivity levels required for the analysis of food and environmental samples. To achieve accurate elemental concentration at ultra-trace levels, appropriate sample pretreatment is always necessary prior to ICP-MS detection for the removal of sample matrix and simultaneous preconcentration of target elements. Solid phase (micro) extraction and liquid phase (micro) extraction are the two most commonly used pretreatment techniques.

2.1 Solid Phase (Micro) Extraction

Solid phase extraction (SPE) is based on the distribution of target analytes between the solid phase and liquid phase. SPE adsorbents determine the analytical performance of the SPE-based method to a great extent in terms of selectivity, enrichment factor, and adsorption capacity. To improve the selectivity of the sorbents, a variety of functionalized or imprinted sorbents have been synthesized. Besides, a series of extraction modes have been proposed to facilitate different analytical purposes, including magnetic solid phase extraction (MSPE), solid phase microextraction (SPME), capillary microextraction (CME), and stir bar sorptive extraction (SBSE).

2.1.1 Trace Elemental Quantification

In conventional SPE, the sorbents are usually packed in a column, which can be easily integrated in a flow injection system for online analysis [4]. In MSPE, magnetic sorbents along with the adsorbed target elements could be easily and rapidly separated from the aqueous solution simply by applying an external magnetic field, while no filtration or centrifugation was necessary. Titania coated magnetic sorbents were synthesized and applied for SPE of trace heavy metal ions from environmental water samples followed by ICP-MS detection [5]. The maximal enrichment factor (EF) of 200-fold could be achieved with the use of 100 mL sample solution. Thiol-grafted magnetic polymer (MPPs-SH) was synthesized by click reaction. MPPs-SH had high S content (5.4 wt.%) and exhibited high adsorption capacity of 254, 64.2, 56.6 mg g^{-1} for Hg(II), Cd(II), and Pb(II), respectively. Based on it, a method of MSPE-ICP-MS was developed for the quantification of trace Hg (II), Cd(II), and Pb(II) in environmental water samples [6]. Three heavy metal ions were extracted from 150 mL aqueous solution in 10 min and eluted by 0.2 mol L^{-1} HNO_3 containing 2% thiourea in 5 min. The method presented low limits of detection (LODs) (0.21, 0.82, and 2.9 ng L^{-1}) for target ions. Erkan et al. [7]

synthesized α-amylase-modified magnetic carbon multi-walled nanotubes (-α-amylase-Fe_3O_4/MWCNTs) and used them for the extraction of arsenic traces from food samples. The composite overcame the problem of inactivation of the free soluble enzyme after extraction, and can be reused at least six times. The microextraction step only takes 15 min. A quantification limit (LOQ) of 47.3 μg kg^{-1} was achieved for the analysis of arsenic in rice and flour samples. With similar strategy, magnetic sorbents doped with different functional groups (such as N, O, S-containing groups) were combined with ICP-MS detection for the analysis of various elements in environmental water samples [8–11].

Besides, imprinting technique is an alternative for the selectivity improvement of the sorbents. Cd(II) imprinted 3-mercaptopropyltrimethoxysilane (MPTS)-silica coated stir bar was prepared by combining sol-gel technique with surface imprinting technique. The imprinted coating exhibited higher selectivity for Cd(II) in the presence of Cu(II) and Ni(II) over non-imprinted coating. The prepared stir bar was employed for SBSE of trace Cd(II) followed by ICP-MS detection. The proposed method was applied for the analysis of trace Cd(II) in rain, lake, and river water samples [12].

2.1.2 Elemental Speciation Analysis

With the use of functionalized sorbents, the selectivity of the method could be improved, sometimes enabling speciation analysis for ICP-MS. For example, 3-(2-aminoethylamino) propyltrimethoxysilane (AAPTS) functionalized MWCNTs were synthesized and used for simultaneous speciation analysis of inorganic As, Cr, and Se in environmental water samples prior to their determination by ICP-MS [13]. At pH 2.2, As(V), Cr(VI), and Se(VI) could be quantitatively retained on the prepared sorbent while As(III), Cr(III), and Se(IV) could not be extracted. The retained As(V), Cr(VI), and Se(VI) were eluted by HNO_3 and determined by ICP-MS. With using $KMnO_4$ as oxidation reagent, the low valence species of As (III), Cr(III), Se (IV) could be transformed into their high valence species (As(V), Cr (VI), and Se(VI)) and the total amount of each element could be determined by the same method. The concentrations of low valence species were then calculated by subtraction. Under the optimized conditions, LODs of 15, 38, and 16 ng L^{-1} for As (V), Cr(VI), and Se(VI) were obtained. Moreover, Cr(III)-imprinted AAPTS functionalized silica gel sorbent was prepared for the speciation analysis of Cr in environmental water samples prior to ICP-MS measurement [14]. The Cr(III)-imprinted silica gel shows the selectivity coefficient of more than 700 for Cr(III) in the presence of Mn(II). Cr(VI) could be adsorbed at low pH by the prepared imprinted silica gel, making it feasible to enrich and determine Cr(VI) at low pH without adding reducing reagents. LODs of 4.43 and 8.30 pg mL^{-1} for total Cr and Cr(VI) were obtained. Compared with most of the analytical methods reported in literature for the speciation of inorganic Cr, the proposed method does not need any reduction/oxidation reagent in the process of separation, which prevented the potential transformation of speciation and contamination.

AAPTS-silica monolithic capillary was used for CME of aluminum fractionation, followed by electrothermal vaporization (ETV)-ICP-MS detection with polytetrafluoroethylene (PTFE) slurry as fluorinating agent [15]. In the pH range of 4–7, labile monomeric Al could be retained quantitatively on the monolithic capillary, while non-labile monomeric Al passed through the capillary directly. The total monomeric Al fraction was determined by AAPTS-silica monolithic CME-fluorination-assisted (FETV)-ICP-MS after the sample solution was adjusted to pH 8.8. Non-labile monomeric Al was obtained by subtracting labile monomeric Al from the total monomeric Al. The method was applied to the fraction of Al in rainwater and fruit juice. This system had advantages over the existing 8-hydroxyquinoline (8-HQ) chelating system for Al fractionation such as wider pH range, higher tolerance of interference, and better regeneration. Besides, a method of dual silica monolithic CME online hyphenated with ICP-MS was developed for sequential determination of inorganic As(III)/As(V) and Se(IV)/Se(VI) in natural waters [16]. The dual CME column was composed of MPTS modified silica monolithic capillary (C1) and AAPTS modified silica monolithic capillary (C2) which both were prepared by sol-gel technology. With the aid of valve-switching, As(III)/Se(IV) retained on C1 and As(V)/Se(VI) retained on C2 were sequentially desorbed by 100 μL of appropriate eluents for subsequent online ICP-MS determination (Fig. 1). Four species of As(III)/As(V) and Se(IV)/Se(VI) could be accurately determined without oxidation/reduction or subtraction, respectively.

Krishna et al. [17] monitored trace inorganic mercury (iHg) and methyl mercury (MeHg) in environmental water and fish tissues by a microcolumn filled with polyaniline (PANI) and online flow injection-chemical vapor generation (CVG)-ICP-MS. Different Hg species were adsorbed into the microcolumn through the N-containing functional groups on the polyaniline and then eluted. Under optimal conditions, the LOD is 2.52 pg for iHg and 3.24 pg for MeHg (in Hg). The developed method was applied to the direct determination of iHg and MeHg in tap, lake, ground, and sea water. Rodolfo et al. [18] adsorbed different iodophenols through various types of solid phase microextraction PDMS fibers. After elution, they were analyzed by gas chromatography (GC)-ICP-MS. The method can extract iodophenol in a variety of water samples effectively.

2.1.3 Nanoparticles Analysis

Metal-containing nanoparticles can be regarded as a specific existing form of the element. Solid phase (micro) extraction combined with ICP-MS detection has been applied for the analysis of metal-containing nanoparticles.

Al^{3+} immobilized Fe_3O_4@SiO_2@iminodiacetic acid (IDA) was prepared and its extraction performance for gold nanoparticles (AuNPs) and gold ions (Au ions) was investigated [19]. AuNPs and Au ions could be simultaneously retained on the adsorbent and their separation was achieved by sequential elution of Au ions and AuNPs with $Na_2S_2O_3$ and $NH_3{\cdot}H_2O$, respectively. Based on it, a strategy by

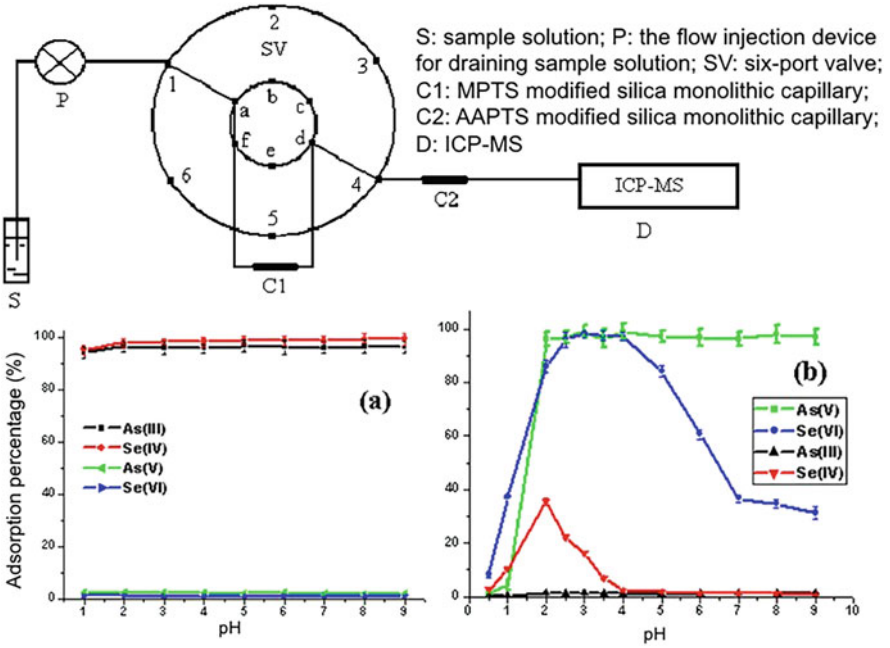


Fig. 1 Effect of pH on the adsorption behavior of inorganic As(III)/As(V) and Se(IV)/Se(VI) on AAPTS-silica monolithic capillary (**a**) and MPTS-silica monolithic capillary (**b**). Reproduced from J. Anal. At. Spectrom., 2009. 24(8): 1051–1061 with permission from the Royal Society of Chemistry

coupling MSPE with ICP-MS was developed for the speciation of AuNPs and Au ions in environmental water samples. AuNPs in a size range of 14–140 nm and with different coatings could be quantitatively determined. The size and shape of the AuNPs were kept unchanged during the extraction process. The method was applied to the analysis of AuNPs in natural water, sewage water, and even sea water samples. Wang et al. [20] synthesized phosphoric acid-functionalized superparamagnetic iron oxide. LOD of the MSPE-ICP-MS method for TiO_2NPs is 17 ng L^{-1} with the enrichment factor of 400. This method was verified to be applicable to the detection of trace TiO_2NPs in river and lake water. Besides, the adsorption mechanism of functionalized adsorbents on TiO_2NPs and titanium ions was investigated by dispersion-corrected density functional theory, which was assumed to be the competitive adsorption of different deprotonated phosphates and OH-anions against TiO_2NPs or aqueous titanium ions (Fig. 2).

Zhang et al. [21] prepared a hydrophilic polymer capillary monolithic column for the separation and enrichment of carboxyl-containing AuNPs and applied this method to the analysis of carboxyl AuNPs in a variety of water samples. The LOD is 24.2 fmol L^{-1}, the adsorption is mainly dependent on the affinity of the carboxyl group and the polymerized monomer on the capillary. Based on the difference in the elution behavior of AuNPs with different particle sizes on the monolithic column

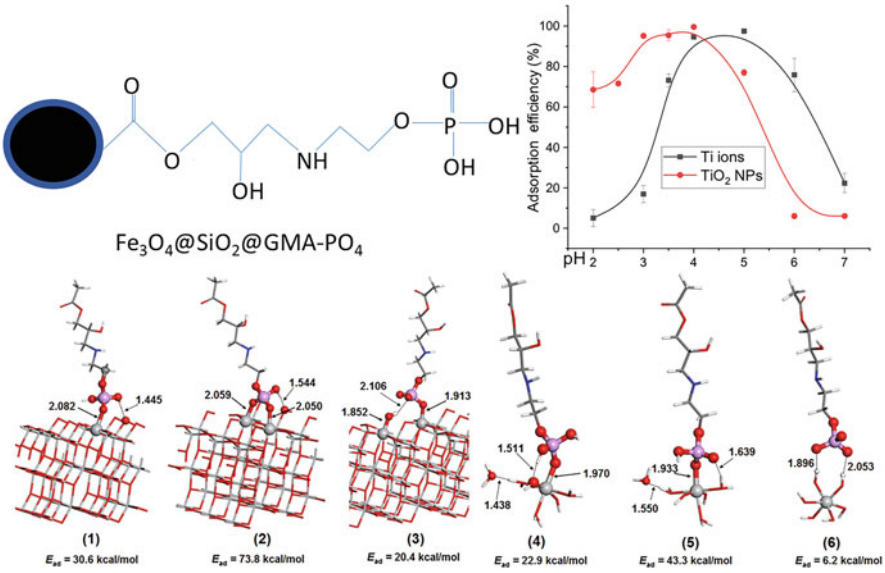


Fig. 2 Phosphoric acid-functionalized magnetic sorbent used for determination of TiO_2 NPs. Reproduced from Sci. Total Environ., 2020. 703: 135464 with permission from 2019 Elsevier *B.V.*

(Fig. 3), Liu et al. achieved the separation of AuNPs with particle size in the range of 3–40 nm and quantification of AuNPs in water sample [22].

2.2 Liquid Phase (Micro) Extraction

Liquid phase (micro) extraction is based on the distribution of target elements or species between aqueous and organic phase, including traditional liquid–liquid extraction (LLE) and miniaturized systems. A variety of liquid phase microextraction (LPME) techniques have been developed, such as single drop microextraction (SDME), hollow fiber liquid phase microextraction (HF-LPME), dispersive liquid-liquid microextraction (DLLME), and solidified floating organic drop microextraction (SFODME) [23]. Cloud point extraction (CPE) based on the cloud point phenomenon of surfactant is also a liquid phase (micro) extraction mode. Compared to solid phase (micro)extraction, the application of liquid phase (micro) extraction in trace/ultra-trace elements especially speciation analysis is much less due to the lack of suitable extraction system, and each mode of LPME has its own drawbacks, such as the instability of solvent drop in SDME, the relatively long extraction time in HF-LPME, and the limited applicable organic solvent for DLLME and SFODME.

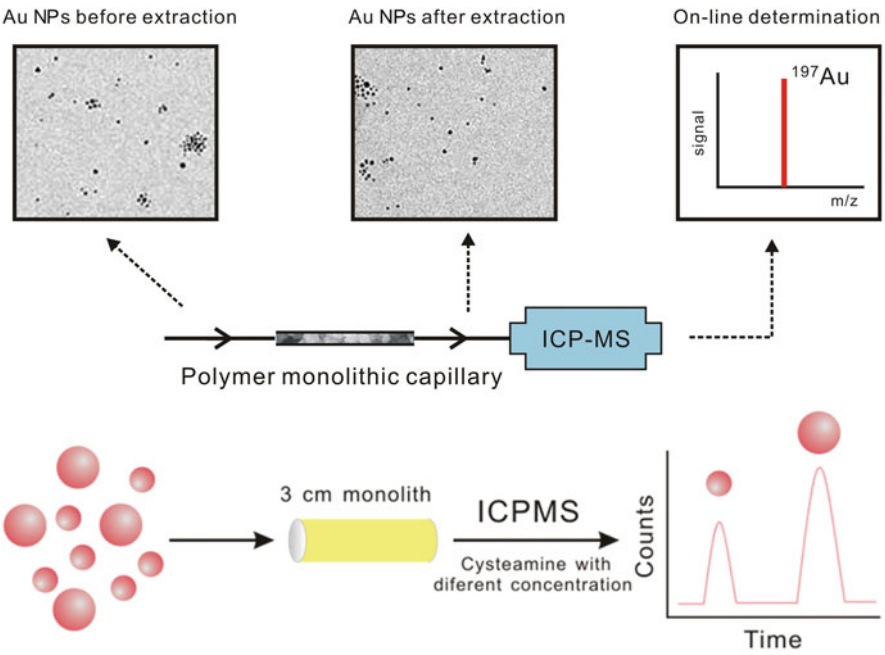


Fig. 3 Analysis of AuNPs with different sizes by polymer monolithic capillary. Reprinted (adapted) with permission from Anal. Chem., 2015. 87(3):1789–1796, and Anal. Chem., 2017. 89(1):560–564. Copyright {2015, 2017} American Chemical Society

2.2.1 Trace Elemental Quantification

In LLE or LPME system for elemental analysis, a chelating reagent is usually necessary to transform the metal ions with strong polarity into nonpolar species, transferring target elements from aqueous solution into organic phase. To facilitate the introduction of organic phase obtained from LPME or LLE into ICP, conventional pneumatic nebulization can be replaced by ETV, which merits low sample consumption, ability of introducing solid, liquid, or organic solvents, and ability of removing sample matrix or solvents with the use of temperature program or chemical modifier.

Xia et al. [24] applied benzoylacetone as the extractant for Be, Co, Pd, and Cd, and developed a method of SDME-ETV-ICP-MS for trace analysis. Benzoylacetone acted as the chemical modifier in ETV simultaneously to improve the vaporization behavior of target analytes. The LODs of the developed methods are 0.12, 0.99, 1.5, and 0.27 pg mL^{-1}, respectively. Phase transfer (PT)-HF-LPME combined with ETV-ICP-MS was developed for the determination of trace Co, Pd, Cd, and Bi in environmental and biological samples [25]. In PT-HF-LPME, an intermediate solvent (1-butanol) was added into the sample solution to ensure the maximum contact area between the target metal ions and the chelating reagent (8-hydroxyquinoline), which accelerated the formation of 8-hydroxyquinoline-metal complexes and their

subsequent extraction by extraction solvent (toluene). The EFs for Co, Pd, Cd, and Bi were 110, 393, 121, and 111-fold, respectively, and the LODs ranged from 3.7 to 8.3 ng L^{-1}. Moreover, SFODME was combined with ETV-ICP-MS for the determination of trace heavy metals in environmental water samples with sodium diethyldithiocarbamate (DDTC) as both chelating reagent in SFODME and chemical modifier in ETV [26]. Chandrasekaran et al. [27] extracted platinum group elements (Pd, Os, Ir, and Pt) and transition elements (Ag, Cd, Ta, and Re) in the form of chlorine and/or fluorine (anion) complexes through DLLME prior to ICP-MS analysis. During the extraction process, the anion complex formed in the presence of 1 ng L^{-1} HCl and 0.5 ng L^{-1} F^- can be extracted into chloroform containing trioctyl methyl ammonium chloride at room temperature, and then the analytes are extracted from the chloroform layer by the mixture of chloric acid and nitric acid for subsequent determination by direct injection in the ICP-MS. The EF for target eight elements was 27–75. This method was applied to the determination of target metal elements in lake water polluted by industrial wastewater and hospital waste.

To further increase the EF, a two-step method involving dispersive solid phase extraction (D-SPE) and DLLME was developed for the preconcentration of 15 rare earth elements (REEs) in environmental water and sediment samples, followed by ETV-ICP-MS measurement [28]. With Chelex 100 as the adsorbent of D-SPE, the target REEs were firstly extracted and then desorbed by 0.1 mol L^{-1} HNO_3. After addition of 125 mmol L^{-1} tris and 40 mmol L^{-1} 1-phenyl-3-methyl-4-benzoylpyrazolone, target REEs were further preconcentrated into CCl_4 by DLLME. The resultant EFs varied from 234- to 566-fold.

2.2.2 Elemental Speciation Analysis

The use of solid phase (micro)extraction or liquid phase (micro) extraction prior to ICP-MS determination can only differentiate a limited number of elemental species, such as Cr(III)/Cr(VI), As(III)/As(V). The speciation of multiple elemental species depends more on chromatographic techniques combined with ICP-MS detection, which will be discussed in Sect. 3. Sometimes, the main contribution of sample pretreatment in speciation analysis is removal of sample matrix and preconcentration of target species, rather than separating interest species.

Ionic liquid-based carrier mediated hollow fiber liquid–liquid–liquid microextraction (IL-carrier mediated HF-LLLME) was combined with high performance liquid chromatography (HPLC)-ICP-MS for the speciation of five phenylarsenic compounds and arsenate in chicken and feed samples [29]. Target arsenic species were extracted from aqueous samples at basic pH into an organic phase (20% (v/v) of methyltrioctylammonium chloride $[MTOA]^+[Cl]^-$ in toluene) impregnated in the pores of a hollow fiber, then back extracted into 10 μL acceptor phase of 0.3 mol L^{-1} NaBr in the lumen of the hollow fiber. The main driving force for the extraction was the gradient concentration of counter ion from the donor phase to the acceptor phase. The LODs for five target phenylarsenic compounds and arsenate were in the range of 1.4–16 ng L^{-1}, with EFs varying from 86 to 372.

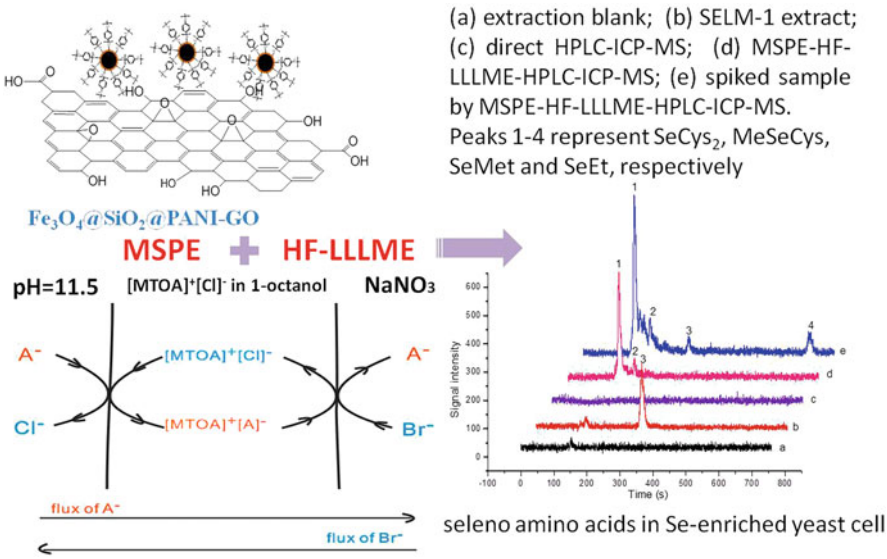


Fig. 4 Speciation analysis of seleno amino acids by MSPE-HF-LLLME. Reproduced from Ref. [30] with permission from the Royal Society of Chemistry

MSPE was further combined with HF-LLLME for the extraction of seleno amino acids and subsequent determination by HPLC-ICP-MS [30]. Cu^{2+} was adsorbed onto the surface of graphene oxide (GO) modified magnetic nanoparticles for the adsorption of seleno amino acids based on the interaction between Cu^{2+} and seleno amino acids. Aqueous ethylenediamine was employed as desorption solvent, and the desorption solution was directly employed as the donor solution in the subsequent HF-LLLME (Fig. 4). After preconcentration by MSPE-HF-LLLME, the EFs for target seleno amino acids ranged from 152 to 278, and the LODs for target seleno amino acids were in the range of 0.0075–0.013 μg L^{-1}. The method was applied to the speciation of seleno amino acids in rice and Se-enriched yeast cell samples.

2.2.3 Nanoparticles Analysis

Surfactant assisted DLLME combined with ETV-ICP-MS was proposed for the analysis of AuNPs in environmental water samples [31]. Effective separation of AuNPs from ionic gold species was achieved by using sodium thiosulfate as a complexing agent. LOD of 2.2 ng L^{-1} was achieved for AuNPs with an EF of 152 and the original morphology of the AuNPs could be maintained during the extraction process. TX-114-based CPE was demonstrated by Liu et al. [32] to be an efficient approach for selective extraction and concentration of trace silver nanoparticles (AgNPs) from environmental water samples without disturbing their sizes and shapes. The highest extraction efficiency for AgNPs was obtained at about

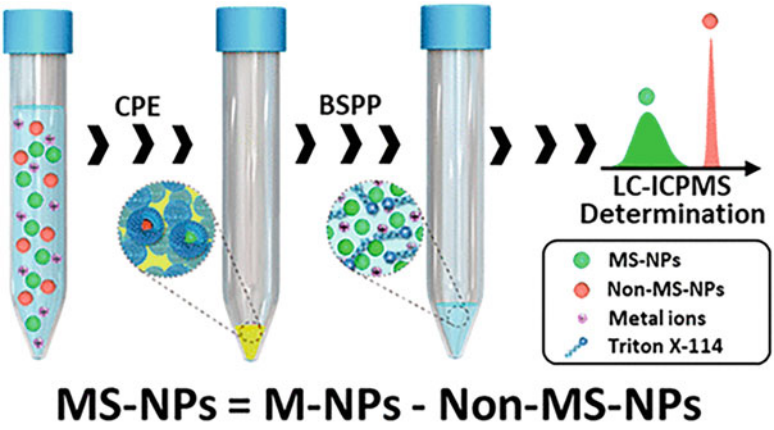


Fig. 5 Separation and determination of different nanoparticles by CPE-LC-ICP-MS. Reprinted (adapted) with permission from Anal Chem, 2020. 92(7):4765–4770. Copyright {2020} American Chemical Society

their zero point charge pH (pH_{PZC}), which was ~3.0–3.5 for the studied AgNPs. Addition of salts such as $NaNO_3$ or $Na_2S_2O_3$ enhanced the phase separation and thus increased the extraction efficiency of AgNPs. Furthermore, $Na_2S_2O_3$ efficiently eliminated the interference of Ag^+ due to the formation of a complex between Ag^+ and $S_2O_3^{2-}$ that was not extracted into the TX-114-rich phase. Similar strategy was applied for the determination of Ag_2S- and ZnS-NPs in environmental water samples (Fig. 5) [33], with the LOD of 8–15 ng L^{-1}.

3 ICP-MS Hyphenated with Chromatographic Techniques

The toxicity or bioavailability of a certain element is mainly attributed to its specific existing forms and relevant concentration. Therefore, not only the total amount of the elements, but also the speciation analysis would provide significant information for environmental pollution monitoring and food safety evaluation. Presently, ICP-MS is the most sensitive element-specific detection technique, but cannot provide species information due to the high temperature of ICP ionization source. With the aid of a highly efficient separation technique, this issue can be solved. So far, this strategy is most widely used for elemental speciation analysis. Various separation techniques have been combined with ICP-MS for the analysis of trace elements and their species in complex environmental and food samples. HPLC can be coupled with online ICP-MS detection easily by introducing the post-column effluent into the nebulizer with a connecting tube as short as possible. The interface between GC and ICP should be thermal insulated and the capillary column for chromatographic separation is inserted into ICP torch replacing the central tube. The interface between capillary electrophoresis (CE) and ICP needs compensation

fluid and grounding. Gel electrophoresis is combined with ICP-MS detection often in an offline mode. The application of ICP-MS hyphenated with chromatographic techniques for the analysis of specific elemental species in food and environmental samples is discussed as follows.

3.1 Chromium (Cr)

Cr is widely used in chemical and material industry, and vast amounts of Cr-rich wastes are discharged into the environment, causing potential pollution. Inorganic Cr species, Cr(III)/(VI), are the main forms of Cr existing in the environment. Cr(III) is an essential trace element in human body and participates in normal glucose and lipid metabolism, while Cr(VI) is a kind of carcinogen with strong oxidation ability. Anion exchange chromatography is often used to separate them. Gao et al. [34] extracted Cr species from river water samples with ZrO diffusive gradients in thin films (DGT) and desorbed them by EDTA. Then Cr(III) and Cr(VI) existing in the form of $[Cr(III)\text{-}EDTA]^-$ and CrO_4^{2-}, respectively, were subjected to HPLC-ICP-MS analysis. The separation and detection were completed within 4.5 min. It should be noted that the speciation analysis of Cr in solid samples, e.g., soil, requires additional pretreatment process, and the transformation of Cr species should not be ignored. Cr(III) was relatively stable under acidic conditions, and the oxidation of Cr (III) into Cr(VI) may occur under alkaline conditions. Quarles et al. [35] extracted Cr species from soil and sludge by hydrochloric acid and nitric acid; after filtration and dilution, the sample was directly analyzed by HPLC-ICP-MS. Drinčić et al. [36] used $NaOH/Na_2CO_3$ system to extract Cr species from river sediment. To avoid transformation of Cr(III) species, a little $MgCl_2$ was used as protective agent. The result for Sava River revealed that Cr(VI) levels are very low even at industrially exposed sites, not representing a considerable environmental burden.

3.2 Mercury (Hg)

Hg can be easily absorbed by plants, enriched in the food chain, and transformed into various organic forms. Hg exists in the environment mainly in the form of Hg(II), methylmercury ($MeHg^+$), ethylmercury ($EtHg^+$), and phenyl mercury ($PhHg^+$). Among them, $MeHg^+$ is highly toxic and humans can be easily exposed to it through seafood. HPLC-ICP-MS is the most commonly used methodology for mercury speciation analysis. With reversed phase C18 column as the stationary phase, various sulfur-containing complexing agents (e.g., 2-mercaptoethanol, L-cysteine) are added into the mobile phase to improve separation and reduce the adsorption of mercury in the HPLC system. Xia et al. [37] applied 2-mercaptoethanol as the complexing reagent in the mobile phase for the separation of three organomercury species. The content of methanol in mobile phase significantly affected the

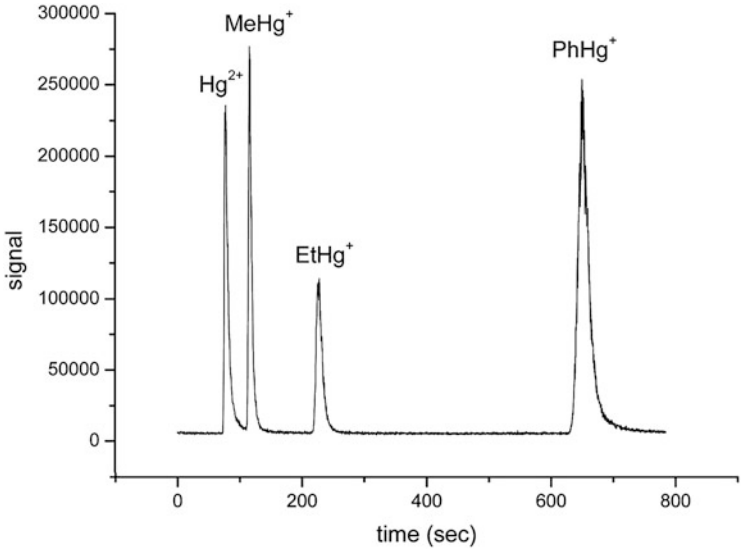


Fig. 6 Chromatogram of four mercury species obtained by HPLC-ICP-MS. Each species of 500 μg L^{-1} (as Hg). Column temperature: 50°C, mobile phase: methanol-buffer solution (2.5 mmol L^{-1} L-cysteine, 12.5 mmol L^{-1} $(NH_4)_2HPO_4$, 0.05% triethylamine, pH 7.0) (8:92), flow rate: 1.5 mL min^{-1}. Injection volume: 8 μL. Reproduced from J. Anal. At. Spectrom., 2015. 30(4):875–881 with permission from the Royal Society of Chemistry

separation resolution of $MeHg^+$ and $EtHg^+$. Under the optimized conditions (mobile phase: methanol/water (7:3, v/v) containing 0.001% 2-mercaptoethanol and 20 mmol L^{-1} buffer solution, pH 6.0; isocratic elution with a flow rate of 1 mL min^{-1}), baseline separation of three organomercury species was realized within 5 min. With L-cysteine as the complexing reagent, Hg(II), $MeHg^+$, $EtHg^+$, and $PhHg^+$ were separated in 12 min (Fig. 6) [38]. With the aid of HF-LLLME, enrichment factors of 120–350 [37] and 221–236-fold [38] were target mercury species, and the quantification limit of the method for target species was significantly improved.

Zhu et al. [39] proposed a MSPE-HPLC-ICP-MS method for the simultaneous speciation of mercury including Hg^{2+}, $MeHg^+$, and $PhHg^+$ in environmental water and fish samples. The functionalization of magnetic sorbents was realized by using γ-mercaptopropyltrimethoxysilane (γ-MPTS), and target mercury species were preconcentrated based on their affinity with sulfhydryl groups. Then, rapid separation of three target mercury species was achieved in 8 min by HPLC. By using 100 mL sample solution and 0.5 mL eluent, enrichment factor of 200-fold was achieved, with LODs of 0.74, 0.67, and 0.49 ng L^{-1} for Hg^{2+}, $MeHg^+$, and $PhHg^+$, respectively. Fe_3O_4@SiO_2@γ-MPTS particles were further packed into the channel on microfluidic chip, and chip-based MSPE was fabricated for the analysis of mercury species in cells (Fig. 7) [40]. It is suitable for the analysis of real sample

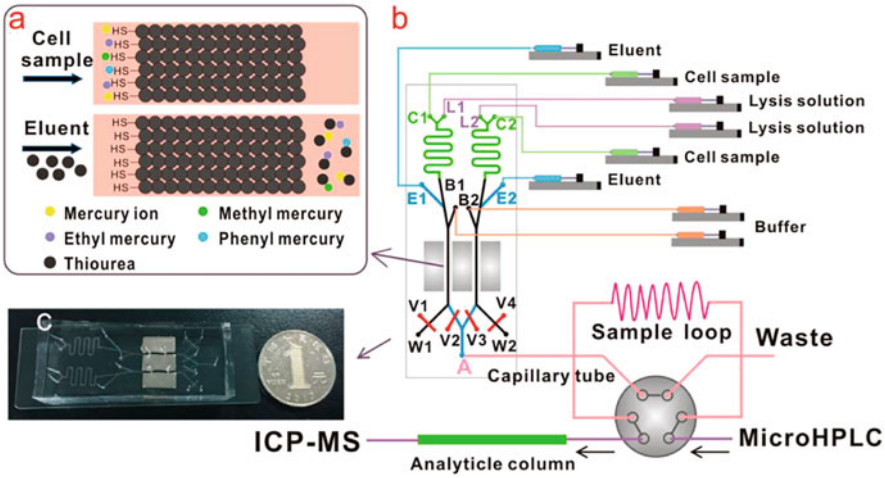


Fig. 7 Diagram of the magnetic packed column, microfluidic chip, and online chip-based MSPME–microHPLC–ICP-MS system. (**a**) Microextraction principle in the magnetic packed column. (**b**) Design sketch of online chip-based MSPME–microHPLC–ICP-MS system: the chip consists of a cell lysis unit (green lines), microextraction unit (black lines), and microvalves (red lines), the blue lines stand for the elution channels; C1 and C2 are the inlets of cell sample, L1 and L2 are the inlet of lysate solution, E1 and E2 are the inlets of eluent, B1 and B2 are the inlet of Fe_3O_4@SiO_2@γ-MPTS and buffer solution, A is the outlet of eluent and is connected with a six-way valve by a capillary tube directly, W1 and W2 are the outlets of waste, and V1, V2, V3, V4 are the gas inlets of microvalves. The width of the microextraction channels is 400 μm, and the microvalves channels are 500 μm wide; all the channels are 50 μm high. (**c**) Photograph of the microfluidic chip. Reprinted (adapted) with permission from Anal Chem, 2016. 88(1):796–802. Copyright {2016} American Chemical Society

with limited volume/amount available, and has been used to explore the antagonism of selenium and mercury [41].

Entwisle et al. [42] extracted Hg species in fish samples using a mild system containing L-cysteine, protease, and esterase. The chromatographic baseline separation of $MeHg^+$ and iHg was achieved in isocratic mode using reverse phase HPLC with a mobile phase containing L-cysteine as a complexing agent. Hg^{2+}, $MeHg^+$ and their isotopic ratio were analyzed by HPLC and CVG-MC-ICP-MS. Song et al. [43] analyzed Pb, Hg species in water by an online SPE-HPLC-ICP-MS method. After treatment with sulfhydryl reagent, target species can be enriched in a C_{18} column and desorbed by mobile phase containing L-cysteine and tetrabutyl ammonium hydroxide solution. Six elemental species were simultaneously analyzed with EFs of 459–2485.

3.3 Arsenic (As)

Arsenic exists as more than 50 chemical species in the environment and biological systems, which can be mainly divided into inorganic arsenic and organic arsenic species. Generally, the toxicity of inorganic arsenic is much higher than that of organic arsenic, and among inorganic arsenic, the toxicity of As(III) is much higher than that of As(V). As species can easily transform through redox or methylation reactions, making their analysis challenging. Letsoalo et al. [44] extracted As species from river sediments by $(NH_4)_2HPO_4$/EDTA assisted with microwave and analyzed them by HPLC-ICP-MS. The analysis of As(III)/(V) and monomethyl arsenic distribution in samples from different areas along Mokolo River showed that As-containing pesticides used in farming activities might be potential sources. Yang et al. [45] optimized the solvent system in DLLME by using solvent with low density and higher compatibility with mobile phase, to extract As species from water. Various As species in river water could be easily and quickly determined by HPLC-ICP-MS. Zhao et al. [46] used protease and hydrochloric acid to digest and extract As species from fish and analyzed them by HPLC-ICP-MS, the matrix interference was effectively reduced. Milani et al. [47] used microwave-assisted extraction with dilute nitric acid to extract As species from herbal tea. Four As species, As(III), As(V), monomethyl arsenic (MMA), and dimethyl arsenic (DMA), were analyzed by HPLC-ICP-MS and it was found that As(V) was the dominate species in Herbal tea from Brazil.

An organic arsenic species, 3-nitro-4-hydroxyphenylarsonic acid (Roxarsone, ROX) has been widely used as a feed additive in poultry industry for decades. However, most metabolites of ROX have not been identified. Peng et al. [48] investigated the arsenic species in chickens fed by ROX-containing food based on HPLC-ICP-MS and ESI-MS (Fig. 8). Methyl-3-nitro-4-hydroxyphenylarsonic acid (methyl-ROX), methyl-3-amino-4-hydroxyphenylarsonic acid (methyl-3-AHPAA), and methyl-3-acetamido-4-hydroxyphenylarsonic acid (or methyl-N-acetyl-ROX, methyl-N-AHPAA) were identified in the chicken livers, and the concentration of methyl-ROX was as high as 90 mg kg^{-1}, even after a five-day clearance period. The formation of these newly discovered methylated metabolites from reactions involving trivalent phenylarsonous acid substrates, S-adenosylmethionine, and the arsenic (+3 oxidation state) methyltransferase enzyme As3MT suggests that these compounds are formed by addition of a methyl group to a trivalent phenylarsenical substrate in an enzymatic process. The IC50 values of the trivalent phenylarsenical compounds were 300–30,000 times lower than those of the pentavalent phenylarsenicals.

Volatile As species play a major role in the transfer of As to the atmosphere, Ilgen et al. [49] developed a cryotrapping-cryofocusing (CT-CF) based method to capture volatile As species in gas samples or in samples after headspace extraction, followed by GC-ICP-MS. Sele et al. [50] used anion exchange column as SPE sorbents to enrich As species in fish oil. GC-ICP-MS analysis showed that As in fat accounted for 13–35% of the total As in fish oil. Comparatively, the application of CE-ICP-MS

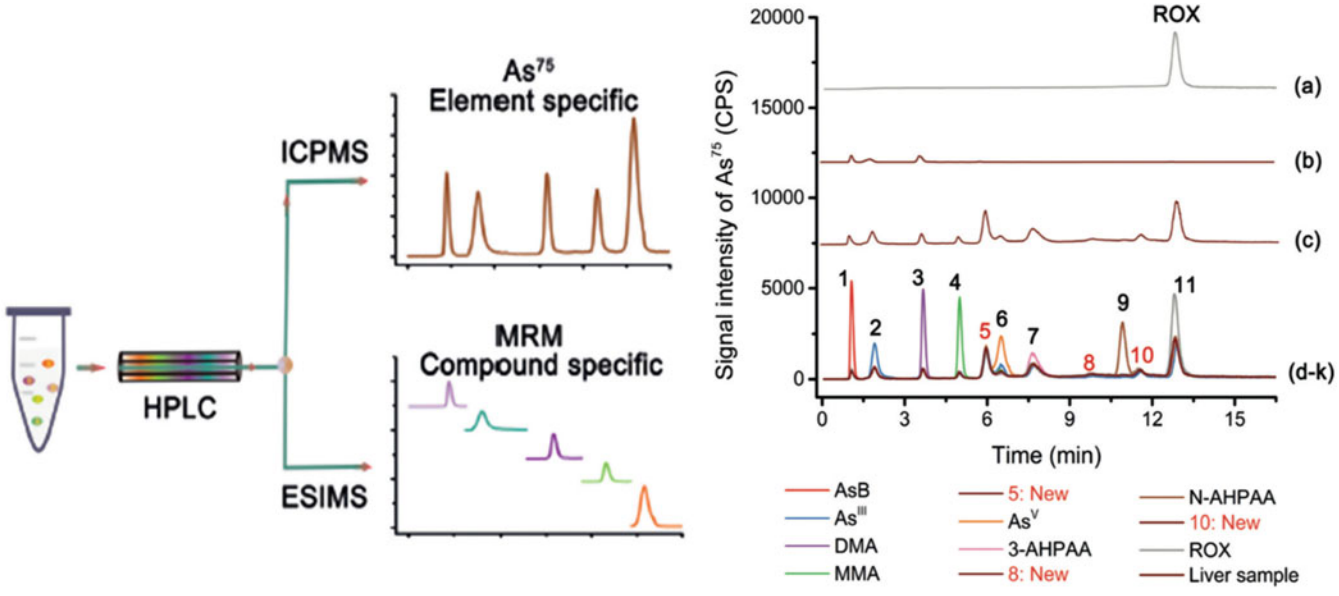


Fig. 8 Chromatograms from HPLC-ICP-MS analyses of ROX and chicken liver samples from a ROX-fed chicken and a control chicken. (**a**) ROX standard. (**b**) A liver sample from a control chicken fed the basal diet. (**c**) A liver sample from a chicken fed the ROX-containing diet. (**d–k**) Analysis of the same chicken liver sample after replicate aliquots were separately spiked with AsB (**d**, peak 1), AsIII (**e**, peak 2), DMA (**f**, peak 3), MMA (**g**, peak 4), AsV (**h**, peak 6), 3-AHPAA (**i**, peak 7), N-AHPAA (**j**, peak 9), and ROX (**k**, peak 11). Peaks 5, 8, and 10 did not correspond to any available arsenic standards. Reproduced from Angew. Chem.-Int. Ed., 2017. 56(24):6773–6777 with permission from John Wiley and Sons (2017)

in trace As speciation analysis is the least although good separation resolution can be allowed [51, 52].

3.4 Selenium (Se)

Se is an essential trace element for human, and various compounds containing Se have also been proved to be beneficial to immunity and metabolism. While the excess intake of Se will cause toxic and side effect, and its normal concentration range in human body is relatively narrow. The analysis of Se species in food can provide important nutritional reference. Piñeiro et al. [53] compared several extraction methods to enrich Se species from crops, including acid and enzyme digestions, followed by HPLC-ICP-MS analysis. It was found that both pressure-assisted and microwave-assisted enzymatic hydrolysis showed good extraction efficiency. The author analyzed Se species in Brazilian golden berries and nuts and found that SeMet was the dominant species (45.2–47.6 μg g^{-1} compared to 0.76–0.81, <0.306, and <0.119 μg g^{-1} for selenocystine ($SeCys_2$), Se(IV), and Se(VI), respectively). Mao et al. [54] combined SBSE with HPLC-ICP-MS for the analysis of seleno amino acids including $SeCys_2$, methylseleno-cysteine (MeSeCys), selenomethionine (SeMet) and selenoethionine (SeEt) and seleno-oligopeptides including γ-glutamyl-Se-methyl-selenocysteine (γ-GluMeSeCys) and selenodiglutathione (GS-Se-SG) in yeast and garlic samples. The partially sulfonated polystyrene-titania organic-inorganic hybrid stir bar coating can directly extract high polar seleno amino acids and seleno-oligopeptides.

With the derivatization by ethyl chloroformate (ECF), Duan et al. [55] proposed a method based on HF-LPME coupling with GC-ICP-MS for the speciation of seleno amino acids in the extracts of garlic, cabbage, and mushroom samples. Under the optimal conditions, the LODs obtained for Se-methyl-selenocysteine (SeMeCys), selenomethionine (SeMet), and selenoethionine (SeEth) were 23, 15, and 11 ng Se L^{-1}, respectively. Moreover, headspace sorptive extraction (HSSE) was combined with GC-ICP-MS for the determination of volatile organo seleno compounds (dimethylselenide (DMSe) and dimethyldiselenide (DMDSe)) in food samples [56]. DMSe and DMDSe retained on the sorptive bar were desorbed by methanol and introduced into GC-ICP-MS for separation and determination. LODs of 33 and 7.1 ng L^{-1} were obtained for DMSe and DMDSe, respectively. The proposed method was applied for the analysis of DMSe and DMDSe in garlic, onion and their juice samples, and a good agreement was obtained between the results obtained by standard addition and external calibration methods.

Duan et al. [57] synthesized phenylalanine derivative (L-HP-Phe, L-N-(2-hydroxy-propyl)-phenylalanine) and the chelate of Cu(II)-$(L\text{-}HP\text{-}Phe)_2$ was used as the chiral selector for the separation of D, L-SeMet. Based on it, a method of SPE-large volume sample stacking (LVSS)-micelle electrokinetic chromatography (MEKC)-UV detection was proposed for the chiral speciation of D, L-SeMet. With the enrichment factor of 1,400 and 1,378, the LODs of 0.44 and 0.60 ng mL^{-1}

for L-SeMet and D-SeMet were obtained, respectively. A certified reference material SELM-1 was analyzed and the results showed that most of SeMet in the selenized yeast sample SELM-1 was L isomer. Zhao et al. [58] used enzyme-assisted extraction method to enrich Se species in rice samples. CE-ICP-MS was used for subsequent analysis with the LOD of 0.1–0.9 ng Se/mL.

3.5 Tin (Sn)

Organotin compounds are now well known as endocrine disruptors and their extensive use as biocides in antifouling paints in the past decades has led to serious environmental problems. Especially in the marine ecosystem, the high toxicity of these compounds toward non-target organisms would cause ecological damage, and influence the human's health through the food chains. Among the organotins, triorganotins are the most biologically active and toxic, which could disturb the functions of mitochondria in tissues and cells; di- and monosubstituted compounds are the main degradation products of triorganotins in the environment. Xiao et al. [59] proposed a method based on headspace single drop microextraction (HS-SDME) in combination with GC-ICP-MS for the speciation analysis of butyltin compounds in seawater and shellfish samples. Sodium tetraethylborate ($NaBEt_4$) and sodium tetrahydroborate ($NaBH_4$) were used as the derivatizing reagent for in situ derivatization of the butyltins; $NaBEt_4$ derivatization was found to be more sensitive and robust than $NaBH_4$, leading to the LODs of 1.4 ng L^{-1} for monobutyltin (MBT), 1.8 ng L^{-1} for dibutyltin (DBT), and 0.8 ng L^{-1} for tributyltin (TBT). The butyltins found in the real-world samples are 31 ng L^{-1} MBT, 79 ng L^{-1} DBT, and 32 ng L^{-1} TBT for seawater, and 11.6–30.4 ng g^{-1} MBT, 11.8–8.9 ng g^{-1} DBT, and 12.8–52.6 ng g^{-1} TBT for different shellfish samples. The accuracy of the method was validated by analyzing certified reference material (CRM) of PACS-2 sediment (Fig. 9).

Hu et al. [60] tried to analyze Hg and Sn species in sea water by combining dynamic headspace in-tube SPME and GC-ICP-MS. Water sample was treated with $NaBPr_4$ [61] to convert Hg and Sn species into gas forms and extract them by a syringe filled with adsorbent material. In this way, TBT, DBT, MBT, $MeHg^+$, and inorganic mercury (iHg) can be analyzed simultaneously.

3.6 Other Elemental Species

Lajin et al. [62] used HPLC-ICP-MS to analyze simultaneously five organophosphorus pesticides including glyphosate in water samples. Nelson et al. [63] used GC-ICP-MS to analyze the organic sulfur, organophosphorus, and organochlorine residues on vegetables, tea, and other crops. Faßbender et al. [64] used CE-MC-ICP-

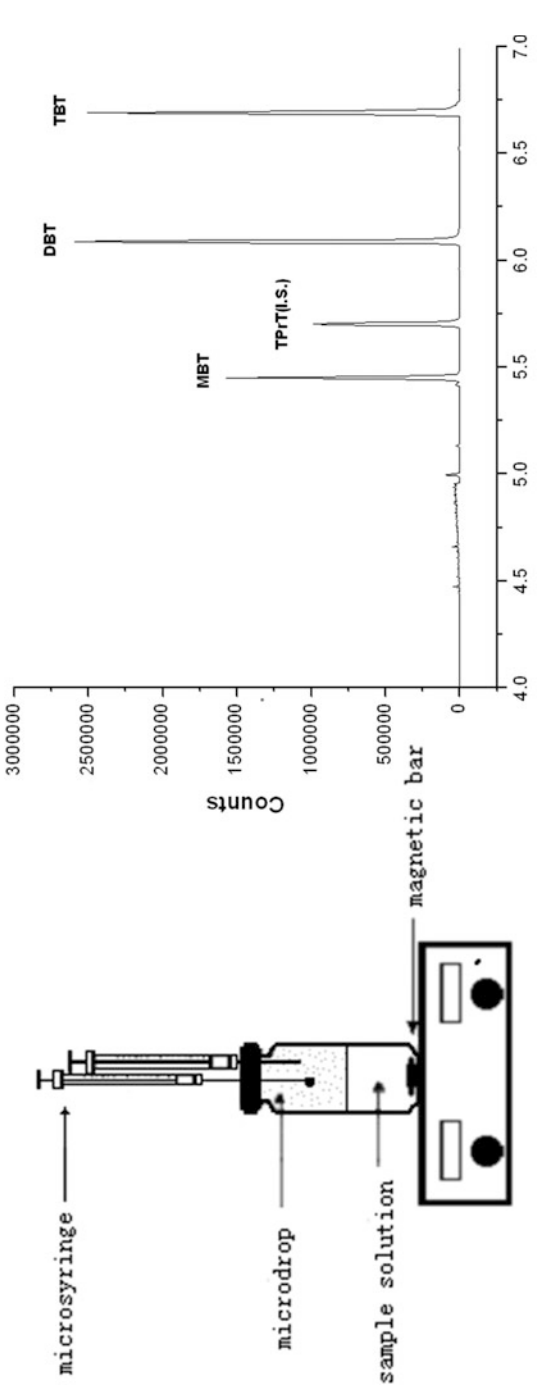


Fig. 9 Chromatogram (right) of the PACS-2 sediment obtained after $NaBEt_4$-HS-SDME (left) with GC-ICP-MS detection. Conditions: 0.5 μg g^{-1} TPrT as internal standard (I.S.), 5 min extraction with 2 μL decane at a stirring rate of 600 rpm, injection volume 1.0 μL. Reproduced from J. Chromatogr. A, 2008. 1211(1–2):135–141 with permission from 2008 Elsevier *B.V.*

MS to detect the S species in river water (mainly sulfates) and compared the difference of S isotope of samples from different rivers.

Polybrominated diphenyl ethers (PBDEs) generally come from the combustion of flame retardants and are exposed to human body in the food or dust. Xiao et al. [65] proposed a sensitive method based on LPME and GC-ICP-MS for the quantification of four PBDEs in human serum, soil, and dust samples. The concentration of PBDEs found in the soil sample was 26.48 ng g^{-1} BDE-28, 172.19 ng g^{-1} BDE-47, 18.74 ng g^{-1} BDE-99, and 87.42 ng g^{-1} BDE-100. The concentration of PBDEs found in the dust sample was 0.28 μg g^{-1} BDE-28, 1.47 μg g^{-1} BDE-47, 1.50 μg g^{-1} BDE-99, and 0.45 μg g^{-1} BDE-100. Novak et al. [66, 67] detected six kinds of PBDEs in sludge from wastewater treatment plants by GC-ICP-MS. The use of potassium bromate as flour modifier has been banned in many countries, but illegal use still exists. Cheng et al. [68] used microchip electrophoresis (MCE) in combination with ICP-MS to analyze Br^- and BrO_3^- in bread. Sodium acetate was used as the electrophoresis buffer, and the separation was completed in 35 s. Circulating volatile methyl siloxane (cVMS) is used in silicon polymer production or some personal care products. It can be easily transported long distances in the atmosphere and finally accumulate in the food chain. The safety of cVMS is under review. River water and fish samples were pretreated by LLE, then the cVMS was analyzed by GC-ICP-MS [69]. Screening results from several rivers in Germany showed that the cVMS content decreased year after year, suggesting a natural degradation. However, the cVMS in fish are still above the safety standard, indicating a risk of cVMS enrichment through the food chain. To a large extent, harmful elements in organism exist in the form of binding with small biological molecules or proteins, and thus continue to accumulate in organisms.

The separation and analysis of metal-binding proteins helps to understand the uptake and transformation pathways of these elements. 2D-gel electrophoresis and LA-ICP-MS were used to investigate the composition of metal-binding proteins in plant roots and stems [70]. Once the protein components were extracted from the homogenate of plant roots and stems, they were separated by 2D-gel electrophoresis. Then LA-ICP-MS was used to imaging Cu, Zn, Na, Mg, K, Ca, Mn, Fe, P, and S, revealing the presence of Cu, Zn, and Mg-binding proteins. Furthermore, these protein components were further digested and analyzed by LC-MS/MS for a discussion about Cu tolerance and accumulation mechanism. Similar methodology was applied to investigate the U-binding proteins in sea shrimp [71]. Eleven kinds of U-containing proteins were found and six of them could correspond to the database, among which ferritin was the main U-containing component. Size exclusion chromatography (SEC) was combined with ICP-MS to analyze the metal components in human whey protein [72]. Mn, Co, Cu, and Se-containing proteins were found. The content of these elements met the reference standard of human milk given by the World Health Organization (WHO).

ZnO NPs are a potential environmental pollutant that has attracted more attention in recent years. Wojcieszek et al. [73] investigated the uptake and transformation of ZnO NPs and $ZnCl_2$ during the growth cycle of lettuce. Tissue samples were extracted by a three-step extraction (ammonium acetate buffer, pectinase, and

cellulase) reaching a extraction efficiency for Zn higher than 90%. The results obtained by SEC-HILIC-ICP-MS showed that the water-soluble Zn-containing fraction was 16%. Further characterization by ESI-MS showed that the nicothiamine-bound state is the main Zn species.

4 Field Flow Fractionation (FFF)-ICP-MS

Field flow fractionation (FFF) is a general hydrodynamic separation technology, which is used to separate macromolecules, colloids, nanoparticles, and particles according to the size or mass. FFF is a high-resolution analytical technology that can provide continuous size classification from 1 nm to 100 μm. According to different applied fields, FFF can be divided into flow field flow separation (Fl FFF), settlement field flow separation (Sd FFF), electric field flow separation (El FFF), and thermal field flow separation (Th FFF). The combination of FFF (high separation) and ICP-MS (high sensitivity) has great application potential to separate nanoparticles in environment and food. At present, the combination of FFF and ICP-MS is mainly divided into Sd FFF-ICP-MS and Fl FFF-ICP-MS.

4.1 Settlement Field Flow Separation (Sd FFF)

Beckett et al. [74] first proposed the coupling of FFF with ICP-MS, and Sd FFF was first used to be combined with ICP-MS. It was used to separate complex particles or macromolecular mixtures and obtain the complete distribution of element composition within the size range of the sample. Taylor et al. [75] used offline Sd FFF and ICP-MS to determine the soil colloidal components and characterize river suspended solids. This hyphenated technique was then applied to monitor Al, Fe, Mg, Rb, Si, and Sr on clay mineral samples and river suspended particles [76]. By drawing appropriate element atomic ratio distribution diagrams (such as Si/Al, Mg/Al), the change of particle composition was evaluated along with the variation of chemical composition of the mixture. The size distribution of specific components can be obtained from complex mixtures by using appropriate tracer elements. Sd-FFF-ICP-MS was widely used in the analysis of trace elements in particulate matter [77, 78], colloid size determination [79], the distribution of metals in colloid [80], and the influence of natural organic matter (NOM) [81].

4.2 Flow Field Flow Separation (Fl FFF)

Fl FFF is the most used separation mode in FFF recently. At present, it is mainly divided into symmetrical flow field flow separation method (F4), asymmetric flow

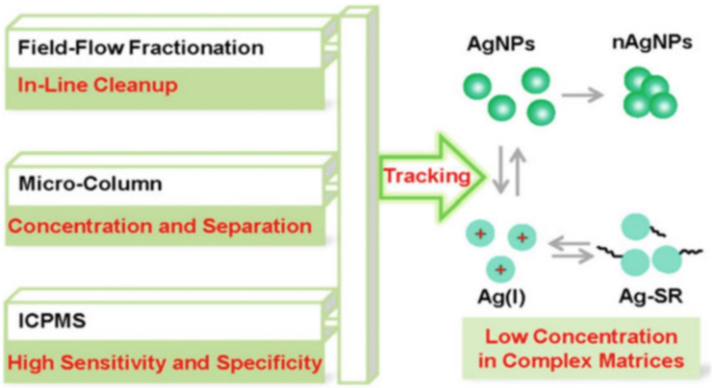


Fig. 10 Hollow fiber flow field-flow fractionation (HF5), microcolumn concentration, and inductively coupled plasma mass spectrometry (ICP-MS) online coupling system for studying the aggregation and chemical transformation behavior of silver nanoparticles. Reprinted (adapted) with permission from Environ. Sci. Technol., 2017. 51(21):12369–12376. Copyright {2017} American Chemical Society

field flow separation method (AF4), and hollow fiber flow field flow separation method (HF5). Among them, AF4 is the most commonly used field flow separation mode. Hoque et al. [82] used both online and offline AF4-ICP-MS, to determine the size and concentration of AgNPs in surface water samples and urban sewage samples. The offline mode can be easily implemented in laboratories without any special requirement. Saenmuangchin et al. [83] used self-made polysulfone hollow fiber membrane to establish a method combining HF5 and ICP-MS for the separation of AgNPs. The effects of carrier liquid and stabilizer on the retention behavior of AgNPs were studied. Tannic acid was added to the carrier solution to modify the surface of AgNPs and the surface of membrane to adjust the retention behavior of AgNPs. The volume of HF5 channel is relatively smaller than that of AF4 or F4 channel, resulting in less dilution of NPs in HF5 separation and more sensitive detection. The aggregation of AgNPs under aqueous conditions was also studied by HF5-ICP-MS [84]. It was found that in the presence of various concentrations of Na^+ and Ca^{2+} ions, the attachment efficiency of AgNPs at concentration of 10 ng mL^{-1} was much higher than that reported in other studies performed at greater concentrations (μg mL^{-1}) (Fig. 10). It proved the sensitivity of HF5-ICP-MS in studying NPs aggregation at low concentrations.

4.3 Application of FFF-ICP-MS in Environment and Food Samples

At present, FFF-ICP-MS is mainly used in NPs and colloids analysis. The application of FFF-ICP-MS in environment and food is introduced below.

4.3.1 AgNPs

Antonio et al. [85] evaluated the behavior of AgNPs in artificial seawater by AF4 SP ICP-MS, and studied the relationship between the aggregation process of AgNPs in water and environmental parameters (e.g., temperature, dissolved organic matter, and salinity) by measuring the particle size distribution in a long culture time. Chang et al. [86] established online AF4-UV vis -ICP-MS to detect the size and concentration of AgNPs in aqueous matrix. The author performed a combination of online and offline AF4-ICP-MS. Both showed similar response ranges, demonstrating the ability of the method for AgNPs analysis in surface water and urban sewage samples.

Loeschner et al. [87, 88] first used AF4-ICP-MS for the separation of AgNP in food matrices. After the enzymatic sample preparation, AF4-ICP-MS was used to separate the NPs from the remaining meat matrix, and the recovery of AgNPs was about 80%. The determination of AgNPs mass fraction and size exhibited acceptable reproducibility, proving the potential of AF4-ICP-MS in the quantitative analysis of NPs in complex food matrices for food monitoring and control.

4.3.2 AuNPs

Meisterjahn et al. [89] established a method of Fl FFF-ICP-MS to study the applicability of the detection, quantification, and characterization of AuNPs in the presence of natural nanoparticles (NNP). The results showed that light scattering detection cannot evaluate mixed samples containing AuNP due to the specific interaction between AuNP and NNP, while FFF-ICP-MS was helpful to distinguish the heterogeneous aggregation and homogeneous aggregation of NPs. Lopez Sanz et al. [90] used AF4-ICP-MS to separate gold nanorods (AuNRs) and nanospheres (AuNSs). Generally, AuNSs are the main impurity in the synthesis of AuNRs. Under the optimal separation conditions, sufficient resolution was achieved between commercially AuNSs and AuNRs. This method can be applied for the analysis of products obtained in the synthesis of AuNRs.

4.3.3 Other NPs

Soto Alvaredo et al. [91] applied online Sd FFF-ICP-MS/MS for the analysis of TiO_2 NPs in lake water samples. The analysis of different water samples (Milli Q water, seawater, and lake samples) showed that the ionic strength had a great impact on the analysis of TiO_2 NPs. Seawater with high salt concentration would lead to obvious aggregation/agglomeration. Li et al. [92] proposed a method of AF4-ICP-MS to characterize the size and quantify the quality of SiO_2 NPs in complex high-fat coffee creamer. By using prechannel calibration with SiO_2 NPs standard and postchannel calibration with ionic silicon standard, good recovery was obtained in the mass content determination of SiO_2 NPs. In addition, AF4-ICP-MS was also used for Cu

NPs in rainwater samples [93], SeNPs in water-based acrylic adhesives for food packaging materials [94], and the behavior of PtNPs in water [95], which proved the application potential of this technology in monitoring NPs in environment and food.

4.3.4 Colloid

Worms et al. [96] used AF4-ICP-MS to analyze the association between metals and colloidal organics from wastewater treatment plants. It was found that most metals such as Ag, Cd, Cu, Cr, Mn, and Zn are only related to colloidal organics with low molecular weight fractions, while Al, Fe, and Pb are related to colloidal organics with high molecular weight fractions. Saito et al. [97] used Fl FFF-ICP-MS to detect the size distribution and elemental composition of nanocolloids in granite groundwater. Geochemical parameters (such as pH value and main element concentration) showed a great influence on the size distribution of organic colloids. Different trace elements revealed different size distributions, reflecting their preferential combination with certain colloidal phases. For example, the distribution of Al, Mg, and Lu was consistent with the particle size distribution of organic colloids, while most other lanthanides and actinides had been proved to be related to organic colloids with small particle size. Moens et al. [98] studied the influence of NOM concentration on the size of Fe-organic carbon colloid and its influence on phosphate absorption by Fl FFF-UV-ICP-MS. The results confirmed that the increasing NOM increased specific surface area by limiting crystal growth and outperformed phosphate adsorption in the competition.

5 Laser Ablation-ICP-MS

Since 1995, LA-ICP-MS has been applied in elemental imaging with high sensitivity. It is a powerful technique for direct analysis of solid samples, enabling microanalysis, depth profiling analysis, and 2-D elemental mapping. Comparatively, much more applications of LA-ICP-MS are found in geological field, rather than that in biological and food samples.

5.1 Plant Samples Analysis

In LA-ICP-MS analysis of the plant samples, most of the current research is focused on (1) the relationship between the biological cycle or physiological function and distribution of some elements and their transporters in plants, (2) transport mechanism and enrichment factors, (3) distribution of some rare or toxic metal elements in plant tissue, and (4) some in-depth studies on the uptake and translocation mechanisms of NPs.

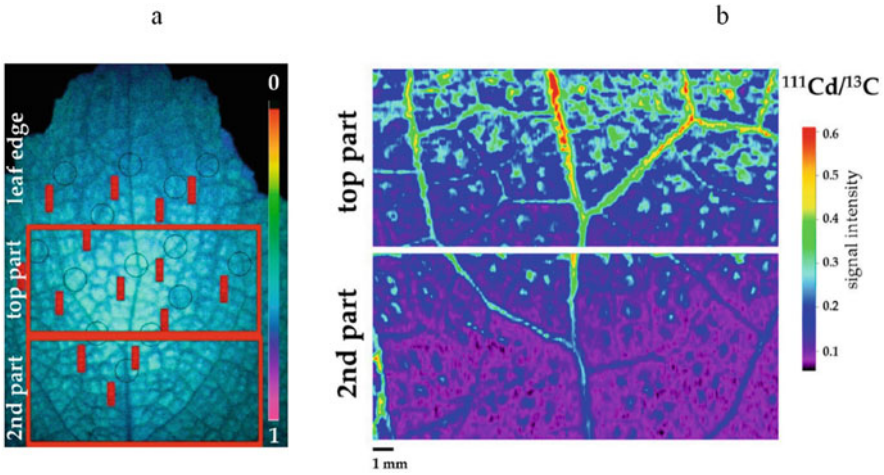


Fig. 11 CA-IF and LA-ICP-MS Image of *Salvia sclarea* leaves exposed to 100 μM Cd. Reproduced from Materials, 2019. 12(18):2953 (Open Access)

The distribution of elemental Cd in the leaves was observed by LA-ICP-MS with high resolution (Fig. 11a), which helped to better understand its distribution and the relationship between the amount of photosynthetic rate and excess energy dissipation [99]. The quantum yield of photosystem II electron transport (Φ_{PSII}) in the leaves of *Salvia sclarea* after 5 days of Cd^{2+} exposure was measured by chlorophyll fluorescence imaging (CF-IA). CF-IA can only vertically divide the leaf into three distinguishable parts and ΦPSII is affected by the fluorescence intensity (Fig. 11b). The combination of CF-IA and LA-ICP-MS helped to identify the effects of heavy metals on plants and provide information on tolerance mechanisms. It also facilitated the alleviation of heavy metal toxicity and photosynthetic tolerance in plants.

In addition to 2D imaging of single elements, 3D imaging and quantification of more than 19 micronutrients and toxic trace elements in wheat and rye grains could be obtained by LA-ICP-MS (Fig. 12) [100]. 3D imaging of the grains with high spatial resolution using the strategy of serial sectioning embedded in epoxy resin and LA-ICP-MS has recorded distribution patterns of several elements. The bulk concentrations of these elements were determined by ICP-MS. Longitudinal sections of the grains were imaged using μ-X-ray fluorescence spectrometry (μ-XRT) to independently validate findings regarding the major element distribution. 3D imaging of the morphology of the grains was performed using X-ray computed tomography (μ-CT). Based on it, the transport mechanisms of toxic trace elements in the grains and the elemental enrichment in some organs were discussed.

Besides the application in distribution of exposed metals, LA-ICP-MS exhibits good potential in the investigation of the absorption, transformation, and translocation of NPs in plants. For instance, other study of the same research team quantified and imaged the distribution of La in leaves and stems after plant absorption of b-La_2O_3 and La_2O_3 NPs by LA-ICP-MS. Since NPs can be observed as sharp peaks,

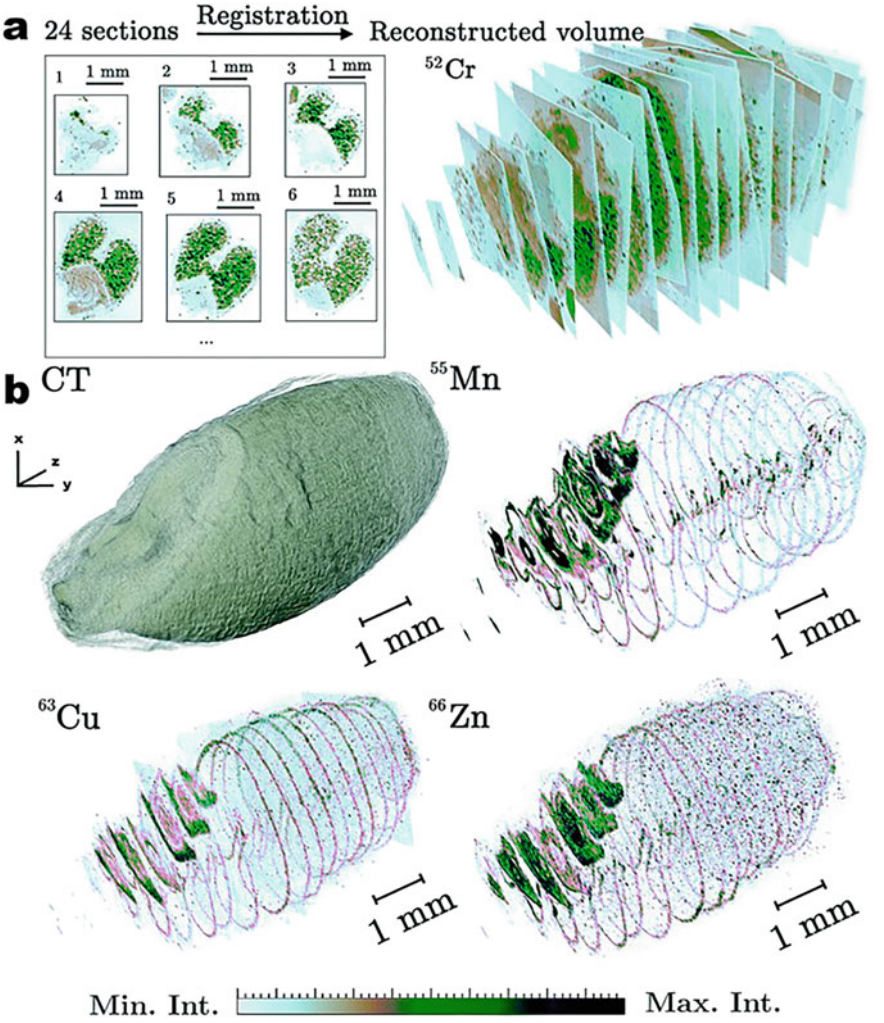


Fig. 12 3D element distributions of the grain from LA-ICP-MS. (**a**) Schematic illustration of the feature-based registration process, showing the transition from a set of 2D images towards a stack of aligned 2D images of the Cr distribution in *S. cereale*. (**b**) 3D distribution of selected micronutrients in *T. aestivum* L. and a micro-CT image of the grain Reproduced from J. Anal. At. Spectrom., 2017. 32(2):289–298 with permission from the Royal Society of Chemistry

LA-ICP-MS could discern b-La_2O_3 and La_2O_3 NPs to some extent [101]. Similar distribution of La in the leaves was observed by μ-XRF analysis, but the sensitivity of this technique is lower than that of LA-ICP-MS that showed better detection and visualization of La distribution in the leaf (Fig. 13). LA-ICP-MS has manifestly become a powerful tool to confirm the uptake, translocation mechanism, and distribution of NPs with minimal sample pretreatment.

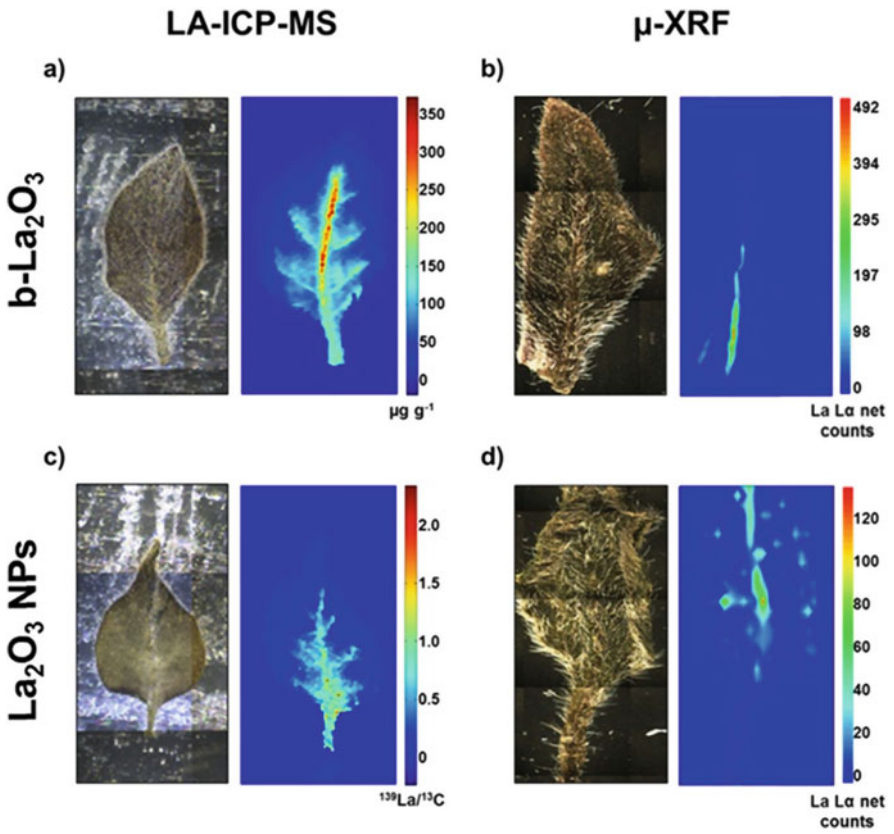


Fig. 13 Lanthanum distribution in leaves of *P. glomerata* cultivated in the presence of 400 mg L^{-1} of b-La_2O_3 and 400 mg L^{-1} of La_2O_3 NPs analyzed by: (**a**, **c**) LA-ICP-MS and (**b**, **d**) μ-XRF. Reprinted (adapted) with permission from Environ. Sci. Technol., 2019. 53(18):10827–10834. Copyright {2019} American Chemical Society

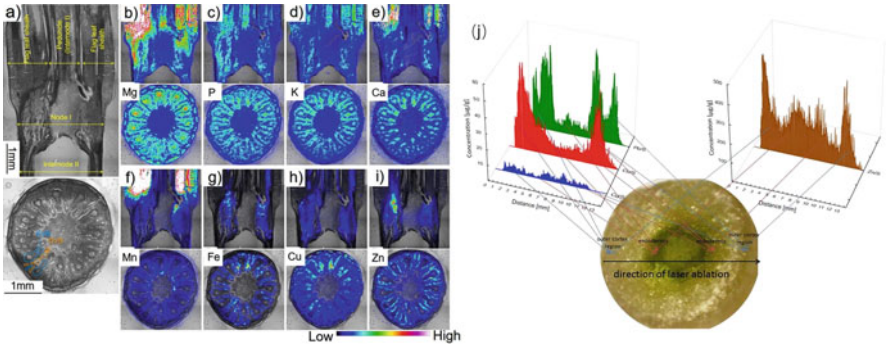


Fig. 14 Element distribution of plant roots by LA-ICP-MS. Reproduced from Plant Journal, 2019. 99(6):1254–1263 (Free Access)

Application of LA-ICP-MS was able to show the metals pathway while entering the pea roots [102]. In Fig. 14, linear ablation scanning was performed from the root surface to the center of plant tissues to obtain the quantitative distribution of Cd, Cu, Pb, and Zn after corresponding incubation. (e.g., cell wall, cell membrane, cytoplasm, vacuole and mitochondris). Analysis with EDX enabled imaging of accumulation of four elements in different plant organs, at the cellular and subcellular level, while confocal microscopy allowed us to indirectly detect the presence of metals through imaging of increased ROS generation. The research is to address oxidative stress and apoptosis-inducing mechanism of cells. As can be seen, both LA-ICP-MS and EDX are robust techniques for analyses of plant tissues in both quantification and imaging.

A method combining "soft ablation technique" with cryo-compound containing element standard followed by LA-ICP-MS, succeed in the quantitative and imaging measurement of eight elements in rice nodes (Fig. 14) [103]. Furthermore, differentially specialized vascular bundles around Si, Zn, and ^{13}C element accumulation gotten the priority mechanism in the Zn distribution in OsHMA2 protein and the transport mechanism of Si transporters. LA-ICP-MS has become a cost-effectiveness tool in linking transporter localization to element distribution at a cellular level.

5.2 Environmental Samples

For the analysis of samples in natural environment, LA-ICP-MS has become a powerful method to study climate signals and their preservation, which may help us further study global issues such as global warming and rising sea levels.

Elemental imaging of some impurities, e.g., Na, Mg, Co, and Sr, in glaciers was investigated [104]. With the introduction of low dispersion ablation pool and fluid dynamics image optimization methods, the results obtained by LA-ICP-MS were much richer, with higher resolution and less image distortion than laser camera. The improved LA-ICP-MS method for ice cores offered not only high scan speeds for single line profiles (around 1 mm s^{-1}) but can also map the localization of impurities at high spatial resolution (35 μm) and without imaging artifacts. This promises not only new insights into the impurity distribution in glacier ice but also lays the ground for an improved understanding of the LA-ICP-MS signal obtained from ice cores. As a result, LA-ICP-MS can deliver an important contribution for retrieving future paleo-environmental records from highly thinned ice layers.

The distribution characteristics of rare earth elements (REEs) have important applications in diagnosis and mineralization, especially in recent years. High-resolution LA-ICP-MS mapping helped to reveal the distributions of major, transition, and REEs at sub-10 μm scale in cross-sections of micronodules from the western tropical North Pacific seafloor, element distributions across the microlaminated rind, and the conditions of its genesis and metal enrichment [105]. The results reflected the existence of various minerals in micronodules and their diagenetic origin. LA-ICP-MS was combined with acid digestion-ICP-MS detection for the quantitative distribution of Ca, P, Zn, and Mn in 0–15 cm depth

soil layer under different fertilizer and planting methods [106]. The image distribution map in vertical direction was obtained, which is an important contribution to characterize and improve cropping systems, fertilizer and herbicide use, different soil types, and root depth versus root length.

Moreover, chlorine in concrete as building material was investigated by LA-ICP-MS. The imaging and quantitative results for common elements such as C, Al, Si, and Ca were obtained [107]. The effective chloride content determined by the Volhard titration overestimated the chloride content in the cement. Compared with that, LA-ICP-MS measurement delivered correct results and as the above described distinction criterion could be used to selectively quantify chloride in the cement phase. This led to correct results, disregarding the chloride concentration in the aggregates and if they are acid-soluble or not.

5.3 Food Samples Analysis

A protocol of grinding and tablet pressings method is imported to analyze the content of nine elements in common food-related Certified Reference Material (CRM) without acid digestion [108]. The photographs of pressed pellets, secondary electron images of laser craters in spot mode and raster mode, and representative time resolved analysis graphs of spot mode and raster mode are shown in Fig. 15.

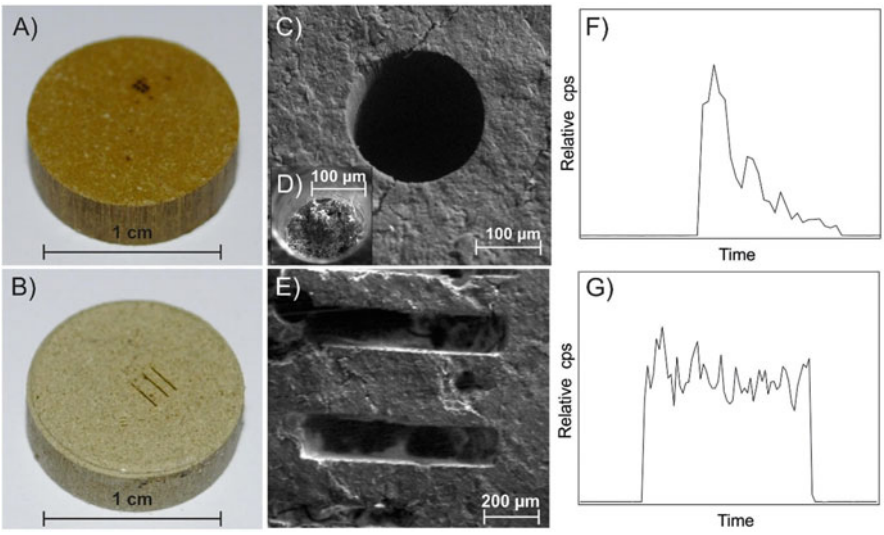


Fig. 15 (**a**, **b**) Photographs of pressed pellets, (**c**–**e**) Secondary electron images of laser craters in spot mode and raster mode, respectively, and (**f**, **g**) Representative time resolved analysis (TRA) graphs of spot mode and raster mode, respectively. Reproduced from Food Chemistry, 2019. 274: 726–732 with permissions from 2018 Elsevier Ltd

Homogenization of the sample and particle size reduction by milling ensured that there are not signal interferences providing more precise results. Raster mode was recommended over spot mode during ablation, as it provided greater accuracy for a wider variety of samples and elements. The work agrees with the recent hot idea of "green chemistry" and maintains extremely high sensitivity and excellent spatial resolution.

5.4 Other Samples

To perform LA-ICP-MS analysis, the sample should be in a form suitable for ablation. A variety of processing methods have been proposed. For example, quantitative analysis of 19 elements was carried out in waste polymer by grinding and laminating [109]. Homogenization of the waste polymers by cryomilling of larger amounts of material and preparation of pressed polymer powder pellets improved the accuracy of measurements and reduced the relative standard deviations.

LA-ICP-MS was used to investigate Cs^{2+} in the wastewater of nuclear power station with high radiation and high risk [110]. The ratio of Cs to its isotope in Fukushima nuclear power plant was determined (Fig. 16). This work demonstrates the applicability of LA-ICP-MS for ^{135}Cs quantitation in a solid sample.

In addition to solving intrinsic problems in environmental and food science, LA-ICP-MS can also serve as a novel crossover technology combining with the booming development of chemometrics [111]. In order to identify counterfeit perfumes, the authors did not start from the perfume itself. They used LA-ICP-MS to quantify 63 elements in a variety of perfume bottles followed by element filtering. The

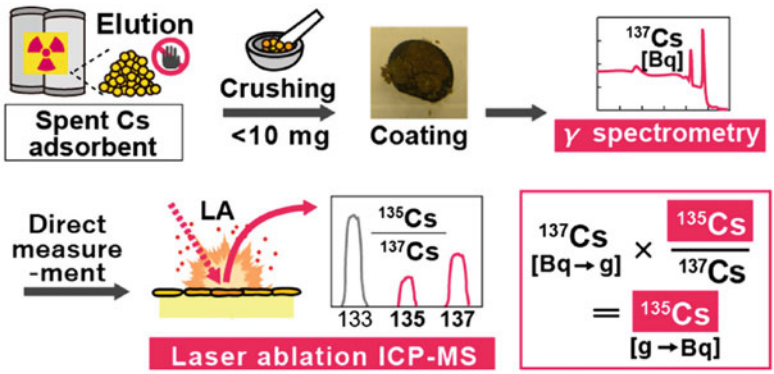


Fig. 16 Cs adsorbent pretreatment process followed by LA-ICP-MS analysis. Reprinted (adapted) with permission from Anal Chem., 2020. 92(4):3276–3284. Copyright {2020} American Chemical Society

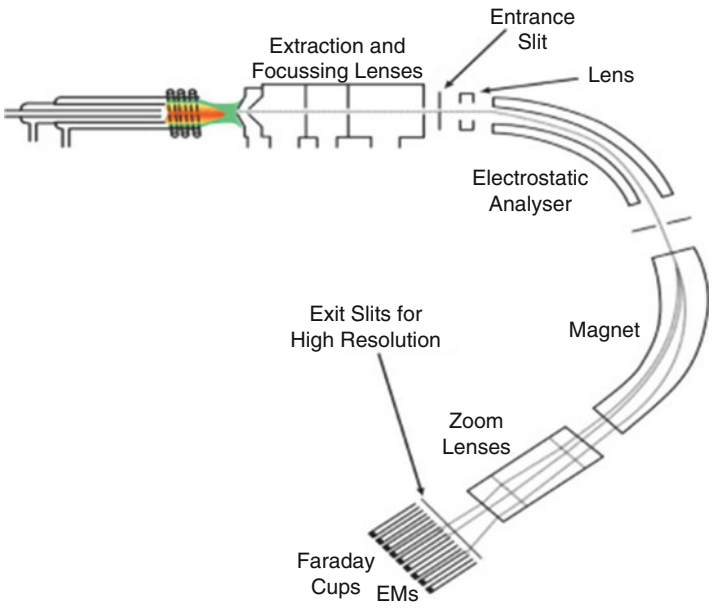


Fig. 17 Schematic diagram of a basic multi-collector-ICP-MS with forward Nier–Johnson geometry. Reproduced from Geosystems and Geoenvironment, 2022. 1(2):100019 (Open Access)

method of combining principal component discerns fake perfume and traces the origin of the perfume, solving common problems in an innovative way.

6 Multiple Collector (MC)-ICP-MS

MC-ICP-MS, first introduced by Walder et al. in 1993 [112–114], is mainly used for the determination of isotope ratios. It consists of ICP source, magnetic sector analyzer, and multiple sets of Faraday cups (Fig. 17) [115]. The sector-shaped magnetic field mass spectrometer involves an electromagnet as the mass analyzer and a magnetic field to separate ions of different mass-to-charge ratios (m/z). The MC part refers to Faraday cups working as receiver, which performs qualitative and quantitative analysis based on the molecular ion peaks of the elements. Sector magnetic field mass spectrometry is the only mass spectrometer which matches multi-receiver detectors. It can achieve static and simultaneous multi-receiving, ensuring high-precision isotope ratio analysis. MC-ICP-MS provides new opportunities for the precise measurement of isotope amount ratios with instrumental precision between 0.1 and $<0.005\%$.

6.1 Interference Correction for MC-ICP-MS

6.1.1 Isotopic Fractionation/Mass Bias Correction

MC-ICP-MS has the advantages of high spatial resolution, high sample throughput, minimal sample preparation, and low contamination. While it suffers from larger isotopic fractionation/mass bias compared to traditional thermal ionization mass spectrometry (TIMS). The isotopic fractionation/mass bias of the instrument is usually attributed to the supersonic diffusion of the ion beam after passing through the sampling cone and the space charge effects after passing through the skimming cone in ICP. To obtain accurate isotope ratios, a variety of conventional isotopic fractionation correction methods (e.g., the power, the exponential and the Russell law) developed originally for TIMS have also been used in MC-ICP-MS. Russell law is the most widely used, while large errors have been reported in relevant research works [116]. It should be noted that traditional isotopic fractionation correction methods were designed to account for mass-dependent fractionation (MDF) as a function of nuclide mass [117]. So far, mass-independent fractionation (MIF) has been recognized to be a genuine and more common phenomenon within MC-ICP-MS. The use of mass-dependent models to correct the MDF-based bias would result in biased isotope amount ratios. Yang et al. [117] proposed a chart (Fig. 18) to inform the selection of isotopic fractionation models for accurate isotope amount ratio results by MC-ICP-MS.

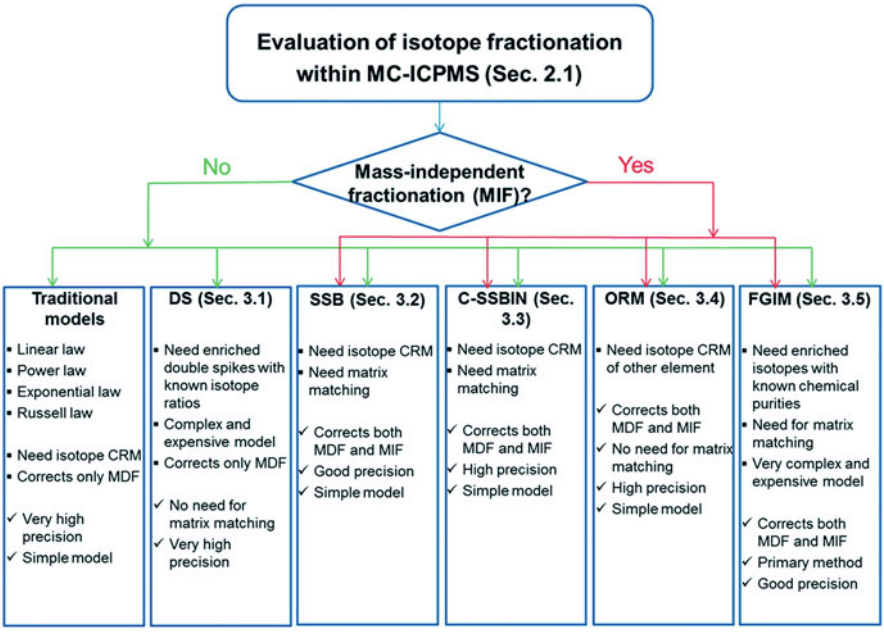


Fig. 18 Flowchart for an appropriate selection of mass bias correction models for the accurate and precise isotope amount ratio measurements by MC-ICP-MS. Reproduced from J. Anal. At. Spectrom., 2018. 33(11):1849–1861 with permission from the Royal Society of Chemistry

6.1.2 Matrix Effect

The presence of large amount of complex matrix in real sample will affect the isotope analysis significantly. In the presence of a high concentration of the matrix, the transmission of ions in the mass spectrometer varies slightly, but the variation is different for different isotopes of the same element. It will result in biased or inaccurate isotopic ratios in MC-ICP-MS measurement. To eliminate or correct matrix effects, traditional solution is the separation of the element from the matrix and the use of a certified isotopic standard of the element for external mass bias correction. Griffiths et al. [118] separated target Pb from seawater by using solid phase extraction with Nobias Chelate PA-1 resin or coprecipitation with $Mg(OH)_2$. As an alternative, internal mass bias correction, using another element close in mass, can be applied [119]. For example, silicon internal standardization could correct matrix effects caused by sodium, chlorine, and calcium in waters for sulfur measurements [120]. The natural silicon content of silicon isotopes with masses 29 and 30 in beer was used for internal mass bias correction. The matrix effect was fully corrected by the internal standardization of silicon to the effect on differential ion transmission [119].

6.2 Application of MC-ICP-MS in Food Science

In recent years, geographical origin and authenticity of food are topics of interest for both consumers and producers. Isotope analysis is one of the important methods for food traceability. It is generally believed that the isotopic composition of a certain element will not change measurably during the process of entering soil from bedrock, entering plant through water, and finally entering organisms along the food chain. Based on it, isotope analysis provides a valuable tool for tracing the origin of commodities, agricultural products, food and even animals.

6.2.1 Geographical Traceability Purposes

Sr is a stable isotope and a mature food tracer. The ratio of $^{87}Sr/^{86}Sr$ can be accurately determined by MC-ICP-MS. The ratio of $^{87}Sr/^{86}Sr$ in soil, water, and food can provide reference value for the geographical origin and authenticity of food such as Lambrusco wines, green coffee beans, and Szegedi paprika [121–124]. The combination of multi-element and Sr isotope fingerprints can also be used, with the help of MC-ICP-MS measurement and the use of multivariate statistical data to ensure the accuracy of the results [122, 123]. It is proposed that the combination of statistical and mathematical tools provides a basis for determining the origin of food using special elements and strontium isotope fingerprints.

6.2.2 Sources of Pollution in the Food

The isotope ratio in the natural environment is relatively stable. Lead isotopic ratio obtained by MC-ICP-MS is helpful to exploring the source of contamination in food. Ndung'u et al. analyzed 58 brands of balsamic vinegar samples to obtain lead concentration and isotopic composition (^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb). The lead isotopic fingerprints of all high-lead content vinegars were found to be mainly anthropogenic. It suggested multiple contamination sources including human factors. The results for organically cultivated grape vinegar indicated that the lead content was mainly attributed to industrial processes carried out post-harvest rather than to the pesticides used during the grapes growth period [125]. Wines from three vineyard locations with different lithology and pollution levels exhibited similar lead isotopic signatures (^{206}Pb/^{207}Pb = 1.16–1.18), although those of the soils where they grew differed significantly (^{206}Pb/^{207}Pb = 1.147–1.22). This fingerprint corresponds to the effect of industrial and urban airborne particulate material both on the soil and on the wine in the studied areas [126].

6.2.3 Isotope Composition in Blood of Different Diet Groups

Isotopic observations in animal samples indicate body accumulation of heavy zinc and light copper throughout life. This hypothesis has never been tested for humans, but the existence of a relationship between blood isotopic composition and age could be promising for age assessment methodologies [127]. Dietary habits can also influence the blood zinc isotope composition, being an additional source of isotopic variation. Significant differences in the isotopic composition of Zn in blood from populations with different feeding habits have been reported and the isotopic composition of Zn has been suggested as a potential dietary proxy [128]. Zinc in products of animal origin, such as meat, fish, eggs, and semi-skimmed milk, is more likely to be enriched in lighter zinc isotopes than in human blood samples. On the other hand, vegetables, grains, dairy products, pasta, and chocolate contain heavier zinc isotopes [129]. The lighter zinc isotopic composition in omnivorous people may be related to the lighter zinc isotopic composition in animal-derived foods. In contrast, the large consumption of grain products and vegetables seems to be related to the relative enrichment of heavy zinc isotopes in the vegetarian crowd. Therefore, diet seems to be the main source of changes in the zinc isotopic composition of human blood.

6.3 Application of MC-ICP-MS in Nanoparticle Analysis

NPs are widely used in industrial, medical, and biological fields due to their unique physicochemical properties. The NPs present in nature would be mixture of NPs of

various chemical compositions, as reflecting the sources or production process, and thus, rapid and sensitive analytical technique for elemental/isotopic ratio measurements in individual NPs was increasingly desired. MC-ICP-MS is an alternative technique for element/isotopic ratio analysis of a single NP. With MC-ICP-MS, multiple isotopes can be monitored without large-scale scanning. This leads to the time efficiency of data collection close to 100%, avoiding the loss of counts for analysis [130]. In order to obtain reliable isotope ratio data from the transient signals from NPs, a multi-collector setup and a fast response (i.e., high temporal resolution) signal monitoring system are needed. A MC-ICP-MS equipped with a high time resolution data acquisition system (HTR-MC-ICP-MS) was used to measure multiple isotope signals from the NPs. Therefore, accurate signal strength data can be obtained from transient signals derived from NPs, and the size and isotope ratio analysis of a single NP can be performed.

6.4 Studies on Isotopes in Human Metabolism

Earlier studies suggested that natural iron isotope ratios in human blood might be a useful tool to assess individual long-term iron absorption efficiency. Compared with the iron in food, human blood is rich in light iron isotopes [131], and the absorption of heavy iron isotopes is lower than the absorption of light iron isotopes when dietary iron is taken in the gastrointestinal tract. MC-ICP-MS was used to screen the tissue samples related to iron metabolism for iron isotope. A lot of isotope fractions were found in the process of intestinal absorption and distributed among body tissues. Compared with feed, the gastrointestinal mucosa is rich in light iron isotopes, and the isotope pattern reflects the main iron-rich sites of intestinal mammals. Studies have shown that when heavy iron isotopes are enriched in the liver, spleen, and bone marrow, light iron isotopes are enriched in red blood cells. Gulson et al. [132] illustrated the use of lead isotopes in a wide spectrum of activities including sources and pathways of lead in diverse environments from urban to mining communities, various applications associated with pregnancy, the contribution of bone lead to blood lead in the elderly, the half-life of lead in blood, and lead in bones and other media.

6.5 Application of MC-ICP-MS in Environmental Fields

6.5.1 Dating of Rocks and Minerals

With the help of MC-ICP-MS, the use of isotopes, especially Pb isotopes, has a wide range of applications in geological research on the dating of the earth's lithospheric rocks and minerals [133, 134]. For example, mean mineral ages with the ^{204}Pb ion beam measured in the multiplier were as follows: apatite, 1,715 $\pm$ 23 m.y.; sphene,

1,789 ± 11 m.y.; and monazite, 1,783 to 1,888 m.y., with relative uncertainties on individual monazite ages of <0.2% but highly reproducible age determinations on single monazite crystals (≪1%).

6.5.2 Isotopes as a Tracer in Earth Science

The development of MC-ICP-MS technology enables rapid and stable ionization of samples in high-temperature plasma ion sources, and improves the accuracy of isotope analysis, making Nd isotope widely used as an indispensable tracer in earth science and cosmic chemistry. It can be used to investigate current and past ocean circulation [135]. The determination of Pb isotopes by MC-ICP-MS has also been used to study various ocean processes, such as surface ocean currents, mesoscale vortices, and the time scale and path of ventilation in deep water bodies. For the study of Hg isotopes, detailed isotope fractionation information can be provided to understand the current or ancient environmental cycle of Hg in the global or local/regional, ecosystem or biota [136].

6.5.3 Other Applications

In recent years, metal stable isotopes have been critically assessed as a tool to obtain qualitative and quantitative integrated information about sources, pathways, and biogeochemical processes of these elements in the environment. There is a significant difference in the isotope ratio of Zn between anthropogenic emissions and natural rocks or soils, which can be used to determine the source of contamination. Zinc isotopes will be fractionated during major man-made processes such as smelting, resulting in artificial fingerprint isotopes that are different from natural isotopic compositions [137]. The U isotope in the surrounding soil samples after the leakage of the Fukushima nuclear power plant was analyzed. The $^{235}U/^{238}U$ isotope ratio can be used as an indicator of U source in environmental research to assess the radioactive element pollution caused by nuclear leakage [138].

7 Future Perspectives and Conclusions

ICP-MS is the most sensitive detection technique for trace elements presently and has been widely used for the quantification of interest elements in food and environmental samples. Due to its inherit characteristics, ICP-MS itself cannot afford information on elemental speciation, distribution, and/or imaging, and cannot meet the requirements in real sample analysis sometimes. So far, ICP-MS involving hyphenated techniques has been developed rapidly to satisfy the needs at different levels.

A variety of sample pretreatment techniques, mainly solid phase (micro)-extraction and liquid phase (micro)extraction, were combined with ICP-MS detection, separating the co-existing interference substances, enriching target element/species, favoring the improvement of the analytical sensitivity and anti-interference ability. Some of the pretreatment can further distinguish different elemental species, helping to achieve speciation analysis. In the following study, simultaneous analysis of species for different elements is expected, and transformation of the species during pretreatment process should be further evaluated and avoided.

Chromatographic separation techniques have been demonstrated with good potential for separating various elemental species, followed by ICP-MS detection. With the aid of ESI-MS, unknown species can be identified along. Besides HPLC, GC, CE, and GE, flow field techniques have been applied for a combination with ICP-MS. These hyphenated techniques provide plenty information on trace elemental species, metal-containing macromolecules, colloids, nanoparticles, and particles.

LA-ICP-MS exhibits good potential in elemental imaging and direct quantification of elements in solid food and environmental samples. It helps to explore the elemental distribution, transportation, pathway, and location in plant and earth.

MC-ICP-MS affords isotope information, which is beneficial to the geological traceability purposes, exploration of the pollution source for the foods, investigation on human metabolism, dating of rocks and minerals, and tracing specific purposes.

Above all, ICP-MS and its variants exhibit significant function in food security, environmental pollution monitoring, and health risk assessment. Relevant methodologies need further improvement to meet the requirements in real sample analysis in terms of anti-interference ability, detection sensitivity, selectivity, sample throughput, and easy operation.

References

1. Aggarwal SK, You CF (2017) A review on the determination of isotope ratios of boron with mass spectrometry. Mass Spectrom Rev 36(4):499–519
2. Montano MD, Olesik JW, Barber AG, Challis K, Ranville JF (2016) Single particle ICP-MS: advances toward routine analysis of nanomaterials. Anal Bioanal Chem 408(19):5053–5074
3. Pozebon D, Scheffler GL, Dressler VL (2017) Recent applications of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for biological sample analysis: a follow-up review. J Anal At Spectrom 32(5):890–919
4. Su SW, Chen BB, He M, Hu B (2014) Graphene oxide-silica composite coating hollow fiber solid phase microextraction online coupled with inductively coupled plasma mass spectrometry for the determination of trace heavy metals in environmental water samples. Talanta 123: 1–9
5. Zhang N, Peng H, Hu B (2012) Light-induced pH change and its application to solid phase extraction of trace heavy metals by high-magnetization Fe3O4@SiO2@TiO2 nanoparticles followed by inductively coupled plasma mass spectrometry detection. Talanta 94:278–283
6. Chen Y, He M, Chen B, Hu B (2021) Thiol-grafted magnetic polymer for preconcentration of Cd, Hg, Pb from environmental water followed by inductively coupled plasma mass spectrometry detection. Spectrochim Acta B At Spectrosc 177

7. Yilmaz E, Erbas Z, Soylak M (2021) Hydrolytic enzyme modified magnetic nanoparticles: an innovative and green microextraction system for inorganic species in food samples. Anal Chim Acta 1178:338808
8. Xu C, Peng C, He M, Chen B, Hu B (2021) Magnetic N-doped porous carbon for analysis of trace Pb and Cd in environmental water by magnetic solid phase extraction with inductively coupled plasma mass spectrometry. Spectrochim Acta B At Spectrosc 184
9. Bao S, Tang L, Li K, Ning P, Peng J, Guo H, Zhu T, Liu Y (2016) Highly selective removal of Zn(II) ion from hot-dip galvanizing pickling waste with amino-functionalized Fe3O4@SiO2 magnetic nano-adsorbent. J Colloid Interface Sci 462:235–242
10. He Y, He M, Nan K, Cao R, Chen B, Hu B (2019) Magnetic solid-phase extraction using sulfur-containing functional magnetic polymer for high-performance liquid chromatography-inductively coupled plasma-mass spectrometric speciation of mercury in environmental samples. J Chromatogr A 1595:19–27
11. Wang Y, Zhou R, Wang C, Zhou G, Hua C, Cao Y, Song Z (2020) Novel environmental-friendly nano-composite magnetic attapulgite functionalized by chitosan and EDTA for cadmium (II) removal. J Alloys Compd 817
12. Zhang N, Hu B (2012) Cadmium (II) imprinted 3-mercaptopropyltrimethoxysilane coated stir bar for selective extraction of trace cadmium from environmental water samples followed by inductively coupled plasma mass spectrometry detection. Anal Chim Acta 723:54–60
13. Peng HY, Zhang N, He M, Chen BB, Hu B (2015) Simultaneous speciation analysis of inorganic arsenic, chromium and selenium in environmental waters by 3-(2-aminoethylamino) propyltrimethoxysilane modified multi-wall carbon nanotubes packed microcolumn solid phase extraction and ICP-MS. Talanta 131:266–272
14. Zhang N, Suleiman JS, He M, Hu B (2008) Chromium(III)-imprinted silica gel for speciation analysis of chromium in environmental water samples with ICP-MS detection. Talanta 75(2):536–543
15. Zheng F, Hu B (2008) Novel bimodal porous N-(2-aminoethyl)-3-aminopropyltrimethoxysilane-silica monolithic capillary microextraction and its application to the fractionation of aluminum in rainwater and fruit juice by electrothermal vaporization inductively coupled plasma mass spectrometry. Spectrochim Acta B At Spectrosc 63(1):9–18
16. Zheng F, Hu B (2009) Dual silica monolithic capillary microextraction (CME) on-line coupled with ICP-MS for sequential determination of inorganic arsenic and selenium species in natural waters. J Anal At Spectrom 24(8):1051–1061
17. Krishna MV, Chandrasekaran K, Karunasagar D (2010) On-line speciation of inorganic and methyl mercury in waters and fish tissues using polyaniline micro-column and flow injection-chemical vapour generation-inductively coupled plasma mass spectrometry (FI-CVG-ICPMS). Talanta 81(1-2):462–472
18. Wuilloud RG, de Wuilloud JCA, Vonderheide AP, Caruso JA (2003) Determination of iodinated phenol species at parts-per-trillion concentration levels in different water samples by solid-phase microextraction/offline GC-ICP-MS. J Anal At Spectrom 18(9):1119–1124
19. Su SW, Chen BB, He M, Xiao ZW, Hu B (2014) A novel strategy for sequential analysis of gold nanoparticles and gold ions in water samples by combining magnetic solid phase extraction with inductively coupled plasma mass spectrometry. J Anal At Spectrom 29(3):444–453
20. Wang Y, Chen B, Wang B, He M, Hu B (2020) Phosphoric acid functionalized magnetic sorbents for selective enrichment of TiO2 nanoparticles in surface water followed by inductively coupled plasma mass spectrometry detection. Sci Total Environ 703:135464
21. Zhang L, Chen B, He M, Liu X, Hu B (2015) Hydrophilic polymer monolithic capillary microextraction online coupled to ICPMS for the determination of carboxyl group-containing gold nanoparticles in environmental waters. Anal Chem 87(3):1789–1796
22. Liu X, Chen B, Cai Y, He M, Hu B (2017) Size-based analysis of au NPs by online monolithic capillary microextraction-ICPMS. Anal Chem 89(1):560–564

23. Hu B, He M, Chen B, Xia L (2013) Liquid phase microextraction for the analysis of trace elements and their speciation. Spectrochim Acta B At Spectrosc 86:14–30
24. Xia L, Hu B, Jiang Z, Wu Y, Liang Y (2004) Single-drop microextraction combined with low-temperature electrothermal vaporization ICPMS for the determination of trace Be, Co, Pd, and Cd in biological samples. Anal Chem 76:2910–2915
25. Guo X, He M, Chen B, Hu B (2012) Phase transfer hollow fiber liquid phase microextraction combined with electrothermal vaporization inductively coupled plasma mass spectrometry for the determination of trace heavy metals in environmental and biological samples. Talanta 101: 516–523
26. Guo X, He M, Chen B, Hu B (2012) Solidified floating organic drop microextraction combined with ETV-ICP-MS for the determination of trace heavy metals in environmental water samples. Talanta 94:70–76
27. Chandrasekaran K, Karunasagar D (2016) Dispersive liquid–liquid microextraction for simultaneous preconcentration of platinum group elements (Pd, Os, Ir, and Pt) and selected transition elements (Ag, Cd, Ta, and Re) at parts per trillion levels in water and their determination by inductively coupled plasma-mass spectrometry. J Anal At Spectrom 31(5):1131–1140
28. Guo XQ, Tang XT, He M, Chen BB, Nan K, Zhang QY, Hu B (2014) Dual dispersive extraction combined with electrothermal vaporization inductively coupled plasma mass spectrometry for determination of trace REEs in water and sediment samples. RSC Adv 4(38):19960–19969
29. Guo X, Chen B, He M, Hu B, Zhou X (2013) Ionic liquid based carrier mediated hollow fiber liquid liquid liquid microextraction combined with HPLC-ICP-MS for the speciation of phenylarsenic compounds in chicken and feed samples. J Anal At Spectrom 28(10):1638–1647
30. Guo X, He M, Nan K, Yan H, Chen B, Hu B (2016) A dual extraction technique combined with HPLC-ICP-MS for speciation of seleno-amino acids in rice and yeast samples. J Anal At Spectrom 31(2):406–414
31. Liu Y, He M, Chen BB, Hu B (2016) Ultra-trace determination of gold nanoparticles in environmental water by surfactant assisted dispersive liquid liquid microextraction coupled with electrothermal vaporization-inductively coupled plasma-mass spectrometry. Spectrochim Acta B At Spectrosc 122:94–102
32. Liu JF, Chao JB, Liu R, Tan ZQ, Yin YG, Wu Y, Jiang GB (2009) Cloud point extraction as an advantageous preconcentration approach for analysis of trace silver nanoparticles in environmental waters. Anal Chem 81(15):6496–6502
33. Zhou XX, Jiang LW, Wang DJ, He S, Li CJ, Yan B (2020) Speciation analysis of Ag2S and ZnS nanoparticles at the ng/L level in environmental waters by cloud point extraction coupled with LC-ICPMS. Anal Chem 92(7):4765–4770
34. Gao L, Gao B, Xu DY, Sun K (2019) In-situ measurement of labile Cr(III) and Cr(VI) in water using diffusive gradients in thin-films (DGT). Sci Total Environ 653:1161–1167
35. Quarles CD, Szoltysik M, Sullivan P, Reijnen M (2019) A fully automated total metals and chromium speciation single platform introduction system for ICP-MS. J Anal At Spectrom 34(2):284–291
36. Drincic A, Zuliani T, Scancar J, Milacic R (2018) Determination of hexavalent Cr in river sediments by speciated isotope dilution inductively coupled plasma mass spectrometry. Sci Total Environ 637:1286–1294
37. Xia LB, Hu B, Wu YL (2007) Hollow fiber-based liquid-liquid-liquid microextraction combined with high-performance liquid chromatography for the speciation of organomercury. J Chromatogr A 1173(1–2):44–51
38. Chen B, Wu Y, Guo X, He M, Hu B (2015) Speciation of mercury in various samples from the micro-ecosystem of East Lake by hollow fiber-liquid–liquid–liquid microextraction-HPLC-ICP-MS. J Anal At Spectrom 30(4):875–881

39. Zhu S, Chen B, He M, Huang T, Hu B (2017) Speciation of mercury in water and fish samples by HPLC-ICP-MS after magnetic solid phase extraction. Talanta 171:213–219
40. Wang H, Chen BB, Zhu SQ, Yu XX, He M, Hu B (2016) Chip-based magnetic solid-phase microextraction online coupled with MicroHPLC-ICPMS for the determination of mercury species in cells. Anal Chem 88(1):796–802
41. Wang H, Chen BB, He M, Yu XX, Hu B (2017) Selenocystine against methyl mercury cytotoxicity in HepG2 cells. Sci Rep 7
42. Entwisle J, Malinovsky D, Dunn PJH, Goenaga-Infante H (2018) Hg isotope ratio measurements of methylmercury in fish tissues using HPLC with off line cold vapour generation MC-ICPMS. J Anal At Spectrom 33(10):1645–1654
43. Song YH, Ma QF, Cheng HY, Liu JH, Wang YC (2021) Simultaneous enrichment of inorganic and organic species of lead and mercury in pg L-1 levels by solid phase extraction online combined with high performance liquid chromatography and inductively coupled plasma mass spectrometry. Anal Chim Acta 1157
44. Letsoalo MR, Godeto TW, Magadzu T, Ambushe AA (2018) Quantitative speciation of arsenic in water and sediment samples from the Mokolo River in Limpopo Province, South Africa. Anal Lett 51(17):2761–2775
45. Yang Y, Liu ZH, Chen H, Li SQ (2019) Low-density solvent-based dispersive liquid-liquid microextraction followed by HPLC-ICP-MS for speciation analysis of phenylarsenics in lake water. Int J Environ Anal Chem 99(1):87–100
46. Zhao F, Liu YM, Zhang XQ, Dong R, Yu WJ, Liu YF, Guo ZM, Liang XM, Zhu JH (2018) Enzyme-assisted extraction and liquid chromatography-inductively coupled plasma mass spectrometry for the determination of arsenic species in fish. J Chromatogr A 1573:48–58
47. Milani RF, de Paiva EL, Peron LI, Morgano MA, Cadore S (2018) Arsenic species in herbal tea leaves and infusions determination by HPLC-ICP-MS. LWT-Food Sci Technol 98:606–612
48. Peng H, Hu B, Liu Q, Li J, Li X-F, Zhang H, Le XC (2017) Methylated phenylarsenical metabolites discovered in chicken liver. Angew Chem Int Ed 56(24):6773–6777
49. Ilgen G, Huang JH (2013) An automatic cryotrapping and cryofocussing system for parallel ICP-MS and EI-MS detection of volatile arsenic compounds in gaseous samples. J Anal At Spectrom 28(2):293–300
50. Sele V, Amlund H, Berntssen MHG, Berntsen JA, Skov K, Sloth JJ (2013) Detection of arsenic-containing hydrocarbons in a range of commercial fish oils by GC-ICPMS analysis. Anal Bioanal Chem 405(15):5179–5190
51. Liu LH, He B, Yun ZJ, Sun J, Jiang GB (2013) Speciation analysis of arsenic compounds by capillary electrophoresis on-line coupled with inductively coupled plasma mass spectrometry using a novel interface. J Chromatogr A 1304:227–233
52. Qu HO, Mudalige TK, Linder SW (2015) Arsenic speciation in rice by capillary electrophoresis/inductively coupled plasma mass spectrometry: enzyme-assisted water-phase microwave digestion. J Agric Food Chem 63(12):3153–3160
53. Moreda-Pineiro J, Sanchez-Pinero J, Manana-Lopez A, Turnes-Carou I, Alonso-Rodriguez E, Lopez-Mahia P, Muniategui-Lorenzo S (2018) Selenium species determination in foods harvested in seleniferous soils by HPLC-ICP-MS after enzymatic hydrolysis assisted by pressurization and microwave energy. Food Res Int 111:621–630
54. Mao XJ, Hu B, He M, Chen BB (2012) High polar organic-inorganic hybrid coating stir bar sorptive extraction combined with high performance liquid chromatography-inductively coupled plasma mass spectrometry for the speciation of seleno-amino acids and seleno-oligopeptides in biological samples. J Chromatogr A 1256:32–39
55. Duan J, Hu B (2009) Separation and determination of seleno amino acids using gas chromatography hyphenated with inductively coupled plasma mass spectrometry after hollow fiber liquid phase microextraction. J Mass Spectrom 44(5):605–612

56. Duan JK, Li X, Yu CH, Hu B (2009) Headspace stir bar sorptive extraction combined with GC-ICP-MS for the speciation of dimethylselenide and dimethyldiselenide in biological samples. J Anal At Spectrom 24(3):297–303
57. Duan J, He M, Hu B (2012) Chiral speciation and determination of selenomethionine enantiomers in selenized yeast by ligand-exchange micellar electrokinetic capillary chromatography after solid phase extraction. J Chromatogr A 1268:173–179
58. Zhao YQ, Zheng JP, Yang MW, Yang GD, Wu YN, Fu FF (2011) Speciation analysis of selenium in rice samples by using capillary electrophoresis-inductively coupled plasma mass spectrometry. Talanta 84(3):983–988
59. Xiao Q, Hu B, He M (2008) Speciation of butyltin compounds in environmental and biological samples using headspace single drop microextraction coupled with gas chromatography-inductively coupled plasma mass spectrometry. J Chromatogr A 1211(1-2):135–141
60. Hu J, Pagliano E, Hou XD, Zheng CB, Yang L, Mester Z (2017) Sub-ppt determination of butyltins, methylmercury and inorganic mercury in natural waters by dynamic headspace in-tube extraction and GC-ICPMS detection. J Anal At Spectrom 32(12):2447–2454
61. Bouchet S, Berail S, Amouroux D (2018) Hg compound-specific isotope analysis at ultratrace levels using an on line gas chromatographic preconcentration and separation strategy coupled to multicollector-inductively coupled plasma mass spectrometry. Anal Chem 90(13):7809–7816
62. Lajin B, Goessler W (2019) Direct speciation analysis of organophosphorus environmental pollutants in water by HPLC-ICPMS/MS. Talanta 196:357–361
63. Nelson J, Hopfer H, Silva F, Wilbur S, Chen JM, Ozawa KS, Wylie PL (2015) Evaluation of GC-ICP-MS/MS as a new strategy for specific heteroatom detection of phosphorus, Sulfur, and chlorine determination in foods. J Agric Food Chem 63(18):4478–4483
64. Fassbender S, Rodiouchkina K, Vanhaecke F, Meermann B (2020) Method development for on-line species-specific sulfur isotopic analysis by means of capillary electrophoresis/multicollector ICP-mass spectrometry. Anal Bioanal Chem 412(23):5637–5646
65. Xiao Q, Hu B, Duan JK, He M, Zu WQ (2007) Analysis of PBDEs in soil, dust, spiked lake water, and human serum samples by hollow fiber-liquid phase microextraction combined with GC-ICP-MS. J Am Soc Mass Spectrom 18(10):1740–1748
66. Novak P, Zuliani T, Milacic R, Scancar J (2016) Development of an analytical method for the determination of polybrominated diphenyl ethers in sewage sludge by the use of gas chromatography coupled to inductively coupled plasma mass spectrometry. Anal Chim Acta 915:27–35
67. Novak P, Zuliani T, Milacic R, Scancar J (2017) Development of an analytical method for the determination of polybrominated diphenyl ethers in mussels and fish by gas chromatography-inductively coupled plasma mass spectrometry. J Chromatogr A 1524:179–187
68. Cheng HY, Han C, Xu ZG, Liu JH, Wang YC (2014) Sensitivity enhancement by field-amplified sample injection in interfacing microchip electrophoresis with inductively coupled plasma mass spectrometry for bromine speciation in bread. Food Anal Methods 7(10):2153–2162
69. Radermacher G, Rudel H, Wesch C, Bohnhardt A, Koschorreck J (2020) Retrospective analysis of cyclic volatile methylsiloxanes in archived German fish samples covering a period of two decades. Sci Total Environ 706
70. Wu B, Susnea I, Chen YX, Przybylski M, Becker JS (2011) Study of metal-containing proteins in the roots of Elsholtzia splendens using LA-ICP-MS and LC-tandem mass spectrometry. Int J Mass Spectrom 307(1-3):85–91
71. Xu M, Frelon S, Simon O, Lobinski R, Mounicou S (2014) Development of a non-denaturing 2D gel electrophoresis protocol for screening in vivo uranium-protein targets in Procambarus clarkii with laser ablation ICP MS followed by protein identification by HPLC-orbitrap MS. Talanta 128:187–195
72. Acosta M, Torres S, Marino-Repizo L, Martinez LD, Gil RA (2018) Novel method for metalloproteins determination in human breast milk by size exclusion chromatography

coupled to inductively coupled plasma mass spectrometry. J Pharm Biomed Anal 158:209–213
73. Wojcieszek J, Jimenez-Lamana J, Bierla K, Asztemborska M, Ruzik L, Jarosz M, Szpunar J (2019) Elucidation of the fate of zinc in model plants using single particle ICP-MS and ESI tandem MS. J Anal At Spectrom 34(4):683–693
74. Beckett R (1991) Field-flow fractionation-icp-ms – a powerful new analytical tool for characterizing macromolecules and particles. At Spectrosc 12:228–232
75. Taylor HE, Garbarino JR, Murphy DM, Beckett R (1992) Inductively coupled plasma mass-spectrometry as an element-specific detector for field-flow fractionation particle separation. Anal Chem 64(18):2036–2041
76. Murphy DM, Garbarino JR, Taylor HE, Hart BT, Beckett R (1993) Determination of size and element composition distributions of complex colloids by sedimentation field-flow fractionation inductively-coupled plasma-mass spectrometry. J Chromatogr 642(1–2):459–467
77. vanBerkel J, Beckett R (1996) Determination of adsorption characteristics of the nutrient orthophosphate to natural colloids by sedimentation field-flow fractionation. J Chromatogr A 733(1–2):105–117
78. Hassellov M, Lyven B, Beckett R (1999) Sedimentation field-flow fractionation coupled online to inductively coupled plasma mass spectrometry – new possibilities for studies of trace metal adsorption onto natural colloids. Environ Sci Technol 33(24):4528–4531
79. Ranville JF, Chittleborough DJ, Shanks F, Morrison RJS, Harris T, Doss F, Beckett R (1999) Development of sedimentation field-flow fractionation-inductively coupled plasma mass-spectrometry for the characterization of environmental colloids. Anal Chim Acta 381(2–3):315–329
80. Chen BL, Hulston J, Beckett R (2000) The effect of surface coatings on the association of orthophosphate with natural colloids. Sci Total Environ 263(1-3):23–35
81. Schmitt D, Taylor HE, Aiken GR, Roth DA, Frimmel FH (2002) Influence of natural organic matter on the adsorption of metal ions onto clay minerals. Environ Sci Technol 36(13):2932–2938
82. Hoque ME, Khosravi K, Newman K, Metcalfe CD (2012) Detection and characterization of silver nanoparticles in aqueous matrices using asymmetric-flow field flow fractionation with inductively coupled plasma mass spectrometry. J Chromatogr A 1233:109–115
83. Saenmuangchin R, Mettakoonpitak J, Shiowatana J, Siripinyanond A (2015) Separation of silver nanoparticles by hollow fiber flow field-flow fractionation: addition of tannic acid into carrier liquid as a modifier. J Chromatogr A 1415:115–122
84. Tan ZQ, Yin YG, Guo XR, Amde M, Moon MH, Liu JF, Jiang GB (2017) Tracking the transformation of nanoparticulate and ionic silver at environmentally relevant concentration levels by hollow fiber flow field-flow fractionation coupled to ICPMS. Environ Sci Technol 51(21):12369–12376
85. Antonio DC, Cascio C, Jaksic Z, Jurasin D, Lyons DM, Nogueira AJA, Rossi F, Calzolai L (2015) Assessing silver nanoparticles behaviour in artificial seawater by mean of AF4 and spICP-MS. Mar Environ Res 111:162–169
86. Chang YJ, Shih YH, Su CH, Ho HC (2017) Comparison of three analytical methods to measure the size of silver nanoparticles in real environmental water and wastewater samples. J Hazard Mater 322:95–104
87. Loeschner K, Navratilova J, Kobler C, Molhave K, Wagner S, von der Kammer F, Larsen EH (2013) Detection and characterization of silver nanoparticles in chicken meat by asymmetric flow field flow fractionation with detection by conventional or single particle ICP-MS. Anal Bioanal Chem 405(25):8185–8195
88. Loeschner K, Navratilova J, Grombe R, Linsinger TPJ, Kobler C, Molhave K, Larsen EH (2015) In-house validation of a method for determination of silver nanoparticles in chicken meat based on asymmetric flow field-flow fractionation and inductively coupled plasma mass spectrometric detection. Food Chem 181:78–84

89. Meisterjahn B, Neubauer E, Von der Kammer F, Hennecke D, Hofmann T (2014) Asymmetrical flow-field-flow fractionation coupled with inductively coupled plasma mass spectrometry for the analysis of gold nanoparticles in the presence of natural nanoparticles. J Chromatogr A 1372:204–211
90. Lopez-Sanz S, Farinas NR, Zougagh M, Martin-Doimeadios RDR, Rios A (2020) AF4-ICP-MS as a powerful tool for the separation of gold nanorods and nanospheres. J Anal At Spectrom 35(8):1530–1536
91. Soto-Alvaredo J, Dutschke F, Bettmer J, Montes-Bayon M, Profrock D, Prange A (2016) Initial results on the coupling of sedimentation field-flow fractionation (SdFFF) to inductively coupled plasma-tandem mass spectrometry (ICP-MS/MS) for the detection and characterization of TiO2 nanoparticles. J Anal At Spectrom 31(7):1549–1555
92. Li B, Chua SL, Ch'ng AL, Yu DY, Koh SP, Phang H, Chiew PKT (2020) An effective approach for size characterization and mass quantification of silica nanoparticles in coffee creamer by AF4-ICP-MS. Anal Bioanal Chem 412(22):5499–5512
93. De la Calle I, Perez-Rodriguez P, Soto-Gomez D, Lopez-Periago JE (2017) Detection and characterization of Cu-bearing particles in throughfall samples from vine leaves by DLS, AF4-MALLS (-ICP-MS) and SP-ICP-MS. Microchem J 133:293–301
94. Palomo-Siguero M, Vera P, Echegoyen Y, Nerin C, Camara C, Madrid Y (2017) Asymmetrical flow field-flow fractionation coupled to inductively coupled plasma mass spectrometry for sizing SeNPs for packaging applications. Spectrochim Acta B At Spectrosc 132:19–25
95. Sanchez-Cachero A, Lopez-Sanz S, Farinas NR, Rios A, Martin-Doimeadios RDR (2021) A method based on asymmetric flow field flow fractionation hyphenated to inductively coupled plasma mass spectrometry for the monitoring of platinum nanoparticles in water samples. Talanta 222
96. Worms IAM, Szigeti ZAG, Dubascoux S, Lespes G, Traber J, Sigg L, Slaveykova VI (2010) Colloidal organic matter from wastewater treatment plant effluents: characterization and role in metal distribution. Water Res 44(1):340–350
97. Saito T, Suzuki Y, Mizuno T (2013) Size and elemental analyses of nano colloids in deep granitic groundwater: implications for transport of trace elements. Colloids Surf A Physicochem Eng Asp 435:48–55
98. Moens C, Smolders E (2021) Suwannee river natural organic matter concentrations affect the size and phosphate uptake of colloids formed by iron oxidation. Geochim Cosmochim Acta 312:375–391
99. Moustakas M, Hanc A, Dobrikova A, Sperdouli I, Adamakis I-DS, Apostolova E (2019) Spatial heterogeneity of cadmium effects on Salvia sclarea leaves revealed by chlorophyll fluorescence imaging analysis and laser ablation inductively coupled plasma mass spectrometry. Materials 12(18)
100. Van Malderen SJM, Laforce B, Van Acker T, Vincze L, Vanhaecke F (2017) Imaging the 3D trace metal and metalloid distribution in mature wheat and rye grains via laser ablation-ICP-mass spectrometry and micro-X-ray fluorescence spectrometry. J Anal At Spectrom 32(2):289–298
101. Neves VM, Heidrich GM, Rodrigues ES, Enders MSP, Muller EI, Nicoloso FT, Pereira de Carvalho HW, Dressler VL (2019) La2O3 nanoparticles: study of uptake and distribution in Pfaffia glomerata (Spreng.) Pedersen by LA-ICP-MS and mu-XRF. Environ Sci Technol 53(18):10827–10834
102. Hanc A, Malecka A, Kutrowska A, Bagniewska-Zadworna A, Tomaszewska B, Baralkiewicz D (2016) Direct analysis of elemental biodistribution in pea seedlings by LA-ICP-MS, EDX and confocal microscopy: imaging and quantification. Microchem J 128:305–311
103. Yamaji N, Ma JF (2019) Bioimaging of multiple elements by high-resolution LA-ICP-MS reveals altered distribution of mineral elements in the nodes of rice mutants. Plant J 99(6):1254–1263
104. Bohleber P, Roman M, Sala M, Barbante C (2020) Imaging the impurity distribution in glacier ice cores with LA-ICP-MS. J Anal At Spectrom 35(10):2204–2212

105. Li D, Fu Y, Liu Q, Reinfelder JR, Hollings P, Sun X, Tan C, Dong Y, Ma W (2020) High-resolution LA-ICP-MS mapping of deep-sea polymetallic micronodules and its implications on element mobility. Gondw Res 81:461–474
106. Zaeem M, Nadeem M, Pham TH, Ashiq W, Ali W, Gillani SSM, Moise ER, Leier H, Kavanagh V, Galagedara L, Cheema M, Thomas R (2021) Development of a hyperspectral imaging technique using LA-ICP-MS to show the spatial distribution of elements in soil cores. Geoderma 385
107. Bonta M, Eitzenberger A, Burtscher S, Limbeck A (2016) Quantification of chloride in concrete samples using LA-ICP-MS. Cem Concr Res 86:78–84
108. Papaslioti EM, Parviainen A, Roman Alpiste MJ, Marchesi C, Garrido CJ (2019) Quantification of potentially toxic elements in food material by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) via pressed pellets. Food Chem 274:726–732
109. Stehrer T, Heitz J, Pedarnig JD, Huber N, Aeschlimann B, Guenther D, Scherndl H, Linsmeyer T, Wolfmeir H, Arenholz E (2010) LA-ICP-MS analysis of waste polymer materials. Anal Bioanal Chem 398(1):415–424
110. Asai S, Ohata M, Hanzawa Y, Horita T, Yomogida T, Kitatsuji Y (2020) Direct quantitation of Cs-135 in spent Cs adsorbent used for the decontamination of radiocesium-containing water by laser ablation inductively coupled plasma mass spectrometry. Anal Chem 92(4):3276–3284
111. von Wuthenau K, Segelke T, Kuschnereit A, Fischer M (2021) Glass authentication: laser ablation-inductively coupled plasma mass spectrometry (LA-ICP-MS) for origin discrimination of glass bottles. Talanta 235
112. Walder AJ, Furuta N (1993) High-precision lead-isotope ratio measurement by inductively-coupled plasma multiple collector mass-spectrometry. Anal Sci 9(5):675–680
113. Walder AJ, Koller D, Reed NM, Hutton RC, Freedman PA (1993) Isotope ratio measurement by inductively-coupled plasma multiple collector mass-spectrometry incorporating a high-efficiency nebulization system. J Anal At Spectrom 8(7):1037–1041
114. Walder AJ, Platzner I, Freedman PA (1993) Isotope ratio measurement of lead, neodymium and neodymium samarium mixtures, hafnium and hafnium lutetium mixtures with a double focusing multiple collector inductively coupled plasma mass-spectrometer. J Anal At Spectrom 8(1):19–23
115. Balaram V, Rahaman W, Roy P (2022) Recent advances in MC-ICP-MS applications in earth and environmental sciences: challenges and solutions. Geosyst Geoenviron 1(2):100019
116. Yang L, Sturgeon RE (2009) High accuracy and precision isotope dilution mass spectrometry: an application to the determination of Mo in seawater. J Anal At Spectrom 24(10):1327–1335
117. Yang L, Tong SY, Zhou L, Hu ZC, Mester Z, Meija J (2018) A critical review on isotopic fractionation correction methods for accurate isotope amount ratio measurements by MC-ICP-MS. J Anal At Spectrom 33(11):1849–1861
118. Griffiths A, Packman H, Leung YL, Coles BJ, Kreissig K, Little SH, van de Flierdt T, Rehkamper M (2020) Evaluation of optimized procedures for high-precision lead isotope analyses of seawater by multiple collector inductively coupled plasma mass spectrometry. Anal Chem 92(16):11232–11241
119. Martinez-Sierra JG, Santamaria-Fernandez R, Hearn R, Gayon JMM, Alonso JIG (2010) Development of a direct procedure for the measurement of Sulfur isotope variability in beers by MC-ICP-MS. J Agric Food Chem 58(7):4043–4050
120. Clough R, Evans P, Catterick T, Evans EH (2006) D(34)S measurements of sulfur by multicollector inductively coupled plasma mass spectrometry. Anal Chem 78(17):6126–6132
121. Durante C, Baschieri C, Bertacchini L, Bertelli D, Cocchi M, Marchetti A, Manzini D, Papotti G, Sighinolfi S (2015) An analytical approach to Sr isotope ratio determination in Lambrusco wines for geographical traceability purposes. Food Chem 173:557–563
122. Tchaikovsky A, Zitek A, Irrgeher J, Opper C, Scheiber R, Moder K, Congiu L, Prohaska T (2019) Chemometric tools for determining site-specific elemental and strontium isotopic fingerprints in raw and salted sturgeon caviar. Eur Food Res Technol 245(11):2515–2528

123. Brunner M, Katona R, Stefanka Z, Prohaska T (2010) Determination of the geographical origin of processed spice using multielement and isotopic pattern on the example of Szegedi paprika. Eur Food Res Technol 231(4):623–634
124. Rodrigues C, Maguas C, Prohaska T (2011) Strontium and oxygen isotope fingerprinting of green coffee beans and its potential to proof authenticity of coffee. Eur Food Res Technol 232(2):361–373
125. Ndung'u K, Hibdon S, Veron A, Flegal AR (2011) Lead isotopes reveal different sources of lead in balsamic and other vinegars. Sci Total Environ 409(14):2754–2760
126. Mihaljevic M, Ettler V, Sebek O, Strnad L, Chrastny V (2006) Lead isotopic signatures of wine and vineyard soils – tracers of lead origin. J Geochem Explor 88(1–3):130–133
127. Jaouen K, Gibert M, Lamboux A, Telouk P, Fourel F, Albarède F, Alekseev AN, Crubézy E, Balter V (2013) Is aging recorded in blood Cu and Zn isotope compositions? Metallomics 5(8):1016–1024
128. Van Heghe L, Engström E, Rodushkin I, Cloquet C, Vanhaecke F (2012) Isotopic analysis of the metabolically relevant transition metals Cu, Fe and Zn in human blood from vegetarians and omnivores using multi-collector ICP-mass spectrometry. J Anal At Spectrom 27(8):1327–1334
129. Costas-Rodriguez M, Van Heghe L, Vanhaecke F (2014) Evidence for a possible dietary effect on the isotopic composition of Zn in blood via isotopic analysis of food products by multi-collector ICP-mass spectrometry. Metallomics 6(1):139–146
130. Yamashita S, Ishida M, Suzuki T, Nakazato M, Hirata T (2020) Isotopic analysis of platinum from single nanoparticles using a high-time resolution multiple collector inductively coupled plasma – mass spectroscopy. Spectrochim Acta B At Spectrosc 169
131. Hotz K, Augsburger H, Walczyk T (2011) Isotopic signatures of iron in body tissues as a potential biomarker for iron metabolism. J Anal At Spectrom 26(7):1347–1353
132. Gulson B (2008) Stable lead isotopes in environmental health with emphasis on human investigations. Sci Total Environ 400(1-3):75–92
133. Willigers BJA, Baker JA, Krogstad EJ, Peate DW (2002) Precise and accurate in situ Pb-Pb dating of apatite, monazite, and sphene by laser ablation multiple-collector ICP-MS. Geochim Cosmochim Acta 66(6):1051–1066
134. Choi JH, Ryu JS, Shin HS, Kim J, Cheong CS (2013) Reevaluation of Th and U concentrations in marine sediment reference materials using isotope dilution MC-ICP-MS: towards the analytical improvements in dose rate estimation for luminescence dating. Geosci J 17(2):123–127
135. Tazoe H, Amakawa H, Suzuki K, Nishioka J, Hara T, Obata H (2021) Determination of Nd isotopic composition in seawater using newly developed solid phase extraction and MC-ICP-MS. Talanta 232
136. Li W, Gou W, Li W, Zhang T, Yu B, Liu Q, Shi J (2019) Environmental applications of metal stable isotopes: silver, mercury and zinc. Environ Pollut 252:1344–1356
137. Araujo DF, Boaventura GR, Machado W, Viers J, Weiss D, Patchineelam SR, Ruiz I, Rodrigues APC, Babinski M, Dantas E (2017) Tracing of anthropogenic zinc sources in coastal environments using stable isotope composition. Chem Geol 449:226–235
138. Murugan R, Kavasi N, Sahoo SK, Omori Y, Sorimachi A, Takahashi H, Aono T (2021) Measurement of uranium isotope ratios in Fukushima-accident contaminated soil samples using multi collector inductively coupled plasma mass spectrometry. J Environ Radioact 232: 106568

Isotopic Mass Spectrometry in Food and Environmental Chemistry

Yolanda Picó and Damià Barceló

Contents

Abstract Isotope ratio mass spectrometry (IRMS) measures small differences in the abundances of light-stable isotopes of elements, mostly carbon, hydrogen, oxygen, sulfur, and nitrogen. The ratio of isotopes is dependent on the environment where the samples are located and therefore the measurement of the isotope signature in food and environmental samples can give information to help to distinguish samples that share the same chemical composition but can be under different conditions. This chapter provides an overview of isotope ratio mass spectrometry (IRMS) in the context of food and environmental sample analysis (e.g., food authenticity, food origin, or environmental contamination). The fundamentals and latest developments of the technique, most important aspects of the instrumentation, analysis of isotope ratio information as well as key applications are described to illustrate the impact of

Y. Picó (✉)
Environmental and Food Safety Research Group-University of Valencia (SAMA-UV), Desertification Research Centre – CIDE (CSIC-UV-GV), Valencia, Spain
e-mail: yolanda.pico@uv.es

D. Barceló
Institute of Environmental Assessment and Water Research (IDAEACSIC), Barcelona, Spain

Catalan Institute for Water Research (ICRA-CERCA), Girona, Spain

Yolanda Picó and Julian Campo (eds.), *Mass Spectrometry in Food and Environmental Chemistry*, Hdb Env Chem (2023) 119: 99–126, DOI 10.1007/698_2022_907,

Published online: 18 October 2022

this rapidly growing field of research especially in terms of the number and type of applications. Finally, applications in nutrition and trophic, environment, soil science, and food authenticity are assessed by giving details on the progresses, advantages, and pitfalls.

Keywords Elemental analysis, Gas chromatography, Isotope ratio mass spectrometry (IRMS), Isotopic abundance, Liquid chromatography, Magnetic sector mass analyzer

1 Introduction

Isotopes are defined "as atoms with equal number of protons but different number of neutrons" [1]. Thus, the number of protons defines the nature of the chemical element (H, C, O, N, etc.), the number of neutrons the isotope of that element, and the sum of protons and neutrons the atomic mass [2]. Most elements are mixtures of isotopes. These, in turn, can be stable or radioactive. The latter undergo radioactive decay at very different rates, from a fraction of a second to tens of billions of years [3].

Stable isotopes can be produced but are never transformed by radioactive decay. They are only transformed by nuclear synthesis reactions. Both heavy and light-stable isotopes take part in chemical, physical, and biochemical reactions but at different rates. Lighter isotopes react faster than heavier isotopes affecting the relative abundance of isotopes in an established medium or compartment depending on the characteristics of the process [2].

Stable isotope composition of the natural materials is used as indicators of physical or chemical (including biological, aging, transformation, etc.) processes and geochemical cycles [4]. They also act as conservative tracers to identify, for example, food sources, nutrient cycling, environmental conditions, etc. [5, 6]. Typically, light elements (traditional stable isotope systems) are used for the analysis: H, C, N, O, or S, but also non-traditional isotopes (Li, B, Mg, Ca, Fe, Mo, etc.) can be useful in some applications.

The natural fluctuations of isotope ratios are minor. These isotope ratios are therefore always reported relative to an isotopic standard to eliminate bias or systematic error in the determinations [7]. Isotopic ratios of samples are reported relative to universal standards and are expressed using the notation delta (δ) with the unit of parts per thousand (‰).

$$\delta^{\mathrm{M}}X = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 1{,}000$$

where R_{sample} is the ratio of the atomic mass of the heavier (commonly the minor) and lighter (commonly the major) isotope for an element in the sample. R_{standard}

Table 1 Initial primary reference materials defining the isotope scales according to IAEA [9]

Element ratio	Primary reference standard	Isotope ratio, *R* Hayes [10] Accepted value $\times 10^6$
Hydrogen $^2H/^1H$	SMOW (*S*tandard *M*ean *O*cean *Water*)	155.76 ± 0.10
Carbon $^{13}C/^{12}C$	PDB (*Pee Dee Belemnite*)	11,237.2 ± 2.9
Nitrogen $^{15}N/^{14}N$	Air nitrogen	3,676.5 ± 8.1
Oxygen $^{18}O/^{16}O$	SMOW	2,005.2 ± 0.43
Sulfur $^{34}S/^{32}S$	(*Canyon-Diabolo-Triolit*)	45,004.5 ± 9.3

is the same ratio but measured in the standard samples with accurately known abundance ratios. The initial standards used for comparison are summarized in Table 1 but many of them are no longer available as their supply has been exhausted. The delta value of a universal standard is zero. For example, carbon isotope ratios were measured against the Pee Dee Belemnite (PDB) standard with $\delta^{13}C$ value 0‰ [8]. Nowadays, new primary reference standard materials (including all the NBS isotope standards) are available from the International Atomic Energy Agency (IAEA) in Vienna, the National Institute of Standards and Technology, and the US Geological Survey (USGS) [9]. Furthermore, and for practical purposes, other secondary reference materials have been calibrated during interlaboratory calibrations headed by the Commission on Isotopic Abundances and Atomic Weights (CIAAW) of the International Union of Pure and Applied Chemistry (IUPAC) [10].

The isotopic composition of the samples studied can be enriched or depleted in the heavy isotope with respect to the standard. This means that they can have both, positive or negative delta values. The $\delta^{13}C$ value of 10‰ means that material is enriched in the heavy isotope (^{13}C) and value of −10‰ means that material is depleted in the heavy isotope with respect to the standard.

Isotope ratio mass spectrometry (IRMS) is one of the relevant techniques used to measure the ratio of stable isotopes. This technique is applied in fields such as archeology, geology, medicine, biology, and forensic science but also in environmental and food sciences, to investigate human and animal diets in the past, for food authentication, to reconstruct past environmental and climatic conditions, etc. IRMS enables to measure ratios of isotope abundances of an element in a sample with very high accuracy. However, this way of using the technique, measuring the isotopic abundance of the whole sample, which is known as bulk-specific isotopic analysis (BSIA), is not responsible for its increasing use. Considering that complex samples have a very large number of compounds (several thousand), this way of measuring isotopic abundance can hide important information about the distribution of isotopes in the different components. Alternatively, compound-specific isotope analysis (CSIA) refers to the measurement of isotope ratios of individual organic compounds extracted from complex mixtures [11, 12]. CSIA commonly needs preparative steps

(pretreatments, extraction, and derivatization) to get amenable chromatographic analytes from bulk samples. These individual organic compounds can be contaminants or natural sample components, such as proteins, lipids, organic matter, etc. CSIA that requires a previous separation of the compounds to determine their specific isotopic distribution is responsible for the multiplication in the last 30 years of the number of applications of this technique.

This chapter outlines the methodological development and application of IRMS while also highlighting the main issues of the technique. In addition, the latest studies on environmental issues as well as on the traceability of the origin and/or authenticity of food products are presented.

2 Stable Isotope Establishment Using Isotope Ratio Mass Spectrometry (IRMS)

The most common technique for stable isotope ratio analysis is IRMS. Figure 1 shows a scheme of the instrument. An IRMS instrument consists of (1) sample introduction system (injector), (2) analytes ionization, (3) mass analyzer, (4) detector array, and (5) data acquisition system. The instrumental configuration has been widely described in many of the books, monographies, and references [3, 8, 13].

Prior to IRMS determination, the sample components must be transformed into the simpler gases CO_2, N_2, H_2, and SO_2, but also others such as O_2, N_2O, CO, CH_3Cl, SF_6, CF_4, and SiF_4 are produced. Most common systems used for sample introduction included elemental analyzer isotope ratio mass spectrometer (EA-IRMS), gas chromatography-isotope ratio mass spectrometry (GC-IRMS), and liquid chromatography-isotope ratio mass spectrometry (LC-IRMS). This offers

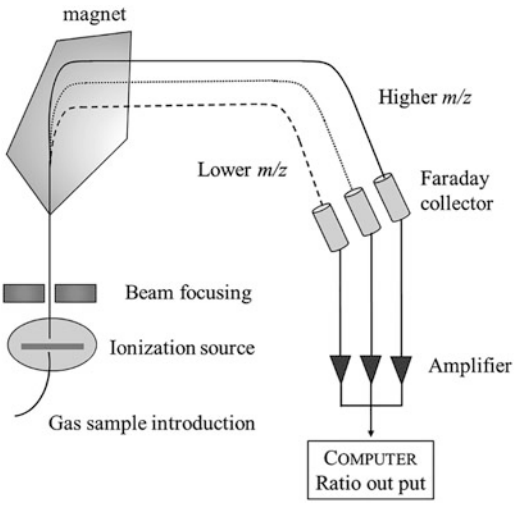


Fig. 1 Basic scheme of IRMS

a vast instrumental array that has been widely exploited in both, environmental and food analysis. The gases are introduced in the mass spectrometer through the inlet system (sample introduction). This inlet system consists of pneumatic valves and could be dual or continuous flow systems. The dual-system involves the alternate switch of the valves to introduce sample and reference standard in the vacuum chamber and the continuous system considers the injected sample as a transient signal and the reference standard as a gas pulse. Globally, continuous flow injection is easier, quicker, and more economical and has specific advantages such as less sample preparation and sample size. As a counterpart, dual inlet injection is more precise and reliable, especially when samples are prepared off-line [8, 13].

The next step is analyte ionization. Most used ionization sources for IRMS are electron impact (EI), thermal ionization mass spectrometry (TIMS), and multi-collector inductively coupled plasma mass spectrometry (ICP-MS). These three ion sources attain determination of almost all the isotopes in the periodical table. Electron ionization (EI) – also electron impact ionization or electron bombardment ionization – was one of the first ionization techniques developed for mass spectrometry and is still widely used. This technique ionizes the gases by a stream of energetic electrons produced by a filament (W, Rh) and accelerated by a difference of potential that interact with atoms or molecules to produce ions. EI is a strong ionization method that produces a large fragmentation of molecules due to the collision with high energy electrons. Thermal ionization mass spectrometry (TIMS) is another method available for high-precision isotopic analysis, in which elements are thermally ionized. The elements are deposited on a metal filament or filaments through which a current is passed at increasing temperature (>1,000°C). The ions created in the filament are accelerated through an electric potential gradient and concentrated into a beam through a series of electrostatically charged slits and plates. Multi-collector ICP-MS (MC-ICP-MS) supplements TIMS with the capacity to determine any type of element that requires higher ionization energies and needs important sample throughput. MC-ICP-MS consists of a robust ICP ion source where the plasma (that provided the ionization) is generated under atmospheric pressure, thus allowing sample introduction by means of continuous nebulization. This ionization source is widely described also in the previous chapter (3. “Elemental Mass Spectrometry”) where many applications are also highlighted. In addition, the combination of laser ablation (LA) —a solid sampling technique that applies a high intensity light to the sample through pulses and is able to transform a solid sample directly into an aerosol—with an MC-ICP-MS has enlarge the possibility to process different types of samples. EI and TIMS present limitations regarding the precise determination of the isotopic composition of elements with high ionization potentials that can hardly be ionized. Instead, MC-ICP-MS achieves determination of almost all compounds of the periodical table [14]. Furthermore, the combination LA-MC-ICP-MS has many advantages mapping compounds [15].

The measurement of the isotope ratios is carried out almost exclusively with magnetic sector type instruments. In a magnetic sector, the mass analyzer uses a magnetic field to separate ions of different m/z values. Once the sample is ionized, the ions are accelerated toward the magnetic sector by applying a high voltage to

them. The ions are generated at the source and travel continuously to the magnetic sector, where the ions are exposed to the magnetic field. The ions are deflected according to Fleming's left-hand rule. The intensity of the deflection depends on the m/z of the ions, so that lighter ions (of the same charge) will experience more deflection and heavier ions less. The magnetic sectors used in IRMS applied multiple detectors (multi-collector deep Faraday cups) to simultaneously monitor and integrate the ion currents of interest. This type of instruments is also named "Nier-type" because Alfred Nier was one of the first designers of the current-type of marketed instruments [5]. The different sample introduction systems are very important because these systems condition the information obtained. This is crucial especially in the case of complex samples. The three most important sample introduction systems are EA-IRMS, GC-IRMS, and LC-IRMS [14].

2.1 Elemental Analyzer Isotope Ratio Mass Spectrometer (EA-IRMS)

The combination of elemental analyzer (EA) with IRMS provides the mean isotopic signal of the bulk sample. In this system, the solid or liquid sample (placed in a tip or silver capsule) is burnt into a combustion or pyrolysis furnace (the AE) at high temperature [13]. This process can take place by combustion under a flow of oxygen (for analysis of C and N) producing different gases (NO_x, CO_2, SO_2, or H_2O) or by thermal conversion at 1,400°C [high-temperature conversion (HTC) in oxygen-free environment (for analysis of H and O) [16]. One pending issue within these systems is the lack of accurate hydrogen isotopic analysis of halogen- and sulfur-bearing organics with traditional HTC because the formation of hydrogen-bearing reaction products other than molecular hydrogen (H_2) is responsible for non-quantitative H_2 yields and possible hydrogen isotopic fractionation. Recently, new chromium-based Elemental Analyzer-Chromium/High-Temperature Conversion Isotope Ratio Mass Spectrometry (EA-Cr/HTC-IRMS) has showed very promising results to overcome these drawbacks [17]. However, some technical and analytical issues concerning halogen- and sulfur-bearing samples remained unsolved and needed further improvement of the systems.

Other problem that affects globally EA-IRMS is the presence of interferents. Numerous gases are formed which can also react with each other and their own reaction products can interfere with the determination of the isotopic abundance of a particular element. Therefore, after combustion, a series of purification steps are required, depending on the analyte to be determined. The most common takes place in the combustion chamber and is the oxidation of the formed gases. The gases formed then pass through a He stream to the reduction chamber where the NOx is reduced to N_2 and the O_2 is retained. The system then has a trap to retain the water (as CO_2 + H_2O can form HCO_2^+ which is an isobaric interference of $^{13}CO_2$), then there is a gas chromatograph which separates CO_2 and N_2 prior to their introduction

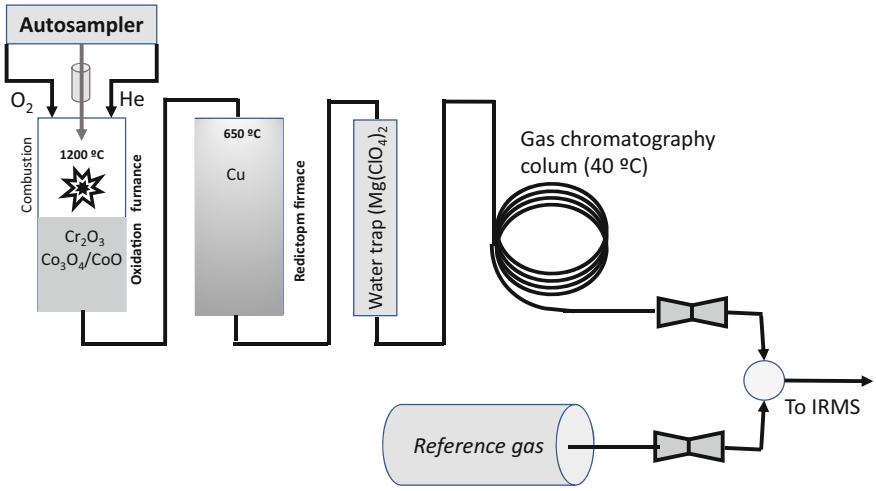


Fig. 2 Basic scheme of the EA sample treatment and purification

into the IRMS [16]. A basic scheme of this sample introduction that illustrated the working flow and purification system is shown in Fig. 2.

The isotope ratios of the gases from the samples are calculated with respect to a standard reference gas, which to provide optimal results must have a similar signal intensity to that of the samples and be analyzed alternatively to the samples. The flow of each reference gas is regulated by an interface system that rapidly alternates between the reference gas and the sample to ensure closeness in time [13].

2.2 *Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS)*

This system enables separation and isolation of specific compounds and then determination of their particular isotopic abundance (certain fatty acids, sterols, proteins, volatile compounds, etc.) [18]. The compounds to be separated must be GC amenable. This means volatile and thermally stable. Very polar compounds (initially non-volatile) can also be separated by GC-C-IRMS after chemical derivatization to transform them in volatile derivatives. The instrument (schematized in Fig. 3) contains three main parts:

1. *Gas chromatograph (GC)* separates the organic compounds of interest using a gas (usually He) as mobile phase and an inert column as stationary phase in an oven at controlled temperature according to their volatility. The compounds are desorbed and volatilized in the analytical column at different times and can be separately analyzed in a combustion chamber. The sample is introduced in the GC using the conventional split/splitless or on-line injector [13, 19]. However,

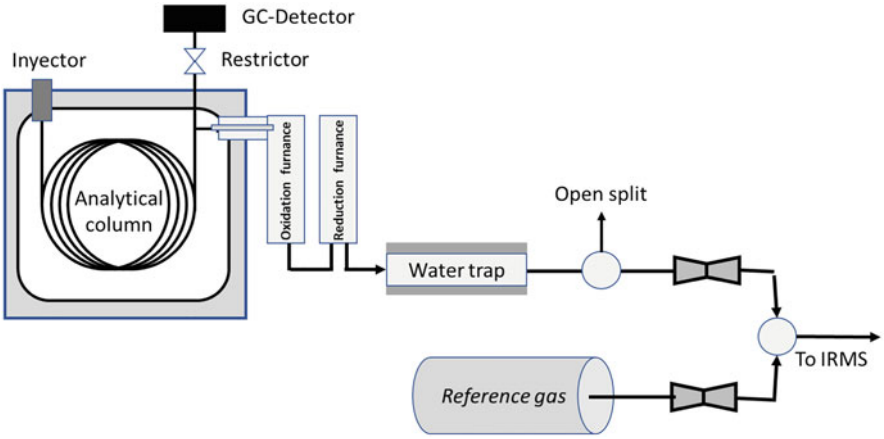


Fig. 3 Basic scheme of the GC sample separation and C-IRMS

modifications and latest advances in the hyphenation of several techniques to GC that improve the capabilities of the system can be applied to these instruments. In this sense, pre-concentration techniques, such as solid-phase microextraction (SPME), prior to the GC have also been reported, particularly for volatile compounds, adding versatility to the methods because these techniques increased several hundred times sensitivity of the determination [20–22]. Using this technique, analytes are adsorbed in a fiber that is thermally desorbed in the GC oven. Furthermore, pyrolysis gas chromatography (Py-GC) has also been incorporated as previous separation step for macromolecules (organic matter, lignins, polysaccharides, plastics, etc.) to get the monomers of the compounds and determine their isotopic signature [23].

In most of these systems, a small part of the eluent is diverted to a conventional GC detector such as flame ionization detector (FID) or MS whereas the main part goes to the next part, the combustion chamber [13].

2. In *C (combustion/conversion)* the analytes after elution of the GC pass to the combustion or high-temperature conversion (HTC) furnaces where they are transform in gases. As reported for EA-IRMS the proper determination of isotopic abundances involved an oxidation or HTC step, a reduction step, and the removal of water.
3. Finally, the gases are sent to the IRMS, where they are ionized in an electron ionization (EI) source, separated in the magnetic sector, and then detected with Faraday cups. The different isotopic combinations detected allow to calculate the isotopic ratios of specific elements in the compounds of interest.

GC-C-IRMS has many fields of use including environmental and food studies. Examples of applications are reported for the analysis of explosives [24], organophosphorus pesticides [25], brominated flame retardants (BFRs) [26], NO_3^- and NO_2^- [27], lignin monomers [28], vanillin [21, 29, 30], acetic acid, acetoin, and

other organic acids [22, 31], bis(methyl-thio)methane [32], different types of sugars [33–36], alcohol [37], and so on.

2.3 *Liquid Chromatography-Isotope Ratio Mass Spectrometry (LC-IRMS)*

The advantage of LC-IRMS over the GC-C-IRMS is that it is able to separate non-volatile compounds without the need of derivatization. LC separates organic-specific components of the sample mostly according to their polarity. This is a complementary technique to GC. This interface is much more recent than the combination GC-C-IRMS [38]. Up to the moment, LC-IRMS applications are typically dedicated to carbon isotope ratio analyses. Nowadays there are two interfaces able to connect and HPLC to IRMS (1) a moving wire interface (still into the prototype state) and (2) a wet-chemical oxidation interface already in use by Thermo (LC IsoLink) and Elementar Analysensysteme GmbH (LiquiFace) with commercial instruments [39]. The scheme of this latter interface is shown in Fig. 4.

The advantage of wet-chemical oxidation is that it converts the individual compounds eluting from the column into CO_2 directly in the mobile phase by a post-column steam of an oxidant (ammonium peroxodisulfate) and a catalyst (silver nitrate). The disadvantage is that the HPLC mobile phase (mostly water) cannot contain organic solvents or redox modifiers because they interfere with the results [39]. This is not a minor disadvantage but an important limitation of the technique considering the role that organic solvents and modifiers play in LC separation improving peak shape and separation power. Once the reagents for the wet oxidation are added to the mobile phase, it passes through a capillary oxidation reactor where the organic compounds are oxidized to CO_2 at a temperature of about 100°C. A

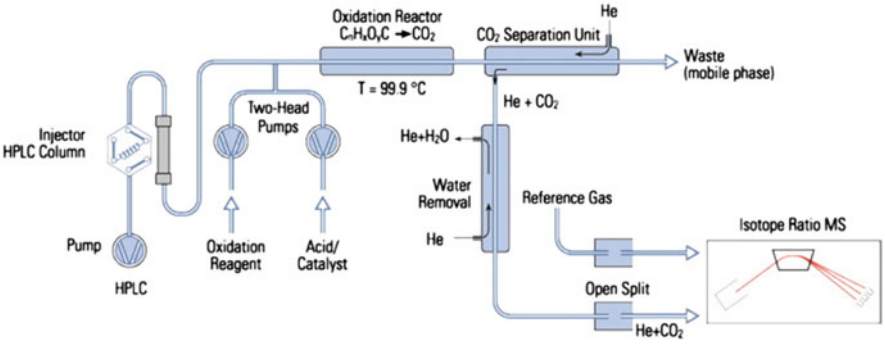


Fig. 4 Principles of the LC-IRMS interface according to [40]. Compounds separated by LC are converted to CO_2 by wet-chemical oxidation. In the separation unit, this CO_2 is transferred from the liquid eluent into a helium flow. Before the CO_2 flow is carried into the IRMS the water vapor is removed. Reproduced from reference [39] with permission from Elsevier

membrane exchanger separates the CO_2 from other gases that are mostly present in the mobile phase. The CO_2 is transferred to the IRMS through a permeable membrane.

3 Applications of IRMS in Food and Environmental Analysis

3.1 Environmental Application

Some applications in environmental analysis can provide good results when bulk material is analyzed by EA-IRMS.

Several applications impact on the use of IRMS to determine the concentration and isotopic composition of atmospheric CO_2, essential for the identification of sources and sinks of anthropogenic CO_2 and the monitoring of various emission reduction techniques, such as geological CO_2 storage [41–43]. Atmospheric particulate matter (PM) is also the subject of intensive environmental investigation as it significantly influences the radiative balance of the Earth and, hence, its surface temperature. The thermal-based EA-IRMS technique offers an opportunity, which can substantially expand our current knowledge of carbonaceous particles. For example, the ratio of ^{13}C and ^{12}C stable isotopes ($\delta^{13}C$) can provide important information about the potential sources and atmospheric processes influencing the characteristics of these particles.

Considering their persistence, plastic waste is an increasing environmental problem, especially in marine ecosystems. Interestingly, Berto et al. [44] performed a preliminary characterization of the stable carbon isotopes ($\delta^{13}C$) of different plastic polymers both conventional (petroleum-derived) and "bio" (plant-derived) in order to increase the data set of isotopic values to characterize the polymers and to apply the technique to marine environmental monitoring. The $\delta^{13}C$ values determined in different food packaging reflect the plant origin of the "bio" materials, while recycled plastic materials showed a $\delta^{13}C$ signature between the plant and petroleum polymer source. The advantages of IRMS over other analytical methods used to characterize the composition of plastic polymers are sensitivity, reduced material required, rapidity of analysis, and low cost. It is not well-established in the presented method but future application will require separation of plastic from the matrix.

The measurement of the $^{18}O/^{16}O$ ratio of water is one of the most important applications of IRMS. Very diverse applications, ranging from studies of effective identification and quantification of groundwater infiltration into sewage collection networks [45] to variations in natural abundance in the ecohydrologic cycle of some forest [46, 47] have been widely studied.

Isotopic abundance ratios can also establish dietary patterns and livestock movements as well as determine nutrient flows in aquaculture systems. Gamboa-Delgado [48] reviewed the most recent applications and findings derived from studies that

have used stable isotope analyses to (1) estimate nutrient assimilation in larval and juvenile organisms, (2) evaluate trophic dynamics and bioremediation potential of macroalgae and invertebrates in integrated multi-trophic aquaculture systems, and (3) investigate the trophic plasticity of aquatic species. The increasing application of CSIA of amino acids and fatty acids is emphasized as it has allowed exploring the physiological fate of specific organic compounds, while also assisting in the definition of nutritional requirements for aquatic species.

In addition to the examples compiled Gamboa-Delgado's [8] review, stable isotope analyses were used to estimate trophic niche width and overlap for flood and dry seasons in an Amazon floodplain lake. Fish ecology is influenced by the bioavailability of food that can be related to the analysis of the values of stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) through the trophic web. The results indicate that seasonal variation in isotopic niche width is specific to feeding habit. Understanding how fish trophic ecology responds to changes in the hydrological regime during the seasons is crucial for sustainable fishery management [49]. The same concept was used by Pouil et al. [50] in aquaculture to establish the fate of commercial pellets and natural resources in the trophic web structure (i.e., plankton of different sizes, macroinvertebrates, periphyton, fish, sediment and commercial pellets) in Indonesian giant gourami (*Osphronemus goramy*) aquaculture ponds. The results showed that, in a typical omnivorous species reared in semi-intensive pond conditions, the endogenous resources of the pond are barely utilized and the diet is mainly based on commercial pellets.

The integrated stable isotope analysis of nitrogen and carbon also provided information on the trophic position of invasive sally lightfoot crab *Percnon gibbesi* and a comprehensive evaluation of its feeding ecology. Results highlighted significant seasonal variation in *P. gibbesi* diet, improving our understanding of its trophic plasticity and potential dietary overlaps with other herbivore species [51].

Thanks to the possibility to extend the isotopic signature to non-conventional stable elements, IRMS can also contribute to understanding of complex Hg metabolism and HgSe nanoparticles formation of giant petrels [52]. This seabird species is one of the largest avian scavengers in the Southern Ocean, highly exposed to methyl mercury (MeHg) through their diet. Species-specific isotopic measurement ($^{\delta 202}$Hg, $^{\delta 199}$Hg) of HgSe nanoparticles in seabirds showed the largest variations between $^{\delta 202}$Hgbulk and $^{\delta 202}$HgSe in muscle and brain tissues. This could be attributed to the higher fraction of Hg present as MeHg in these tissues. Hg-biomolecules screening highlights the importance of the isotopic characterization of these (unknown) complexes.

One of the hot topics in environmental research is to elucidate the intimate relationships between plant roots, the rhizosphere, and the soil. These relationships are favored by the release of organic compounds from the plant to the soil through various forms of rhizodeposition and the simultaneous uptake of nutrients from the soil to the plant. Denis et al. [15] spatially tracked and mapped the migration of plant-derived carbon (C) through roots into the rhizosphere and surrounding soil using LA-IRMS. This technique, in conjunction with a ^{13}C tracer, allows the spatially resolved quantification of recent photosynthate in plant–soil microcosms at $>>$ 100 mm resolution. Using the measured $^{\delta 13}C$ values, the fraction of recent

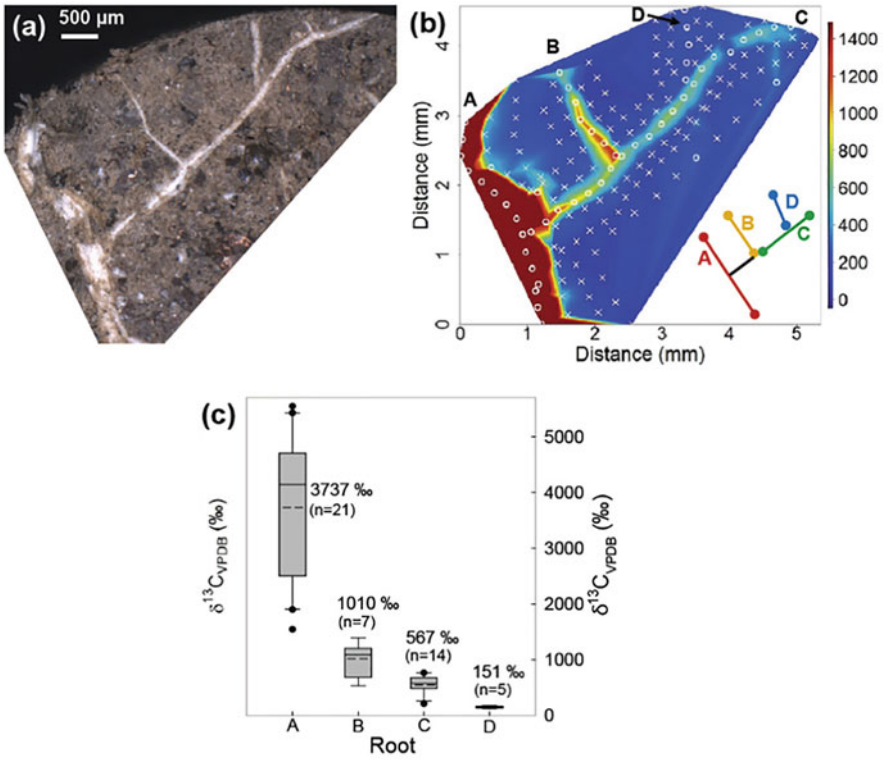


Fig. 5 LA-IRMS tracks variable carbon flow through different roots. (**a**) Light image of area of soil pellet ablated. (**b**) Isotopic heat map of root "o" and rhizosphere-soil "x" ablation spots (50 mm spot size). Dark red indicates ablation spots with C isotope values greater than 1,500%. Bottom right diagram shows the segment associated with each root letter, where the black line represents root that connects root A to branch point of root B and C. (**c**) Box plots of isotope values of the four roots with the mean listed (dashed line). Means are significantly different ($P < 5\%$). Reproduced from [15] with permission of Wiley

plant-derived C at a given location in the root, rhizosphere, or bulk soil can be calculated. This work demonstrates the utility of LA-IRMS for mapping the extent of ^{13}C-label migration and root exudation into rhizosphere and soil. The relatively straightforward sample preparation does not require addition of any embedding medium which may confound isotopic measurements. Photosynthate distribution within the sampled roots displayed significant heterogeneity and distinct branch points where roots receiving high versus low photosynthate allocation diverged (Fig. 5).

SCIA using GC-IRMS has also been widely used in environmental analysis. The isolation of compounds from the high complex mixture that constitute the matrix is extremely important.

The compounds isolated for SCIA have been in an important proportion environmental contaminants. One interesting application is the compound-specific

analysis of stable C, N, and O isotopes in explosives contaminants and characterized biodegradation of 2,4,6-trinitrotoluene (TNT) and dinitrotoluene isomers in the soil of a contaminated site [24]. The technique provides information on the biodegradation of explosives in the environment and helps to assess the risk management of military ranges. Other is the CSIA of organophosphorus pesticides with different chemical properties [25] using $^{\delta 13}C$ and $^{\delta 2}H$ analysis. This study particularly compares HTC and Cr/HTC units to explore the limitations of hydrogen isotope analysis of heteroatom bearing compounds. The method showed potential for investigations regarding the fate of these pesticides. However, the enrichment and clean-up of organophosphorus pesticides from environmental samples with residual levels of micrograms are still an important challenge because only high concentrations are detected by IRMS.

Other contaminants whose degradation mechanism has been studied by SCIA are polybrominated diphenyl ethers (PBDEs). In an interesting study, Wang et al. [26] demonstrated that microbial reduction processes of PBDEs are one of the most important degradation mechanisms in an anaerobic environment such as most of the wetland ecosystems and established that the progressive debromination of BDE-47 and BDE-153 is the mechanism responsible for their degradation [26].

Going a step further, Shan et al. [53] examined biomarkers, along with stable carbon (δ13C) and hydrogen (δD) isotopic compositions of n-alkanes in a set of condensates collected from the East China Sea shelf basin to establish their organic matter input, depositional conditions and assess their thermal maturity. The combination of GC × GC-TOFMS and CSIA analyses provided additional information concerning the maturity, environment, and post-depositional processes involved in the production of these condensates and enabled them to be distinguished from each other.

SCIA has also been applied to the natural compounds such as lignin that are difficult to transform in their monomers. Wang et al. [28] developed a novel method to measure the $^{\delta 18}O$ of the three oxygens (O-3, O-4, and O-5) attached to the aromatic ring of the monomeric units (bearing no oxygen in their side chains) releasable by highly selective tungsten carbide supported by activated carbon-catalyzed hydrogenolysis of lignin. O-4 is obtained by measuring the $^{\delta 18}O$ of H-type monomeric unit, while O-3 and O-5 can be calculated following isotope mass balance between different types of monomeric units measurable simultaneously with GC-IRMS. This study has application in plant physiological, metabolic, lignin biosynthetic, and climate studies.

The $^{18}O/^{16}O$ (and $^{15}N/^{14}N$) ratio of natural nitrate (NO_3^-) and nitrite (NO_2^-) can be used to extract valuable information about their source and fate as environmental contaminants, their metabolism as macronutrients in plants and animals, and their behavior in the N biogeochemical cycle. Wang et al. [27] developed a method to selectively isolate and purify nitrate and nitrite from natural water, soil, air, and plant materials by strong anion exchange for low- to normal-salinity samples or strong cation exchange for high-salinity samples, followed by quantitative conversion to their respective benzyl esters, which can be separated and individually analyzed for $^{\delta 18}O$ (and potentially $^{\delta 15}N$) by GC-IRMS. The method compares favorably with the

bacterial denitrification and chemical reduction methods, in terms of sensitivity and reliability, and has the potential to simultaneously measure $^{\delta 15}N$ and $^{\delta 18}O$ of nitrate and nitrite from natural samples of various origins.

Furthermore, as a link with the next section of food application, there are a number of studies that relate the current situation of water scarcity, drought, and climate change to characteristics of produced food. Recently, Spangenber and Zufferey [54] reported that the carbon isotope composition of the solid residues obtained by freeze-drying white and red wines ($^{\delta 13}C$) could be used for tracing the water status of the grapes used to produce them. These authors compared different methods using $^{\delta 13}C$ values of other wine components (particularly those of whole wine obtained by EA-IRMS) and of wine ethanol obtained by GC-IRMS, for their suitability to assess the vine water status. For all wine varieties, both $^{\delta 13}C$ values were linearly correlated with the predawn leaf water potential (Ψ pd) and therefore serve as reliable indicators of vine water status. An evaluation of the advantages and drawbacks of the different methods showed that the $^{\delta 13}C$ analysis of wine ethanol by GC-C-IRMS is the most appropriate.

Drought stress is a major limiting factor for banana production and the incidence of drought spells is expected to increase with climate change. Climate smart practices and varieties are needed, but evaluation in the field is hampered by a lack of reliable physiological drought stress indicators. Vantyghem et al. [55] investigated the potential of using stable carbon isotope ratios ($^{\delta 13}C$) and leaf temperature and its derived Degrees above Non-Stressed Canopy index (DANS) as proxies for drought stress in banana. Leaf samples were taken for $^{\delta 13}C$ analysis and leaf temperature was monitored throughout the day in a field trial with different banana plant stages (mother and daughter plants) under two irrigation treatments (rain fed and irrigated) during the dry season in Arusha, Tanzania. The results showed that $^{\delta 13}C$, leaf temperature, and DANS were highly sensitive proxies for drought stress in banana and soil volumetric water content had a significant effect on both $^{\delta 13}C$ values and DANS.

Similarly, the stable isotope and photosynthesis response of tea (*Camellia sinensis*) were determined under different light and temperature conditions. The results showed that isotopes of young tea leaves were more enriched with increasing light intensity. However, the value of $^{\delta 13}C$ and $^{\delta 15}N$ seemed depleted, while $^{\delta 2}H$ and $^{\delta 18}O$ became enriched as temperature increasing from 15 to 35°C. Pearson's correlation showed a negative correlation between isotopes ($^{\delta 13}C$, $^{\delta 15}N$, and $^{\delta 2}H$) and photosynthetic parameters that provided new insights which could be used to predict tea isotope responses arising from subtle seasonal or climate change conditions [56].

3.2 Food Applications

Food scientists have long recognized the need for analytical techniques that can provide data on issues such as traceability, authenticity, and origin of foods and beverages. Two recent literature reviews cover globally the state of the art of IRMS

in this area. In this sense, van Leeuwen et al. [57] demonstrated that GC-C-IRMS is one of the most powerful techniques available to detect fraudulent, illegal, or unsafe practices in the food and beverages industries. This technique is able to provide data on key food components such as aroma compounds, sugars, amino acids, and carbon dioxide (in carbonated beverages). Such data can be used to determine synthetic and natural ingredients, substitution of one ingredient for another, the use of synthetic or organic fertilizers, and origin of foods and food ingredients, including carbon dioxide. IRMS is gaining wider acceptance in food control laboratories due to its increasing versatility. This is largely due to the new possibilities that now exist to hybridize various diverse instruments, as the reliability of instrumentation and array of "on-line" techniques. These combinations are consolidating the use of IRMS analyzers as "another detection system". The IRMS has ultimately become a tool to unequivocally prove food adulteration. This explains its increasing routine use in control laboratories to prosecute fraud, enforce the law and thus ensure food safety [19]. An interesting example investigated the fraudulent addition of sugars to coconut water by carbon isotope ratio ($^{13}C/^{12}C$, δ13C, ‰) using EA-IRMS. The $^{\delta 13}C$ can detect the addition of one or more sugars in percentages higher than 2%. This study shows that of randomly sampled commercial coconut waters in Thailand approximately 50% were adulterated by the addition of sugars [33].

Another example of the usefulness of this technique to detect adulteration is the identification of the addition of acetic acid to rice vinegar by SCIA. The specific vinegar compounds for which the stable carbon isotope ratio is measured by GC-C-IRMS are acetic acid and acetoin. The method detected the adulteration of rice vinegar when it contains more than 10% synthetic acetic acid [31].

Vanilla flavor is one product also highly susceptible to food fraud since the main component "vanillin" is obtained from vanillin pods (naturally) but can easily be produced by much cheaper synthetic processes. Commonly origin of vanillin is identified because the $^{\delta 13}C$ ranges for synthetic vanillin from petroleum and C3 plants are depleted in comparison with the reported $^{\delta 13}C$ range for vanillin from vanilla orchids. Wide et al. [29] reported a preparation procedure that includes a head space SPME clean-up and GC-IRMS to determine the isotopic carbon ratio of vanillin in food products. The method was highly specific to identify vanillin source but was not always an easy task, especially when very low concentrations are incorporated in complex food matrices. The synthetic vanillin can nowadays be considered as biosynthetic when it is obtained using different precursors (mainly lignin and guaiacol) or by enzymatic bioconversion (biovanillin) from natural precursors (mainly ferulic acid, eugenol and isoeugenol, and turmeric acid). The $^{\delta 13}C$ value for biovanillin was higher compared to vanillin from vanilla pods [30]. One fraud being perpetrated to obtain the $^{\delta 13}C$ range of vanillin from vanilla pods is combining vanillin derived from inexpensive sources. Schipilliti et al. [21] proposed also headspace SPME and GC-IRMS to determine the carbon isotope ratio ($^{\delta 13}C$) of vanillin extracted from vanilla pods, commercial flavored foodstuff, and in vanillin samples of different origins. Biovanillin can be easily characterized and distinguished from the synthetic ones. However, with the invention of new biosynthetic pathways, vanillin overlapping with the characteristic $^{\delta 13}C$ range reported for vanillin from vanilla pods can be produced [21]. Interestingly, a recent study

demonstrated that bulk and site-specific analysis by GC-IRMS of stable carbon and hydrogen isotope ratios of vanillin derived from ex-glucose can be easily identified by 2D plot of the $^{\delta 13}$CBulk vs. $^{\delta 2}$HBulk values of the combined data [30]. This is an important step ahead to detect this fraud.

Similarly, bis(methyl-thio)methane (BMTM), the molecule which provides "white truffle-like" flavor can be synthetic or of natural origin. The molecule can be extracted from various raw materials using anhydrous methanol. $^{\delta 13}$C values established by GC-C-IRMS define the range of $^{\delta 13}$C values of synthetic BMTM. Measurements by 1H NMR made on synthetic BMTM and BMTM extracted from "white truffle-like flavor" confirmed that the method determined the isotopic distribution of ^{13}C/^{12}C ratio in two characteristic sites of this molecule [32].

Other important case of adulteration that affects different types of food is the undeclared addition of exogenous sugar products in food and beverages. The isotope analysis of the carbon-bound non-exchangeable (CBNE) hydrogen in mono and disaccharides has demonstrated its capacity to detect these added sugars in fruit juices and honey. The procedure utilizes a simple one-step reaction, with the derivatizing agent N-methyl-bis-trifluoroacetamide, to substitute the exchangeable hydroxyl-hydrogens with trifluoroacetate derivatives that are sufficiently volatile to be separated and measured by a GC-IRMS [34].

The sugar-replacement compound xylitol has also gained increasing attention because of its use in commercial food products, dental-hygiene articles, and pharmaceuticals. The traditional "birch xylitol" is considered a premium product, in contrast to xylitol produced from agricultural by-products such as corn husks or sugarcane straw. BSIA and CSIA of chewing-gum extracts by LC-IRMS were used to determine the $^{\delta 13}$C isotope signatures for xylitol. The analyzed chewing gum could be clearly related to the raw-material plant class [35]. The chromatographic separation of a chewing-gum extract by the optimized LC-IRMS method is shown in Fig. 6. This application required the development of an organic-solvent-free

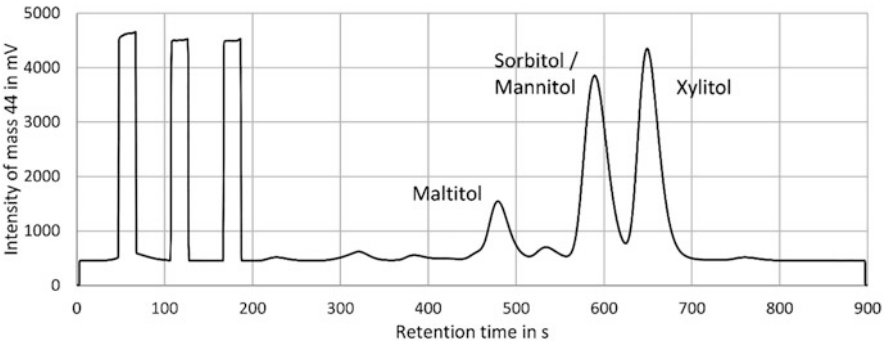


Fig. 6 Chromatographic separation of xylitol from an aqueous extract of a chewing-gum sample (MSQ) containing xylitol, mannitol, sorbitol, maltitol, aspartame, maltitol syrup, sucralose, and acesulfame potassium. Reproduced from ref. [35] with permission of American Chemical Society

extraction protocol and HPLC method for the separation of xylitol from different artificial sweeteners and sugar-replacement compounds.

Sake is a traditional Japanese alcohol that has been adulterated by the addition of alcohol and sugar obtained as by-products in the brewing industry. Sutto and Kawashima [58] analyzed the $^{\delta 13}C$ values of the ethanol and glucose in Sake by LC-IRMS to check brewers' alcohol and sugar addition. The method was able to calculate the percentage of brewers' alcohol in commercial sake.

Similarly, the production of "Polish Vodka" is restricted by law to the ethyl alcohol of agricultural origins obtained from rye, wheat, barley, oat, triticale, and potatoes grown on the territory of the Republic of Poland. Unfortunately, genuine "Polish vodka" is counterfeited through the illegal practice of adding cheaper and more accessible corn liquor, which reduces the production costs of the spirit. Quantitative NMR and IRMS of deuterium and $^{\delta 13}C$ allows to distinguish alcohol from different raw materials. Both approaches led to the same conclusions regarding the percentage of maize-derived ethanol addition [37].

Non-concentrated juice (NFC) is a higher priced product than juice reconstituted from concentrate due to its higher quality and nutritional value. However, a growing problem in the industry is the adulteration of NFCs by the addition of water and sugar. Wu et al. [59] investigated the carbon and oxygen stable isotope ratios ($^{\delta 13}C$ and $^{\delta 18}O$ values) of the bulk juice and different juice components, and qualitatively and quantitatively analyzed the addition of water and sugar in NFC juices. Sugar-specific isotope analysis (SSIA) technology was used to determine the $^{\delta 13}C$ values of different sugars (sucrose, glucose, and fructose) and carbon content to qualitatively infer C3 plant sugar addition. The addition of water to NFC juice can also be determined by comparing $^{\delta 18}O$ values of extraneous water, pulp, and filtered juice. Thus, the determination of stable isotope ratios allows the detection of adulteration of NFC juice. Related to this fraud, other study compared commercial and directly-pressed orange and apple juices using both, IRMS ($^{\delta 13}C$ and $^{\delta 18}O$) and ICP-MS (Ni, Cr, Ca, Sr, Fe, K, Mg, and Na). The concentrations of the different metals provide interesting information to distinguish agricultural practices, whether the juice is orange or apple juice or whether it is natural or not. However, only the isotopic abundances demonstrated that most commercial juices come from concentrates and that in some cases they contain fraudulent addition of corn or cane sugars (since they are C4 plants) [36].

The establishment of the geographical origin is also an important potential of these techniques that has been applied to many different foods. The addition of sugars to honey is detected by the AOAC 998.12 that detects the addition of sugar from C4 plants. However, this method is not applicable to honey with non-extractable protein. The authenticity of pure honeys of various botanical and geographical origins with non-extractable proteins was studied on the basis of the stable carbon isotope ratio characterization of total honey, proteins, and the main sugars. Thirty-five honeys from which protein could not be extracted were all identified as adulterated with C-4 or C-3 sugars. The findings obtained supplemented the AOAC 998.12 C-4 sugar method, about honeys from which protein cannot be extracted [60].

Determining the geographical origin of wines is a major challenge in wine authentication, but little information is available regarding non-parametric statistical approaches for wines. Interestingly, a study on Chardonnay wines both vinified on a small scale from grapes cultivated in Japan, and imported from 8 countries, which analyzes the oxygen stable isotope and 18 elements applying non-metric multidimensional scaling (NMDS), kernel principal component analysis (KPCA) and principal component analysis (PCA). The non-parametric methods, NMDS and KPCA, separated domestic from imported Chardonnay wines better than the parametric method, PCA. Of 19 variables, 18 were important for geographical discrimination, with the $^{\delta 18}O$ value being the most significant in all statistic methods [61]. Similarly, GC-IRMS combined with SPME was able to measure the $^{\delta 13}C$ values of six typical volatiles commonly occurring in wine (isoamyl acetate, 2-octanone, limonene, 2-phenylethanol, ethyl octanoate, and ethyl decanoate). The $^{\delta 13}C$ of these volatile compounds is able to distinguish the real imported wines [20]. Recently, Suto and Kawashima [62] developed a method to determine the $^{\delta 13}C$ values of various organic acids in wine and sake that involves solid-phase separation (SPE) combined with LC-IRMS. This method detected the addition of organic acids (tartaric, malic, lactic, succinic) from different sources to sake and wine. The application of the method to different samples showed that the addition of lactic acid to sake is a common practice while organic acids in wine usually come from grapes.

A curious comparison of the use of conventional IRMS and SPME-GC-MS and non-conventional analytical techniques (Electronic Nose) to characterize and discriminate origin, drying, and age of saffron was reported by Rochi et al. [22]. The IRMS analysis of the stable carbon and nitrogen isotopes was reliable in discriminating the geographical origin of saffron. The SPME-GC-MS was able to discriminate the different origin, drying, and age based on the isolation of volatile compounds. An E-Nose was used as alternative and rapid tool to characterize the complex aroma patterns and to exploit authenticity of saffron samples. Results of this study could contribute to select and identify routine quality control methods for quality and authentication of saffron.

Within the cocoa market (*Theobroma cacao L.*), quality and prices are often determined by geographical origin, making traceability indispensable. Therefore, to investigate possibilities of tracing by analytical methods, 48 carefully selected cocoa samples from 20 countries have been profiled using a combination of stable IRMS and proton nuclear magnetic resonance (^{1}H NMR). Chemometric analysis of combined data sets from both, stable isotope data ($^{\delta 13}C$, $^{\delta 15}N$, $^{\delta 18}O$, $^{\delta 2}H$) and ^{1}H NMR fingerprints, achieved good separation with increased classification rates compared to classification with data of the isolated methods. IRMS contributed primarily to discrimination between countries, while ^{1}H NMR significantly contributed to separation of varieties, but also the regions within individual countries. This study thus demonstrates that combination of two analytical methods is an effective tool to enhance both, accuracy and precision, in authenticity testing of cocoa [63].

Another case where geographical identification is important is in the case of shiitake mushrooms to distinguish those grown in other countries (such as Korea or the USA) on imported Chinese inoculated medium from those grown in China.

In this regard, Chung et al. [64] developed a CSIA discriminant model of the $^{\delta 15}N$ amino acids aspartate and glutamate capable of distinguishing between Korean shiitake, Korean shiitake grown in Chinese inoculated medium, and Chinese shiitake with 100% accuracy.

Flaxseed is an economically important oilseed crop whose geographic origin is of significant interest to producers and consumers because every region may exhibit particular quality characteristics. To improve the discrimination rate and the geographical traceability of this crop, the chemical profiles of the flaxseed samples were characterized via lipids/fatty acids, stable isotopes, and antioxidant capacity. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) attained a satisfactory discrimination rate after combining fatty acids, stable isotopes, and antioxidant capacity to trace the origin of flaxseed from five regions in northern China [65].

Not all the studies performed have been successful, a study that comprehensively analyzed different kinds of pure honey which was produced in various areas in China according to $^{\delta 13}C$-EA-IRMS (AOAC method 998.12) and $^{\delta 13}C$-LC-IRMS (proposed by the Intertek laboratory in Europe) methods. As for the $^{\delta 13}C$-EA -IRMS method, the study confirmed the C4 sugar of all authentic honey samples. However, about 70% samples had $^{\Delta\delta 13}C$ (‰) values outside the range, indicating that a large proportion of pure honey in China cannot pass the $^{\delta 13}C$-LC-IRMS test, although these honeys were extracted from unadulterated sources. Based on the findings, the $^{\delta 13}C$-LC-IRMS method seems not appropriate to reliably detect adulterated honeys with C3 sugars in China [66].

Instead, the stable isotope ratios of carbon, nitrogen, hydrogen, oxygen, and mineral elements and their stoichiometric methods were examined as possible factors that could certify Chinese tea based on its production years. In this sense, a total of 43 multi-element stable isotope ratios of Xiangzhujing Pu'er tea in five production years were determined through ICP-MS and EA-IRMS methods. Two unsupervised learning techniques (PCA and hierarchical clustering analysis) and three supervised learning techniques (partial least squares discriminant analysis [PLS-DA], back-propagation artificial neural network [BP-ANN], and linear discriminant analysis [LDA]) were used on the basis of 18 statistically significant multi-elemental stable isotope ratios to build authentication models for Pu'er tea. The three supervised models correctly separated the corresponding production years of the samples [67].

Reliable identification of the origin of economically important animal species is essential to preventing fraud. Remarkably, Xu et al. [68] investigated the efficacy of stable isotope and multi-element analyses in claw meat, abdominal meat, gills, and the hepatopancreas of swimming crabs (*Portunus trituberculatus*) using specimens from three discrete production areas in China and developed an origin discrimination model using stepwise discriminant analysis. Stable isotope ratios ($^{\delta 13}C$, $^{\delta 15}N$) and the contents of 14 elements (Na, Mg, Al, K, Mn, Fe, Co, Cu, Zn, As, Se, Rb, Ag, and Ba) varied significantly by tissue and by sampling location. Obvious isotopic fractionation effect was observed among different tissues, the muscle tissue and gills isotope ratios were more effective geographical origin indicators than hepatopancreas. Although the accumulation effects of the elements differed between

tissues, the discriminatory correctness was generally consistent across tissues. This method supports the geographical traceability and origin identification of *P. trituberculatus* [68].

Other species such as Octopus play an increasingly important role in ocean ecosystems and global fisheries, but provenance authentification techniques are very scarce. Martino et al. [69] investigated whether chemical profiling can distinguish geographical origins of octopus on international and domestic scales in samples consisted of wild-caught octopus from south-east Asia and southern Australia. To this end, these researchers used a combination of stable carbon ($^{\delta 13}C$) and oxygen ($^{\delta 18}O$) isotope analyses by EA-IRMS of internal calcified structures called statoliths, with elemental analyses (X-Ray Fluorescence using Itrax) of soft-tissue. Multivariate profiles exhibited distinctive regional signatures, even across species, with high classification success back to region of origin. This study validates isotopic and multi-elemental profiling as an effective provenance tool, which could be used to support transparency and accountability of seafood supply chains and thus encourage sustainable use of ocean resources [69].

Growing demand for organically-grown food has resulted in the establishment of multiple standards, certification, and labeling of organic products that were successfully established, thanks to the use of BSIA and CSIA. Mihailova and Kelly [70] reviewed innovative IRMS techniques to ascertain organically-grown food, identifying three growing techniques (1) stable sulfur isotopes (providing additional information about regional agricultural practices and fertilization strategy), (2) CSIA of plant-derived nitrate (distinguishing between crops grown with synthetic and organic fertilizers), and (3) stable magnesium isotope analysis which can be performed using MC-ICP-MS. Independently of these promising techniques, the natural abundance of stable isotope ^{15}N ($^{\delta 15}N$) in production systems has already become an important technique to distinguish organic products from conventional ones. Trapp et al. [71] evaluated the use of $^{\delta 15}N$ values recorded for nitrogen fertilizers, soil and plant tissue in order to set the differences between organic and conventional agricultural production systems applied to rice, potatoes, apple, and banana crops. Values recorded for $^{\delta 15}N$ in food from organic farms were higher than those recorded in conventional farms. Results have allowed for differentiation between production systems.

Similarly, O'Sullivan et al. [72] reviewed the scientific literature on stable isotope analysis for authentication of milk and dairy products. Through numerous examples, these authors demonstrate the usefulness of the technique for identifying geographical origin as well as the influence of processing and seasonality on the results especially using SCIA of caseins. Furthermore, these authors identified the need of complete datasets for comparison and statistics as well as promising areas for the future, such as the analysis of less used elements.

Other field of application of IRMS is animal nutrition that ultimately ensures the quality and safety of food. One of these applications is to obtain time-integrated estimation of assimilated and not just ingested nutrients. This feature turned possible its application to evaluate the effects of dietary nucleotides and glutamate on carbon turnover ($^{\delta 13}C$) in the brain of weanling piglets. The carbon turnover verified for

cerebral tissue was faster for diet containing glutamate in comparison with other diets, supporting the fact that glutamate contributed to develop the piglets' brain, due to the fastest incorporation of ^{13}C-atoms in this tissue at post-weaning phase, despite the energy deficit experienced by them [73, 74]. Other application of IRMS is to determine whether animals consume animal by-products (ABP), a widely prohibited practice because there is a risk of transmitting scrapie – a fatal prion disease in human beings. Da Silva et al. [75] used IRMS for $^{\delta 13}$C and $^{\delta 15}$N to trace animal proteins in the serum of sheep. This method is biologically and economically relevant for the veterinary field because it can track protein over time or make a point assessment of animal feed with high sensitivity and resolution, providing a low-cost analysis coupled with fast detection.

The use of SCIA within food analysis has many more fields of application even though the most important have been described. Stimulatingly, Llana-Ruíz-Cabello et al. [23] described the results obtained by hyphenating pyrolysis-gas chromatography (Py-GC) with carbon IRMS for the analysis of a polylactic acid based bio-plastic extruded with variable quantities of a natural plant extract or oregano essential oil. The chemical structural information of pyrolysates was first determined by conventional Py-GC and, then, the measure of $^{\delta 13}$C in specific compounds was done by Py-GC-C-IRMS. PyCSIA is shown as a promising tool to study such materials, providing not only a fingerprinting, but also valuable information about the origin of the materials, allowing the traceability of additives and minimizing sample preparation [23].

4 Conclusions

IRMS is considered a well-established technique. However, new instrumentation is being developed to solve the existing problems in the technique, such as, isotope analysis in multi-element molecules. Furthermore, new ways of applying it to specific case studies are still being explored and are opening new horizons within the field. Among them, the on-line combination of IRMS with numerous other techniques, a higher degree of automation, and better standardization will improve the reliability of the technique. The future objectives of this technique will also focus on those objectives that dominate the analytical chemistry landscape, which are basically to increase the speed of analysis and reduce its economic cost while simultaneously obtaining higher resolution and precision data and developing methods and protocols that are within the principles of green chemistry, i.e. that are respectful of the environment.

IRMS is now part of a versatile platform with a wide range of possibilities as it can be coupled with various systems for preparation, separation, and isolation of sample components. The most suitable system will depend on the type of sample to be analyzed and the objective of the study. For solid, non-volatile samples, such as food, amino acids, or fatty acids, EA-IRMS is the technique of choice, although the result it provides is the mean value of the isotope ratio for the whole sample, even

though samples can contain several thousands of compounds. However, in complex environmental and food samples, CSIA of some sample components provides a greater amount of information than BSIA. As complementary techniques to determine the isotope ratio in each component of the sample, GC-IRMS or LC-IRMS can be used to separate compounds by GC or LC, respectively, prior to IRMS.

Whichever method of sample introduction is combined with IRMS shows many applications in the environment and foodstuffs. In the environmental field IRMS has shown great versatility to increase the knowledge of carbonaceous materials, identify organic contaminants, establish the water origin, or elucidate relations between different interfaces. In the food field the applications to establish food authenticity, geographical origin, organic food practices, or most common food fraud have grown enormously. A noticeable gap in the market exists for compound-specific isotope ratio standards, although several suppliers are available for bulk isotope ratio standards such as polyethylene, sugar, and flour.

Acknowledgments This work has been supported by Grant RTI2018-097158-B-C31 funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe."

References

1. Fry B (2006) Isotope notation and measurement. In: Fry B (ed) Stable isotope ecology. Springer, New York, pp 21–39
2. Barrie A, Prosser SJJM. s. o. s. NYMD (1996) Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. 1–46
3. Taylor PDP, De Bièvre P, Valkiers S (2004) Chapter 41 – the nature and role of primary certified isotopic reference materials: a tool to underpin isotopic measurements on a global scale. In: de Groot PA (ed) Handbook of stable isotope analytical techniques. Elsevier, Amsterdam, pp 907–927
4. (2009) Variations of stable isotope ratios in nature. In: Hoefs J (ed) Stable isotope geochemistry. Springer, Berlin, pp 93–227
5. Danezis GP, Papachristidis CA, Georgiou CA (2017) Elemental fingerprinting. In: Food authentication, pp 83–116
6. Picó Y (2017) Organic foods. In: Food authentication, pp 431–451
7. Skrzypek G (2013) Normalization procedures and reference material selection in stable HCNOS isotope analyses: an overview. Anal Bioanal Chem 405(9):2815–2823. https://doi.org/10.1007/s00216-012-6517-2
8. Gröning M (2004) International stable isotope reference materials. In: Handbook of stable isotope analytical techniques. Elsevier, pp 874–906
9. Brand WA, Coplen TB, Aerts-Bijma AT, Böhlke J, Gehre M, Geilmann H, Gröning M, Jansen HG, Meijer HA, Mroczkowski SJ, Qi H, Soergel K, Stuart-Williams H, Weise SM, Werner RA (2009) Comprehensive inter-laboratory calibration of reference materials for δ18O versus VSMOW using various on-line high-temperature conversion techniques. Rapid Commun Mass Spectrom 23(7):999–1019
10. Hayes JM (1983) State of the art of high-precision stable isotopic analysis in organic materials, pp 450–453
11. Elsner M, Imfeld G (2016) Compound-specific isotope analysis (CSIA) of micropollutants in the environment – current developments and future challenges. Curr Opin Biotechnol 41:60–72

12. Jochmann MA, Schmidt TC (2015) Compound-specific stable isotope analysis. R Soc Chem
13. Muccio Z, Jackson GP (2009) Isotope ratio mass spectrometry. Analyst 134(2):213–222. https://doi.org/10.1039/b808232d
14. Mahoney JB, Weis D, Keiffer B, Hanano D, Williams G, Pretorius W, Barling J, Scoates J, Friedman R, Goolaerts A, Mattielli N, Maerschalk C (2003) Comprehensive and systematic MC-ICP-MS/TIMS isotopic characterization of USGS standards. 2003:V32C–V1044C
15. Denis EH, Ilhardt PD, Tucker AE, Huggett NL, Rosnow JJ, Moran JJ (2019) Spatially tracking carbon through the root–rhizosphere–soil system using laser ablation-IRMS. J Plant Nutr Soil Sci 182(3):401–410. https://doi.org/10.1002/jpln.201800301
16. Kaklamanos G, Aprea E, Theodoridis G (2020) 11 – Mass spectrometry: principles and instrumentation. In: Pico Y (ed) Chemical analysis of food2nd edn. Academic Press, pp 525–552
17. Gehre M, Renpenning J, Geilmann H, Qi H, Coplen TB, Kümmel S, Ivdra N, Brand WA, Schimmelmann A (2017) Optimization of on-line hydrogen stable isotope ratio measurements of halogen- and sulfur-bearing organic compounds using elemental analyzer–chromium/high-temperature conversion isotope ratio mass spectrometry (EA-Cr/HTC-IRMS). Rapid Commun Mass Spectrom 31(6):475–484. https://doi.org/10.1002/rcm.7810
18. Rodrigues C, Maia R, Lauteri M, Brugnoli E, Máguas C (2013) Chapter 4 – stable isotope analysis. In: de la Guardia M, Gonzálvez A (eds) Comprehensive analytical chemistry, vol 60, Elsevier, pp 77–99
19. Kelly SD (2013) Using stable isotope ratio mass spectrometry (IRMS) in food authentication and traceability. In: Food authenticity and traceability, pp 156–183
20. Jin X, Zhang L, Wu S, Huang M, Yu W, Zhang S (2021) Developing an authentication approach using SPME-GC-IRMS based on compound-specific δ13C analysis of six typical volatiles in wine. Food Qual Saf 5:fyaa031. https://doi.org/10.1093/fqsafe/fyaa031
21. Schipilliti L, Bonaccorsi IL, Mondello L (2017) Characterization of natural vanilla flavour in foodstuff by HS-SPME and GC-C-IRMS. Flavour Fragr J 32(2):85–91. https://doi.org/10.1002/ffj.3364
22. Rocchi R, Mascini M, Faberi A, Sergi M, Compagnone D, Di Martino V, Carradori S, Pittia P (2019) Comparison of IRMS, GC-MS and E-nose data for the discrimination of saffron samples with different origin, process and age. Food Control 106:106736. https://doi.org/10.1016/j.foodcont.2019.106736
23. Llana-Ruíz-Cabello M, Pichardo S, Jiménez-Morillo NT, González-Vila FJ, Guillamón E, Bermúdez JM, Aucejo S, Camean AM, González-Pérez JA (2017) Pyrolysis-gas chromatography–isotope ratio mass spectrometry for monitoring natural additives in polylactic acid active food packages. J Chromatogr A 1525:145–151. https://doi.org/10.1016/j.chroma.2017.10.023
24. Wang Q, Zhu Y, Zhao Y, Zhang H, Zhao S, Liu X (2021) In: Novel GC-IRMS-based method for compound-specific isotope analysis (CSIA) of explosives contaminants in soil
25. Wu L, Kümmel S, Richnow HH (2017) Validation of GC–IRMS techniques for δ13C and δ2H CSIA of organophosphorus compounds and their potential for studying the mode of hydrolysis in the environment. Anal Bioanal Chem 409(10):2581–2590. https://doi.org/10.1007/s00216-017-0203-3
26. Wang G, Liu Y, Wang X, Dong X, Jiang N, Wang H (2022) Application of dual carbon-bromine stable isotope analysis to characterize anaerobic micro-degradation mechanisms of PBDEs in wetland bottom-water. Water Res 208:117854. https://doi.org/10.1016/j.watres.2021.117854
27. Wang B, Zheng S, Huang Y, Wang Y, Zhu Z, Ma R, Zhao Y, Yin X, Su J, Xiong J, Zhang B, Zhou Y (2020) Novel GC/Py/GC/IRMS-based method for Isotope measurements of nitrate and nitrite. I: converting nitrate to benzyl nitrate for δ18O analysis. Anal Chem 92(18):12216–12225. https://doi.org/10.1021/acs.analchem.0c01403
28. Wang Y, Zhu Z, Xia Y, Zhong M, Ma R, Zhao Y, Yan Q, Miao Q, Wang B, Ma Y, Yin X, Zhou Y (2021) Accessing the position-specific 18O/16O ratios of lignin monomeric units from higher

plants with highly selective hydrogenolysis followed by GC/Py/IRMS analysis. Anal Chim Acta 1171:338667. https://doi.org/10.1016/j.aca.2021.338667
29. Wilde AS, Hansen AMS, Fromberg A, Lauritz Frandsen H, Smedsgaard J (2020) Determination of δ13C of vanillin in complex food matrices by HS-SPME-GC-C-IRMS. Flavour Fragr J 35(4):387–393. https://doi.org/10.1002/ffj.3573
30. Wilde AS, Frandsen HL, Fromberg A, Smedsgaard J, Greule M (2019) Isotopic characterization of vanillin ex glucose by GC-IRMS – new challenge for natural vanilla flavour authentication? Food Control 106:106735. https://doi.org/10.1016/j.foodcont.2019.106735
31. Fang CJ, You HC, Huang ZL, Hsu CL, Tsai CF, Lin YT, Kao YM, Tseng SH, Wang DY, Su NW (2020) Simultaneous analysis of the stable carbon isotope ratios of acetoin and acetic acid by GC-C-IRMS for adulteration detection in brewed rice vinegar products. J Agric Food Chem 68(48):14252–14260. https://doi.org/10.1021/acs.jafc.0c05674
32. Bononi M, Tateo F, Benevelli F, Pennetta A, Benedetto GD (2018) Gc-c-irms characterization of synthetic bis(methyl-thio)methane in truffle flavorings. Ital J Food Sci 30(4):752–761
33. Meepho SA, Kamdee K, Saengkorakot C, Thapprathum P, Judprasong K (2021) Comparison between isotope ratio mass spectrometry (IRMS) and cavity ring-down spectroscopy (CRDS) for analysing the carbon isotope ratio and detection of adulteration in coconut water. Int J Food Sci Technol 56(12):6611–6617. https://doi.org/10.1111/ijfs.15321
34. Abrahim A, Cannavan A, Kelly SD (2020) Stable isotope analysis of non-exchangeable hydrogen in carbohydrates derivatised with N-methyl-bis-trifluoroacetamide by gas chromatography – chromium silver reduction/high temperature conversion-isotope ratio mass spectrometry (GC-CrAg/HTC-IRMS). Food Chem 318:126413. https://doi.org/10.1016/j.foodchem.2020.126413
35. Köster D, Wolbert JB, Schulte MS, Jochmann MA, Schmidt TC (2018) Origin of xylitol in chewing gum: a compound-specific Isotope technique for the differentiation of corn- and wood-based xylitol by LC-IRMS. J Agric Food Chem 66(8):2015–2020. https://doi.org/10.1021/acs.jafc.7b05212
36. Cristea G, Dehelean A, Voica C, Feher I, Puscas R, Magdas DA (2021) Isotopic and elemental analysis of apple and orange juice by isotope ratio mass spectrometry (IRMS) and inductively coupled plasma–mass spectrometry (ICP-MS). Anal Lett 54(1–2):212–226. https://doi.org/10.1080/00032719.2020.1743717
37. Ciepielowski G, Pacholczyk-Sienicka B, Frączek T, Klajman K, Paneth P, Albrecht Ł (2019) Comparison of quantitative NMR and IRMS for the authentication of 'Polish Vodka'. J Sci Food Agric 99(1):263–268. https://doi.org/10.1002/jsfa.9168
38. Mai Z, Lai B, Sun M, Shao J, Guo L (2019) Food adulteration and traceability tests using stable carbon isotope technologies. Trop J Pharm Res 18(8):1771–1784. https://doi.org/10.4314/tjpr.v18i8.29
39. Perini M, Bontempo L (2022) Liquid chromatography coupled to isotope ratio mass spectrometry (LC-IRMS): a review. TrAC Trends Anal Chem 147:116515. https://doi.org/10.1016/j.trac.2021.116515
40. Krummen M, Hilkert AW, Juchelka D, Duhr A, Schlüter HJ, Pesch R (2004) A new concept for isotope ratio monitoring liquid chromatography/mass spectrometry. Rapid Commun Mass Spectrom 18(19):2260–2266. https://doi.org/10.1002/rcm.1620
41. Yu EJ, Lee KS (2020) Improved method for simultaneous determination of the carbon isotopic composition and concentration of atmospheric CO2 using CF-IRMS. Int J Mass Spectrom 452:116327. https://doi.org/10.1016/j.ijms.2020.116327
42. Yao P, Ni H, Paul D, Masalaite A, Huang RJ, Meijer HAJ, Dusek U (2022) An automated method for thermal-optical separation of aerosol organic/elemental carbon for 13C analysis at the sub-μgC level: a comprehensive assessment. Sci Total Environ 804:150031. https://doi.org/10.1016/j.scitotenv.2021.150031
43. Major I, Gyökös B, Túri M, Futó I, Filep Á, Hoffer A, Furu E, Timothy Jull AJ, Molnár M (2018) Evaluation of an automated EA-IRMS method for total carbon analysis of atmospheric aerosol at HEKAL. J Atmos Chem 75(1):85–96. https://doi.org/10.1007/s10874-017-9363-y

44. Berto D, Rampazzo F, Gion C, Noventa S, Ronchi F, Traldi U, Giorgi G, Cicero AM, Giovanardi O (2017) Preliminary study to characterize plastic polymers using elemental analyser/isotope ratio mass spectrometry (EA/IRMS). Chemosphere 176:47–56. https://doi.org/10.1016/j.chemosphere.2017.02.090
45. Heiderscheidt E, Tesfamariam A, Marttila H, Postila H, Zilio S, Rossi PM (2022) Stable water isotopes as a tool for assessing groundwater infiltration in sewage networks in cold climate conditions. J Environ Manage 302:114107. https://doi.org/10.1016/j.jenvman.2021.114107
46. Castro-López JA, Robles-Morua A, Méndez-Barroso LA, Garatuza-Payan J, Rojas-Robles OA, Yépez EA (2022) Water isotope variation in an ecohydrologic context at a seasonally dry tropical forest in northwest Mexico. J Arid Environ 196:104658. https://doi.org/10.1016/j.jaridenv.2021.104658
47. Wang T, Xu Q, Zhang B, Gao D, Zhang Y, Ren R, Jiang J (2022) Effects of understory removal and thinning on water uptake patterns in Pinus massoniana Lamb. Plantations: evidence from stable isotope analysis. For Ecol Manage 503:119755. https://doi.org/10.1016/j.foreco.2021.119755
48. Gamboa-Delgado J (2022) Isotopic techniques in aquaculture nutrition: state of the art and future perspectives. Rev Aquac 14(1):456–476. https://doi.org/10.1111/raq.12609
49. Azevedo LS, Pestana IA, Almeida MG, Bastos WR, Souza CMM (2022) Do fish isotopic niches change in an Amazon floodplain lake over the hydrological regime? Ecol Freshw Fish 31(1):72–80. https://doi.org/10.1111/eff.12609
50. Pouil S, Mortillaro JM, Samsudin R, Caruso D, Kristanto AH, Slembrouck J (2022) Fate of commercial pellets and role of natural productivity in giant gourami ponds using stable isotope analyses. Aquaculture 547:737484. https://doi.org/10.1016/j.aquaculture.2021.737484
51. Bada N, Da Ros Z, Rindi F, Busi S, Azzurro E, Derbal F, Fanelli E (2022) Seasonal trophic ecology of the invasive crab Percnon gibbesi (Brachyura, Plagusiidae) in the southwestern mediterranean: insights from stomach contents and stable isotope analyses. Mar Environ Res 173:105513. https://doi.org/10.1016/j.marenvres.2021.105513
52. Queipo-Abad S, Pedrero Z, Marchán-Moreno C, El Hanafi K, Bérail S, Corns WT, Cherel Y, Bustamante P, Amouroux D (2022) New insights into the biomineralization of mercury selenide nanoparticles through stable isotope analysis in giant petrel tissues. J Hazard Mater 425:127922. https://doi.org/10.1016/j.jhazmat.2021.127922
53. Shan C, Ye J, Scarlett A, Grice K (2019) Molecular and isotopic characteristics of mature condensates from the East China Sea Shelf Basin using GC×GC-TOFMS and GC-IRMS. J Earth Sci 30(2):376–386. https://doi.org/10.1007/s12583-018-1001-3
54. Spangenberg JE, Zufferey V (2019) Carbon isotope compositions of whole wine, wine solid residue, and wine ethanol, determined by EA/IRMS and GC/C/IRMS, can record the vine water status – a comparative reappraisal. Anal Bioanal Chem 411(10):2031–2043. https://doi.org/10.1007/s00216-019-01625-4
55. Vantyghem M, Merckx R, Stevens B, Hood-Nowotny R, Swennen R, Dercon G (2022) The potential of stable carbon isotope ratios and leaf temperature as proxies for drought stress in banana under field conditions. Agric Water Manag 260:107247. https://doi.org/10.1016/j.agwat.2021.107247
56. Xia W, Li C, Nie J, Shao S, Rogers KM, Zhang Y, Li Z, Yuan Y (2022) Stable isotope and photosynthetic response of tea grown under different temperature and light conditions. Food Chem 368:130771. https://doi.org/10.1016/j.foodchem.2021.130771
57. van Leeuwen KA, Prenzler PD, Ryan D, Camin F (2014) Gas chromatography-combustion-isotope ratio mass spectrometry for traceability and authenticity in foods and beverages. Compr Rev Food Sci Food Saf 13(5):814–837. https://doi.org/10.1111/1541-4337.12096
58. Suto M, Kawashima H (2019) Compound specific carbon isotope analysis in sake by LC/IRMS and brewers' alcohol proportion. Sci Rep 9(1):17635. https://doi.org/10.1038/s41598-019-54162-6

59. Wu H, Zhou X, Chen H, Jin B, Yan Z, Xie L, Zhou H, Rogers KM, Lin G (2022) A comparative authentication study of fresh fruit and vegetable juices using whole juice and sugar-specific stable isotopes. Food Chem 373:131535. https://doi.org/10.1016/j.foodchem.2021.131535
60. Dong H, Xiao K, Xian Y, Wu Y (2018) Authenticity determination of honeys with non-extractable proteins by means of elemental analyzer (EA) and liquid chromatography (LC) coupled to isotope ratio mass spectroscopy (IRMS). Food Chem 240:717–724. https://doi.org/10.1016/j.foodchem.2017.08.008
61. Akamatsu F, Shimizu H, Hayashi S, Kamada A, Igi Y, Koyama K, Yamada O, Goto-Yamamoto N (2022) Chemometric approaches for determining the geographical origin of Japanese Chardonnay wines using oxygen stable isotope and multi-element analyses. Food Chem 371: 131113. https://doi.org/10.1016/j.foodchem.2021.131113
62. Suto M, Kawashima H (2020) Carbon isotope ratio of organic acids in sake and wine by solid-phase extraction combined with LC/IRMS. Anal Bioanal Chem. https://doi.org/10.1007/s00216-020-03003-x
63. Bindereif SG, Brauer F, Schubert JM, Schwarzinger S, Gebauer G (2019) Complementary use of 1H NMR and multi-element IRMS in association with chemometrics enables effective origin analysis of cocoa beans (Theobroma cacao L.). Food Chem 299:125105. https://doi.org/10.1016/j.foodchem.2019.125105
64. Chung IM, Kim YJ, Moon HS, Han JG, Kong WS, Yarnes CT, Kim SH (2022) Improved accuracy of geographical origin identification of shiitake grown in sawdust medium: a compound-specific isotope model-based pilot study. Food Chem 369:130955. https://doi.org/10.1016/j.foodchem.2021.130955
65. Liang K, Zhu H, Zhao S, Liu H, Zhao Y (2022) Determining the geographical origin of flaxseed based on stable isotopes, fatty acids and antioxidant capacity. J Sci Food Agric 102(2):673–679. https://doi.org/10.1002/jsfa.11396
66. Xu JZ, Liu X, Wu B, Cao YZ (2020) A comprehensive analysis of 13C isotope ratios data of authentic honey types produced in China using the EA-IRMS and LC-IRMS. J Food Sci Technol 57(4):1216–1232. https://doi.org/10.1007/s13197-019-04153-2
67. Liu H, Zeng Y, Zhao X, Tong H (2020) Chemometric authentication of pu'er teas in terms of multielement stable isotope ratios analysis by EA-IRMS and ICP-MS. J Food Drug Anal 28(2): 248–260. https://doi.org/10.38212/2224-6614.1059
68. Xu Y, Peng K, Jiang F, Cui Y, Han D, Liu H, Hong H, Tian X (2022) Geographical discrimination of swimming crabs (Portunus trituberculatus) using stable isotope and multi-element analyses. J Food Compos Anal 105:104251. https://doi.org/10.1016/j.jfca.2021.104251
69. Martino JC, Mazumder D, Gadd P, Doubleday ZA (2022) Tracking the provenance of octopus using isotopic and multi-elemental analysis. Food Chem 371:131133. https://doi.org/10.1016/j.foodchem.2021.131133
70. Mihailova A, Kelly S (2012) Organic food authenticity – recent advances in isotope ratio mass spectrometry. Food Sci Technol 26(1):35–37
71. Trapp T, Inácio CDT, Ciotta MN, Hindersmann J, Lima AP, dos Santos TS, Ferreira GW, Morais GP, de Conti L, Comin JJ, Loss A, Giacomini SJ, Lourenzi CR, Rozane DE, Brunetto G (2022) Natural abundance analysis of the role played by 15N as indicator for the certification of organic-system deriving food. J Sci Food Agric 102(1):330–340. https://doi.org/10.1002/jsfa.11362
72. O'Sullivan R, Schmidt O, Monahan FJ (2022) Stable isotope ratio analysis for the authentication of milk and dairy ingredients: A review. Int Dairy J 126:105268. https://doi.org/10.1016/j.idairyj.2021.105268
73. Saleh MAD, Dos Santos LS, Berto DA, Amorim AB, Costa VE, Ducatti C (2018) Timing carbon turnover (δ13C) in weaned piglet's brain by IRMS. An Acad Bras Cienc 90(2): 2469–2478. https://doi.org/10.1590/0001-3765201820170861
74. Anton Dib Saleh M, Sousa dos Santos L, Antonio Berto D, Borges Amorim A, Lívio Panhoza Tse M, Eliodoro Costa V (2019) IRMS as a tool to obtain the carbon turnover (δ 13 C) in organs

of weaned piglets fed glutamic acid and nucleotides. J Anim Physiol Anim Nutr 103(3): 906–914. https://doi.org/10.1111/jpn.13089

75. da Silva DAF, Biscola NP, dos Santos LD, Sartori MMP, Denadai JC, da Silva ET, Ducatti C, Bicudo SD, Barraviera B, Ferreira Jr RS (2016) Detecting animal by-product intake using stable isotope ratio mass spectrometry (IRMS). Vet J 217:119–125. https://doi.org/10.1016/j.tvjl.2016.10.002

Liquid and Gas Chromatography–Mass Spectrometry Methods in Food and Environmental Safety

Yelena Sapozhnikova

Contents

Abstract This chapter describes recent advances in applications of gas chromatography (GC) and liquid chromatography (LC) coupled with mass spectrometry (MS) for analysis of contaminants in the field of environmental and food safety during the last decade (2011–2021). Most employed MS analyzers with unit-resolution, different ionization modes, and improvements in liquid and gas chromatography techniques are discussed. Regulatory compliance for GC-MS/(MS) and LC-MS/(MS) identification as outlined by regulatory agencies is presented. Examples of innovative uses of state-of-the-art methods for analysis of diverse contaminants in the last decade are provided, and an opinion on future trends in the field is offered.

Y. Sapozhnikova (✉)
United States Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA, USA
e-mail: Yelena.Sapozhnikova@usda.gov

Yolanda Picó and Julian Campo (eds.), *Mass Spectrometry in Food and Environmental Chemistry*, Hdb Env Chem (2023) 119: 127–148, DOI 10.1007/698_2022_883,

Published online: 7 October 2022

Keywords Environmental analysis, Food safety, Gas chromatography, Liquid chromatography, Mass spectrometry

1 Introduction

In environmental and food safety, contaminants analyzed by gas chromatography–mass spectrometry (GC-MS/(MS)) methods are non-polar, semi-polar, volatile, and semi-volatile compounds. These include pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and other flame retardants, persistent organic pollutants (POPs), i.e. chemicals identified by the Stockholm convention as persistent, bioaccumulative, and toxic, and emerging contaminants, such as chlorinated paraffins, organophosphate flame retardants, plasticizers, and many others. Contaminants analyzed by liquid chromatography–mass spectrometry (LC-MS/(MS)) methods are semi-polar to polar and non-volatile. Examples are some pesticides, pharmaceuticals, personal care products, natural toxins such as mycotoxins, veterinary drugs, polar flame retardants, and many others.

Since pesticides are the most studied contaminants in food and environmental safety, a quick look into the past publication records provides a glance into when mass spectrometry coupled with LC and GC started to be routinely used in analytical laboratories. A search in Web of Science for "LC-MS pesticides" returned 1 paper published in 1989, describing confirmation of pesticides by LC-MS, then 2 hits for 1991 on applications of LC-MS for pesticide analysis. It was not until 2010 that 102 papers reporting LC-MS for pesticide analysis were published. A similar search for "GC-MS pesticides" in the Web of Science engine returned 1 paper published in 1990 on an analysis of 50 pesticides in water by GC-MS, growing to 100 papers per year published in 2003. At that early time of GC- and LC-MS analysis, most MS detectors were single quadrupole or ion traps. Since the 2010s, triple quadrupoles (QqQ) became more common tools in analytical laboratories. Additionally, different ionization techniques associated with both GC- and LC-MS instrumentation were developed to cover a wide range of polarity for analytes of interest (Fig. 1).

This chapter is covering advantageous developments in applications of GC-MS/(MS) and LC-MS/(MS) methods in analysis of small molecule organic contaminants (under 1,000 Da) in environmental and food safety with a focus on the last decade (2011–2021), with the emphasis on unit-resolution MS instrumental applications, with high-resolution MS applications described in a separate chapter.

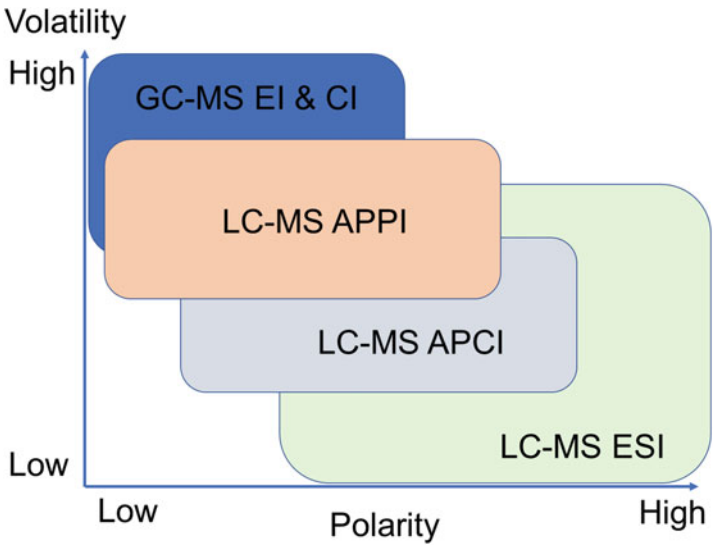


Fig. 1 Instrumental analysis and ionization modes based on analytes' volatility and polarity

2 Recent Advances in Gas Chromatography–Mass Spectrometry Analysis

2.1 GC-MS Analyzers

GC-MS/(MS) analyzers utilized for analysis of residual levels of contaminants in environmental and food safety encompass single and triple quadrupoles, ion trap, Orbital ion trap (Orbitrap), time of flight (TOF), and hybrid systems: quadrupole (Q)–ion trap (Q-trap), Q-TOF, Q-Orbitrap. In the last 10 years (2011–2021), GC coupled with triple quadrupole (QqQ) analyzers was most often applied for targeted analysis of contaminants and became a gold standard for environmental and food safety testing. Indeed, a Web of Science search for "pesticide analysis GC-MS" with different MS analyzers for 2011–2021 showed 59% of the published articles used GC-MS/MS with QqQ analysis, followed by 15% using GC-MS with single quadrupole MS. The remaining 12% and 15% of the published articles used TOF and ion trap MS, respectively. Starting from the 2010s, many laboratories transitioned from GC-MS in single ion monitoring (SIM) mode to GC-MS/MS analysis operated in multiple/selected reaction monitoring (MRM/SRM) mode, which provided greater sensitivity, selectivity, and specificity compared to GC-MS with single quadrupole analyzers.

Main vendors of GC-MS/(MS) instruments are Agilent Technologies, Thermo Fisher Scientific, LECO, and Shimadzu. The biggest advances in GC-MS instruments in the last decade are improvements in MS analyzers' sensitivity and scan speed (Da/s). The latest GC-MS/MS QqQ instruments provide instrument detection

limit (IDL) sensitivity for octafluoronaphthalene (OFN) < 0.4 fg, MRM speed of up to 800 MRM transition/s, minimum MRM dwell time of 0.5 s, and scan speed ≤20,000 Da/s. In addition, to reduce down time needed to vent the instrument for ion source cleaning and/or GC column change, Agilent 7010D QqQ offered an automated, self-cleaning ion source, and Thermo Fisher Scientific TSQ 9000 enabled changing ion source and GC column without venting the instrument.

Recent improvements in detectors' scan speed and acquisition rates translate to more acquired MRMs per unit time, resulting in a greater number of contaminants analyzed in a single run, thus increasing the scope of the method. For example, a multi-residue method was developed and validated for 192 pesticides in animal feed by GC-MS/MS [1] with 2 MRM transition for each analyte in a 22 min GC run time. A total of 32 contaminants including PCBs, PBDEs, PAHs, and organochlorine pesticides were analyzed by GC-MS/MS in mussels and clams with 3 MRM transitions/analytes in a 45 min GC run [2]. A multi-class method for pesticides, PCBs, PAHs, PBDEs, and other flame retardants in meats, poultry [3], and catfish [4] covered 232 analytes with 3 MRMs/analytes in a 10 min fast low pressure GC run. In a recent study, 4 MRM transitions/analytes were applied for analysis of 400 pesticides in food samples in a 60 min GC run [5]. These examples demonstrate how modern GC-MS analyzers provide wide scope of analysis by covering hundreds of contaminants and multiple classes of analytes in a single GC-MS/(MS) run, thus increasing throughput and laboratory efficiency.

Taking advantage of improvements in MS detector's speed, 3 MRMs instead of 2 MRMs/analytes are acquired to improve selectivity and provide more confidence in the identification based on additional MRM transitions and their ion ratios (see Sect. 4), thus increasing identification reliability and minimizing false negative findings in complex samples with matrix interferences.

Additional advantage in acquisition of multiple MRMs is software improvements for GC-MS/MS with dynamic/scheduled MRM algorithms, allowing the user to specify retention time and time window for acquiring selected MRMs compared to laborious division of MRMs into time segments.

Modern MS analyzers provide great sensitivity, and each next version is at least 2–3 times more sensitive than the previous version. Agilent 7000 MS/MS QqQ introduced in 2014, for example, has IDL ≤4 fg while 7010B version, introduced in 2016, has IDL ≤0.5 fg for 2 fg OFN injected, thus offering an eight-fold sensitivity increase in just 2 years. Increased sensitivity provides greater signal to noise (S/N) ratio and lower limits of detection (LODs), allowing to measure contaminants at lower levels. This is especially valued in the environmental field where a general rule of thumb is to measure the lowest quantities possible. In the food safety arena, most chemical contaminants are regulated and have established tolerances or maximum residue limits (MRL). For example, for most pesticides, MRLs are 10 ng/g, thus the lowest amounts needed to be quantified are ½ MRL, or 5 ng/g, and the lowest calibration curve point is usually at 0.5–1 ng/g. In this case, increase in MS sensitivity permits injection of more diluted final extracts without resulting in increased LODs, decreased matrix effects, and improved robustness. This advantage can result in fewer sample preparation steps, sample dilution instead of

concentration/evaporation, smaller sample equivalent injected on column, and consequently, less instrument maintenance.

2.2 GC-MS/(MS) Ionization Modes

Electron ionization (EI) is most commonly used with GC-MS analyzers. The advantage of EI is its universal applicability. Mass spectra generated in EI mode with 70 eV are highly reproducible with any GC-MS/(MS) instrument and are used for identification and confirmation with existing commercial EI spectral libraries. One of the most utilized spectral databases is the NIST/EPA/NIH EI-MS library containing 306,643 compounds (2020 release), including pesticides, industrial chemicals, petrochemicals, surfactants, drugs and metabolites, toxins, etc. The main disadvantage of EI is in its harsh ionization causing extensive fragmentation, and in most cases lack of a diagnostic molecular ion. Softer ionization techniques, such as chemical ionization (CI) in positive (PCI) or negative (NCI) mode, atmospheric pressure chemical ionization (APCI), and cold EI overcome this challenge and provide highly diagnostic molecular ion.

GC-MS with NCI in selected ion monitoring (SIM) mode using methane as a chemical reagent was used for determination of organochlorine pesticides and PBDE congeners in air particulate matter [6]. In another study, a multi-residue method for 51 pesticides in green coffee beans was developed with GC-MS-NCI [7], and high MS selectivity was achieved, while matrix effects were high for this difficult food matrix. Gonzalez-Gago et al. compared sensitivity of GC-MS-EI with GC-MS-NCI for PBDE congeners and concluded that GC-MS-NCI had lower LODs, especially for higher brominated congeners [8]. Ayala-Cabrera et al. compared GC-MS with positive (PCI) and negative chemical ionization (NCI) modes and EI mode for the determination of fluorotelomer olefins, fluorotelomer alcohols, perfluoroalkyl sulfonamides, and sulfonamido-ethanols in water [9]. For most of the analytes, PCI worked the best generating protonated molecules and low fragmentation compared to high fragmentation observed in EI and NCI modes.

Applications of APCI in GC-MS(/MS) published by 2020 are summarized in three recent reviews [10–12]. These reviews highlighted APCI as an advantageous soft ionization technique for generating spectral data with protonated molecular ions for improved identification and low LODs. Li et al. discussed advantages and drawbacks of GC-MS-APCI [12], while Fang et al. [10] reviewed its applications for the analysis of persistent organic pollutants (POPs) and Niu et al. [11] covered applications in non-targeted analysis and targeted analysis for pesticides, flame retardants, PAHs, PCBs, dioxins and furans, sterols, esters, pharmaceuticals, and cannabinoids. Cherta et al. first studied application of GC-MS/MS-APCI for 25 pesticides selected based on high fragmentation and low/no molecular ion in EI mode [13]. Under APCI conditions with water as a modifier, abundant protonated ions $[M + H]^+$ were observed for most of the selected pesticides and used as precursors for MS/MS, thus resulting in increased selectivity and sensitivity. Among pesticides,

pyrethroids are especially difficult to analyze with EI due to the lack of molecular ion and extensive fragmentation. GC-MS/MS-APCI was shown to improve analysis of pyrethroids [14] based on the formation of highly abundant protonated molecular ions with low LODs. One disadvantage of APCI is a strong interference of matrix components leading to matrix-induced suppression or enhancement. In the study of pyrethroids in fruits and vegetables, average signal reduction was 55% [14]. GC-QTOF-APCI was used in a non-targeted study to investigate chemicals migrating from food packaging in combination with GC-TOF-EI to increase confidence of identification by using two complementary ionization modes [15]. Other soft ionization techniques with high potential for future applications in GC-MS/(MS) analysis are atmospheric pressure photo-ionization (APPI) [16], dielectric barrier discharge ionization (DBDI) [17], and supersonic molecular beam (SMB) also known as cold EI [18].

2.3 Improvements in Gas Chromatography

Modern analytical laboratories demand fast sample turnaround time and higher throughput to analyze as many samples as fast as possible. Typical GC run with the most often used analytical column: (5%-phenyl)-methyl-polysiloxane, 30 m × 0.25 mm internal diameter (i.d.) × 0.25 μm film thickness can take 30–60 min depending on the selected analytes and GC conditions. Increasing the speed of GC separation while reducing total run time is important to achieve greater productivity.

Zocalli et al. reviewed GC-MS techniques from the last decade [19] and Pico et al. reviewed recent innovations in GC-MS for pesticide analysis [20], and both reviews presented several fast GC approaches and their applications. One way to reduce GC run time is to alter GC column dimensions, i.e. reduce length, internal diameter (i.d.), and film thickness. To discuss reduction of i.d. here, classification of GC capillary columns was based on the following parameters [21] – microbore: i.d. 0.1–0.2 mm, narrow bore: i.d. 0.2–0.3 mm, wide bore i.d. 0.3–0.5 mm, and megabore: i.d. ≥0.5 mm. Use of microbore column (20 m × 0.18 mm i.d. × 0.18 μm) and pulsed pressure injection was reported for analysis of 356 pesticides by GC-MS/MS [22]. Analysis time was 18 min, plus significantly higher and narrower peaks were observed, resulting in greater signal to noise (S/N) and lower sensitivity. In another study with GC × GC-MS, 10 m × 0.1 mm × 0.1 μm column was used for cryogenic modulation [23] to evaluate rapid two-dimensional comprehensive GC × GC analysis with single quadrupole MS for cosmetic allergens. Low polarity 8.9 m × 0.1 mm × 0.1 μm and medium polarity 1.1 m × 0.1 mm × 0.1 μm GC columns were used in first and second dimension, respectively, with analysis time of 11.4 min. The well-known drawbacks of microbore columns are reduced sample capacity and method robustness.

In contrast to microbore column, narrow bore GC columns (i.d. 0.2–0.3 mm) are widely used in environmental and food safety applications. GC runtime of 12 min

was reported for the analysis of PBDEs and their methoxylated metabolites by Cruz et al. [24] with 10 m × 0.25 mm × 0.1 μm GC column and triple quadrupole MS/MS using EI and NCI modes. In another study GC separation <10 min for pesticides and PCBs was achieved with 15 m × 0.25 mm × 0.25 μm by GC-MS/MS-APCI [25]. Several factors were optimized to reduce GC analysis run time: short GC column (15 m), high starting oven temperature (120°C), fast rate of temperature programming (30°C/min), and high flow rate (6 mL/min).

High flow rate in addition to increased velocity of carrier gas is the main characteristic of low pressure (LP) vacuum outlet GC-MS/(MS) technique [26]. LPGC-MS/(MS) uses short (5–15 m) megabore (i.d. > 0.5 mm) analytical column connected to a restrictor at the inlet and vacuum at the MS outlet. By extending vacuum conditions through LPGC column, carrier gas (helium) linear velocity becomes 10 times greater compared to under atmospheric pressure, thus speeding up GC separation. In addition, higher flow rates, rapid heating, thicker film thickness provide faster separation, more sensitivity, high sample capacity, greater ruggedness, and less degradation of thermally labile compounds [26, 27]. Khan et al. compared LPGC-MS/MS and conventional GC-MS/MS for analysis of 259 pesticides in tobacco [28]. A fast separation of 14 min was achieved for LPGC-MS/MS analysis with 15 m × 0.53 mm × 1 μm column and flow rate of 4 mL/min compared to 42 min using conventional GC-MS/MS with 30 m × 0.25 m × 0.25 μm column. Additionally, low sensitivity with limit of quantitation (LOQs) < 2 ng/g was demonstrated for all pesticides. Recent studies using LPGC-MS/MS with 15 m × 0.53 mm × 1 μm LPGC column connected to 5 m × 0.18 mm guard column and carrier gas at 2 mL/min for analysis of >200 pesticides, PCBs, PBDEs, and other flame retardants had a total run time of 10 min with LOQ ≤ 5 ng/g, and low matrix effects in complex samples of meat, poultry, and fish [3, 4]. Figure 2 illustrates fast separation of PBDE and PCB congeners relevant to environmental and food safety using LPGC-MS/MS in 7 min. On the other hand, disadvantages of LPGC-MS/(MS) are decreased separation efficiency, greater potential for leaks, and typically only MS-based detection since vacuum conditions are required.

Among other recent developments in GC-MS worth mentioning is column backflushing GC-MS/MS method [29] to eliminate unwanted matrix components from GC column by reversing the column flow with a pressure-controlled tee device. Fast analysis of pesticides in dietary supplements with column backflushing resulted in increased sample throughput (50%), decreased instrument maintenance, and greater ruggedness [29]. In another study, column backflushing was used for analysis of nitrosamines in bacon [30] with reporting limits of 0.1 ng/g, and backflushing was demonstrated to be rugged for long-term use with minimal maintenance.

Another advantageous technique for analysis of challenging complex samples is multi-dimensional GC, which offers improved resolution for analytes with greater separating power, higher peak capacity, improved identification, and lower detection limits compared to one-dimensional GC separation. A recent review outlined the use of comprehensive two-dimensional (2D) GC in environmental analysis in targeted and non-targeted applications [31]. In non-targeted screening of surface water by GC × GC-TOFMS, over 3,000 chemicals were detected, including pharmaceuticals

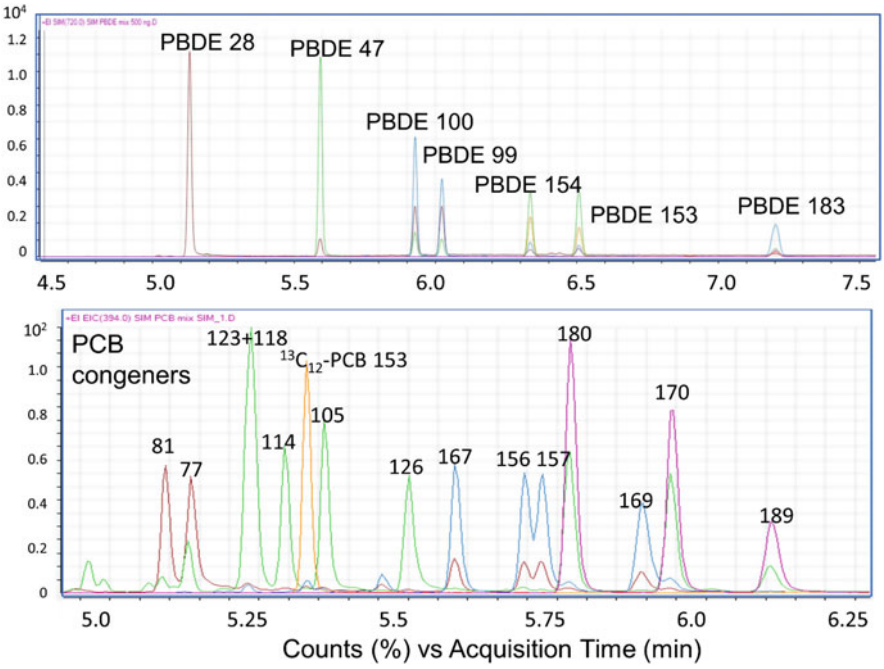


Fig. 2 Example of fast separation of PBDE and PCB congeners using low pressure vacuum outlet GC-MS/MS

and personal care products (PPCPs), sunscreens, pesticides, hormones, fragrances, and emerging endocrine disrupting chemicals [32]. In another study, 327 persistent and bioaccumulative compounds were identified in blubber bottlenose dolphins by GC × GC-TOFMS non-targeted analysis, with 280 identified chemicals not typically monitored in environmental surveys [33]. In food safety applications, non-targeted analysis with GC × GC-TOFMS was used to screen for chemicals migrating from food packaging with 91 chemicals identified [34].

While most GC × GC-MS/(MS) studies to date used EI to utilize common MS databases, some reports have emerged with alternative ionization modes. Pulsed flow modulation GC × GC-MS with cold EI (supersonic molecular beam) was reported for pesticide analysis in agricultural products [35]. The identification with this technique improved NIST library identification probabilities. Another study explored milder EI conditions, e.g. 20 eV vs. 70 eV for GC × GC – quadrupole MS for analysis of pesticides, sterols, linear alkanes, etc. [36]. Lower energy resulted in increase of the relative abundance of higher-mass diagnostic ions and fragments. GC × GC coupled with TOF with APCI was evaluated for analysis of flame retardants and plasticizers with direct probe [37].

GC × GC-TOFMS is a powerful tool for targeted and especially non-targeted analysis in complex and difficult matrices which can tolerate little/no sample preparation, however tentative identification of thousands of compounds can be a long

and overwhelming process, and software improvements are expected in the future to streamline and improve data analysis.

3 Liquid Chromatography–Mass Spectrometry

3.1 Analyzers

Just as for GC-MS/(MS) analysis, the most common LC-MS/(MS) analyzers are single and triple quadrupoles, ion trap, Orbitrap, TOF, and hybrid instruments combined with a quadrupole: QTRAP, Q-TOF, Q-Orbitrap. Based on Web of Science search for papers on "pesticide analysis LC-MS" with different MS analyzers in the last decade, 57% used QqQ, followed by 19% TOF, 16% ion trap, and 8% single quadrupole. Major vendors of LC-MS/(MS) instruments are Agilent Technologies, Shimadzu, Sciex, Thermo Fisher Scientific, and Waters.

LC-MS/MS with QqQ instruments have become "workhorses" of modern laboratories for analysis of LC-amenable contaminants. Common characteristic of modern LC-MS/MS QqQ (based on Agilent QqQ 6495C) are: MRM speed is 500 MRM transitions/second, minimum MRM dwell time is 0.5 s, polarity switch <25 ms, collision cell clearance time <0.5 s, scan speed ~17,000 Da/s, and IDL sensitivity is <0.6 fg based on reserpine on column. Other advances in the last years, besides improved sensitivity and speed, are improvements in ion source design and diversity of ion source ionization modes.

3.2 LC-MS/(MS) Ionization Sources

The most frequently used ionization sources in LC-MS(MS) analysis are electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photo-ionization (APPI). ESI dominates in the published LC-MS/(MS) methods for LC-amenable contaminants due to high selectivity, sensitivity, and efficient ionization for a wide range of diverse analytes. ESI can be operated in positive (+) and negative (−) modes, and ESI (+) is generally preferred mode based on most compounds ionization efficiency producing $[M + H]^+$ or other adduct ions, resulting in wider scope of analysis. ESI (−), on the other hand, creates deprotonated ions $[M\text{-}H]^-$ and is characterized by lower background noise [38]. Liigand et al. [39] challenged the assumption that most compounds are better suited for ESI (+) by comparing ionization efficiency of 33 compounds ionized in both ESI(+) and ESI (−) modes. Their findings showed that ESI (−) provided better sensitivity for almost half of the selected analytes. To take advantage of both ESI polarities in one LC run, polarity switching is employed. A recent study demonstrated simultaneous analysis of 52 multi-class illegal dyes in food [40] with ESI polarity switching in one LC-MS/MS run of 12 min for acidic, neutral, and basic

analytes. Another interesting investigation of this study was a 100-fold dilution of the extracts then taking advantage of the instrument sensitivity while eliminating matrix effects. In another study, ESI polarity switching was applied to acquire 27 and 710 scheduled MRM transitions in ESI(−) and ESI (+), respectively, in 10 min LC run to determine pesticides, veterinary drugs, and their metabolites in catfish [41].

Four ionization sources: ESI, heated ESI (HESI), APPI with and without dopant, and multi-mode source with ESI and APCI were compared for analysis of 40 pesticides in tomato and garlic [42]. The lowest LODs were achieved with ESI and HESI, and ESI was significantly less affected by matrix effects compared to HESI.

Softer, but currently less utilized ionization sources are APCI and APPI. The applicability of three ionization sources: ESI, APCI, and APPI was tested for analysis of five pharmaceuticals in wastewater samples [43], and ESI provided the best ionization efficiency, lower LODs, greater S/N, and lower matrix effects. Tokumura et al. compared analysis of 14 organophosphorus flame retardants by LC-MS/MS with APCI and ESI, as well as by GC-EI-MS and GC-NCI-MS [44]. LC-MS/MS with APCI afforded the lowest LOQs for 12 analytes. In contrast, Silva et al. compared efficiency of ESI and APCI ionization sources for analysis of 22 pesticides in food and found two- to four-fold lower LOQs for ESI vs. APCI, and greater matrix effects in APCI [45]. These examples highlight the differences between ionization efficiency of various chemicals, depending on their properties and the impact of matrix effects for different ionization mechanisms.

Ramirez et al. reported the determination of 28 parent and 15 alkylated PAHs in environmental waters by LC-MS/MS-APPI with chlorobenzene as a dopant on a polymeric C18 column [46]. Brecht et al. reported the development of a fast-switching dual source operating ESI and APCI simultaneously or in switched mode [47] with potential for routine use in LC-MS/(MS) analysis in the future. Similarly, Galani et al. evaluated a new ionization interface, UniSpray, in comparison with ESI [48] for 81 pesticides in food and water samples. The UniSpray was shown to achieve better sensitivity and improved S/N, but overall, LODs were similar to ESI, while signal suppression from matrix effects was lower with UniSpray compared to ESI.

3.3 Improvements in LC Analysis

Since ultra-high performance (UHP) LC instruments were introduced in 2004, they became routine LC instruments in analytical laboratories. UHPLC instruments operate at high pressure (up to 1,500 bars) with small column particle size (typically <2–5 μm) and high flow rates (up to 5 mL/min), which allows short separation time (typically 10–15 min) with high efficiency and resolution [49]. Among LC developments in the last decade, the introduction of sub-2 μm particles, novel monolithic columns, superficially-porous (core-shell) particles, and elevated temperatures are some of the most significant advances to meet demands for efficient separation in multi-class, multi-residue analytical methods.

Hundreds of UHPLC-MS(MS) applications have been reported for pharmaceuticals, personal care products, pesticides, drugs, flame retardants, and many other emerging contaminants. Kachhawaha et al. reviewed recently developed LC-MS/(MS) methods for detection and quantitation of PPCPs in environmental waters [50]. UHPLC-MS/MS with ESI was the most extensively used analysis for PPCPs at trace levels (ng/L). UHPLC-MS/MS methods for brominated flame retardants in food [51] and for organophosphorus flame retardants/plasticizers in mussels [52] were reported, achieving low detection limits at sub ng/g levels. Quantitative LC-MS/MS method was reported for 295 bacterial and fungal metabolites, including mycotoxins, in food using "dilute and shoot" approach [53]. The method LOQs for all analytes were below the established regulatory tolerance levels of these contaminants.

To speed up LC analysis, flow injection (FI) coupled to MS was tested in various FI-MS/(MS) applications. FI-MS(MS) eliminates LC separation taking advantage of modern MS detectors used as a "separation" tool. Nanita and co-workers pioneered FI-MS/MS high-throughput analysis of pesticide residues in foods, biological matrices, and water [54], and Mol and van Dam developed an FI-MS/MS method for polar pesticides not amenable to multi-residue methods [55] with run time of 30–60 s. In a recent study, FI-MS/MS was evaluated for simultaneous analysis of selected pesticides and mycotoxins in food and feed samples in 2 min [56]. Despite high complexity of the samples, the method achieved LODs below the established regulatory values. The main advantages of FI coupled with MS are speed, simplicity, high throughout, and low cost, however, it can suffer from high ion suppression caused by matrix interferences in absence of chromatography. FI-MS/MS was utilized for the analysis of organophosphate esters (OPEs), used as flame retardants and plasticizers to avoid background contamination from common LC solvents [57]. Garcia-Ac et al. compared FI-MS/MS with ESI, APCI, and APPI [43] for analysis of five pharmaceuticals in wastewater and found that ESI performed the best among the three in terms of S/N ratio and peak areas.

With increasing complexity of samples and greater demand to analyze more chemicals, comprehensive two-dimensional LC (LC × LC) is becoming a more popular and attractive approach to increase separation power with enhanced peak capacities. Selectivity can be dramatically increased when different retention mechanisms are employed for two dimensions. These reviews outlined recent developments and successful applications in LC × LC [58, 59], however, no practical applications can be found for environmental or food safety applications, but applications in polymers, peptides, natural medicine, metabolomics [58], and food composition are described [60].

Another interesting approach to meet the challenge for complex samples is LC column backflushing. This approach helps to avoid ghost peaks, reduce matrix effects in high-throughput methods, and reduce instrumental maintenance. Michlig et al. [61] applied LC column backflushing for 3 min followed by 3 min re-equilibration between every injection in the analysis of pesticides in complex samples of hemp and hemp products.

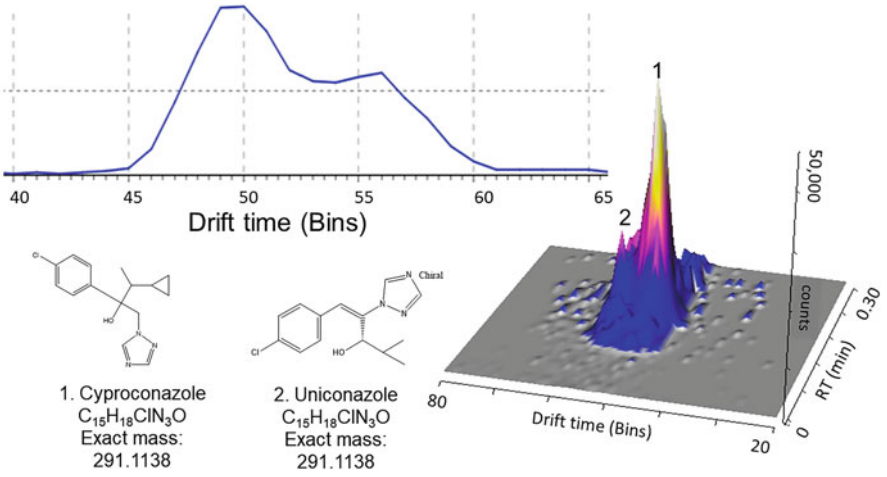


Fig. 3 Example of partial separation of structurally isomeric pesticides – cyproconazole and uniconazole with flow injection ion mobility TOF-MS in parent trap MS mode for $[M + H]^+$ ion 292.112

Additionally, ion mobility (IM) spectrometry should be mentioned as an additional dimension to LC-MS, as its hyphenation to LC-MS is drawing more interest and attention recently [62]. Applications of IM for environmental analysis of perfluorocarbons, PAHs, pesticides, terpenes, chlorophenols, etc. in air, water, and soil were reviewed [63]. UHPLC-(Q)TOFMS with traveling-wave ion mobility spectrometry was evaluated for >200 pesticides in fish feed [64] and for screening of multi-class pesticides in fruits and vegetables [65]. In another study, FI-IM-TOFMS was evaluated for separation of structurally isomeric pesticides [56]. Figure 3 shows an example of partial separation of two pesticides used in agriculture, cyproconazole and uniconazole using FI-IM-TOF [56]. Both pesticides have the same formula $C_{15}H_{18}ClN_3O$ and exact mass 291.1138, which challenges their differentiation. However, their identification and accurate measurement are extremely important in food trade as two pesticides have different regulatory values in different countries. Thus, cyproconazole has MRL = 0.05–0.2 mg/kg in the EU, and MRL = 0.05 mg/kg for corn and wheat grain in the USA, while uniconazole has MRL = 0.01 mg/kg in the EU, and no regulatory value in the USA. False positives and other incorrect results in the analysis of these or other structurally isomeric pesticides can lead to detrimental economic and health consequences, and advanced techniques such as ion mobility in this case can increase confidence of identification and reduce false findings.

4 Regulatory Compliance for Identification with GC-MS/(MS) and LC-MS/(MS)

In regulatory testing, compliance with the identification criteria established by governing agencies is required [66, 67]. Table 1 summarizes identification criteria for liquid and gas chromatography with various MS analyzers to meet regulatory compliance as established by some regulatory agencies worldwide in environmental and food safety. For retention time (RT) compliance, most criteria require RT within 6–12 s of RT in midpoint calibration standard in the same sequence, although the International Organization for Standardization (ISO) criteria is different, and RT tolerance highly depends on RT range [68]. Ions selected for MS should be characteristic or structurally significant, and $\geq$3 ions should be selected for MS, and $\geq$2 product ions with S/N $\geq$3 should be selected for MS/MS, and analyte peaks from all product ions in the extracted ion chromatogram must overlap. For ion ratios, most regulators use relative tolerance of $\pm$30%, while others use absolute tolerance, and ISO uses a formula: [$<\pm(0.1 \times I_{std} + 10)\%$] to calculate relative tolerance, where I_{std} is a relative ion intensity for calibration standard (Table 1).

Recently, Angeles and Aga pointed out that not all official methods require ion ratio tolerance [69]. For example, the EPA methods 542 and 1694 for PPCPs and method 537.1 for selected per-and polyfluorinated alkyl substances by LC-MS/MS do not have ion ratio requirements, but methods 8270E and 8260B for semi-volatile and volatile compounds by GC-MS/MS do have ion ratio tolerance. The authors attributed this to the lack of the established guidelines, which are still developing and evolving.

In summary, regulatory compliance parameters for identification of analytes by GC- and LC-MS/(MS) are presented in Table 1. In any testing, regulatory or not, the use of these criteria should be implemented to increase the confidence of identification.

5 Orthogonal Applications of GC-MS and LC-MS

In an interesting study, Schürmann et al. reported a false positive finding of pesticide sebuthylazine in tarragon sample [76] with LC-MS/MS analysis based on retention time, 2 MRM transitions and their ratios. A co-eluting matrix interference resulted in a false positive by producing product ions corresponding to 2 MRM transitions of the compound. However, a 3rd LC-MS/MS MRM transition and a separate analysis by orthogonal GC-MS/MS revealed the false positive findings. Many chemicals are both GC- and LC-amenable (Fig. 1) and can be routinely analyzed by both instrumental techniques. Thus, GC-MS and LC-MS can be not only complementary, but also confirmatory for these compounds. For example, pesticides and veterinary drugs in animal feed were analyzed by GC-MS/MS (192 analytes) and LC-MS/MS (187 analytes) with >50 overlapping analytes analyzed by both techniques

Table 1 Regulatory compliance with retention times and ion ratios for identification by GC-MS and LC-MS (single quadrupole, ion trap, TOF) and GC- and LC-MS/MS (MS/MS triple quadrupole, ion trap, Q-Trap, Q-TOF) with unit mass resolution

		Criteria		
Regulatory source	Guidelines for	Chromatography: retention time (RT)	Mass spectrometry: # of required ions	Ion ratio tolerance, %
US EPA methods	8270E: semi-volatile organic compounds (PAHs, PCBs, pesticides) [70] 8260B: volatile organic compounds [71]	*GC:* RT within ±10 s of RT in midpoint calibration standard from the same sequence; or within ±10 s relative to the shift of the associated internal standard (IS) (delta RT of the IS ±10 s)	≥2 ions	MS/MS: ±30% (relative) of expected ion ratio in reference spectrum
EU SANTE 12682/2019 (2020) [72]	Pesticides in food and feed	*GC & LC:* RT ± 12 s	MS: 3 ions MS/MS: ≥2 product ions with S/N ≥ 3	±30% (relative) of average of calibration standards from same sequence
US FDA Office of regulatory affairs (ORA) [73]	ORA-LAB.5.4.5. (2020): methods, method verification and validation	Not specified	MS: 3 ions MS/MS: ≥2 product ions with S/N ≥ 3	± 30% of average of calibration standards from same sequence
USA: FDA guidance for industry 118 confirmation of identity of animal drug [74] residues (2003)	Animal drug residues	LC: RT ≤ 5% GC: RT ≤ 2%	MS: ≥3 ions	3 ions ±10% (absolute)
				≥ 4 ions: ±15% (absolute)
				2 product ions: ±10% (absolute)
			MS/MS: ≥ 2 product ions	≥3 product ions: ±20% (absolute)
USDA Agricultural Marketing Service Science & Technology Pesticide Data Program (2021) [75]	Pesticides in agricultural commodities	*GC & LC:* RT ± 6 s if an external standard is used	MS: 3 ions MS/MS: ≥2	±30% (relative) when compared to the same relative

		RRT ± 0.1 min if an internal standard is used	product ions with S/N ≥ 3	abundances observed from a standard solution injection made during the same analytical run
The International Organization for Standardization (ISO) 22,892:2006 (confirmed in 2016) [68]	Contaminants in soil	*GC:* RT < 500 s: ±1 s RRT = 500–5,000 s, ±0.2% RT > 5,000 s, ±6 s	MS: 3 ions MS/MS: ≥2 Product ions	<±(0.1 × I_{std} + 10)% (relative)

RT retention time, *RRT* relative retention time, *S/N* signal to noise, I_{std} relative ion intensity for calibration standard

[1]. Similarly, 302 contaminants, including pesticides, PAHs, PCBs, PBDEs were analyzed in catfish, with 128 and 219 by UHPLC-MS/MS and GC-MS/MS, respectively, and 45 overlapping contaminants were analyzed by both. Orthogonal applications of GC-MS and LC-MS provide an additional degree of confidence by employing different chromatographic mechanisms and different ionization modes and are especially important when analyzing complex samples where matrix interferences can result in false negatives or false positives.

6 Future Trends

Evolving challenges to analyze more contaminants faster, more efficiently, with low detection limits and to reduce false positive/false negative rates in complex samples place high demands on future improvements in LC- and GC-MS/(MS) instrumentation and techniques.

Just like in the past, improvements in MS detectors' sensitivity, speed, selectivity, specificity, and wider dynamic linear range are expected. We may see new features aimed to reduce instrumental downtime needed for maintenance, and new and improved ionization sources and interfaces, plus combinations of existing ion sources used simultaneously.

With continually improving modern powerful LC- and GC-/MS(MS) instruments, 1,000 contaminants from different classes with various properties may be covered in one analytical method providing a wide scope of analysis. While triple quadrupole instruments have been "workhorses" in analytical laboratories in the last decade, a transition to high-resolution MS systems will likely occur to avoid shortcomings associated with QqQ instruments in terms of developing ion transitions, their optimization, limited amount of transitions due to the scanning speed of QqQ. Kaufmann et al. conducted direct comprehensive comparison of LC-MS/MS and LC-HRMS [77], and found that selectivity of LC-HRMS at 50,000 FWHM was superior to LC-MS/MS. Analysis with modern HRMS instruments allows virtually unlimited amount of analytes based on the full scan data, plus retrospective analysis. Recent improvements in sensitivity of HRMS instruments put them on par with modern MS/MS.

Other trends expected and desired by the analytical community in the future are software improvements that make it easier for analytical chemists to deal with an enormous amount of generated data. Software packages with streamlined and flexible workflows for different types of applications are expected.

Disclaimer Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

References

1. Kumar A, Bhattacharyya A, Shinde R, Dhanshetty M, Elliott CT, Banerjee K (2020) Development and validation of a multiresidue method for pesticides and selected veterinary drugs in animal feed using liquid- and gas chromatography with tandem mass spectrometry. J Chromatogr A 1627
2. Chiesa LM, Nobile M, Malandra R, Pessina D, Panseri S, Labella GF, Arioli F (2018) Food safety traits of mussels and clams: distribution of PCBs, PBDEs, OCPs, PAHs and PFASs in sample from different areas using HRMS-Orbitrap® and modified QuEChERS extraction followed by GC-MS/MS. Food Addit Contam A 35:959–971
3. Sapozhnikova Y (2018) High-throughput analytical method for 265 pesticides and environmental contaminants in meats and poultry by fast low pressure gas chromatography and ultrahigh-performance liquid chromatography tandem mass spectrometry. J Chromatogr A 1572:203–211
4. Han L, Sapozhnikova Y (2020) Semi-automated high-throughput method for residual analysis of 302 pesticides and environmental contaminants in catfish by fast low-pressure GC–MS/MS and UHPLC-MS/MS. Food Chem 319:126592
5. Feng C, Xu Q, Qiu X, Jin YE, Ji J, Lin Y, Le S, Wang G, Lu D (2020) Comprehensive strategy for analysis of pesticide multi-residues in food by GC–MS/MS and UPLC-Q-orbitrap. Food Chem 320:126576
6. Beristain-Montiel E, Villalobos-Pietrini R, Arias-Loaiza GE, Gomez-Arroyo SL, Amador-Munoz O (2016) An innovative ultrasound assisted extraction micro-scale cell combined with gas chromatography/mass spectrometry in negative chemical ionization to determine persistent organic pollutants in air particulate matter. J Chromatogr A 1477:100–107
7. Pizzutti IR, de Kok A, Cardoso CD, Reichert B, de Kroon M, Wind W, Righi LW, da Silva RC (2012) A multi-residue method for pesticides analysis in green coffee beans using gas chromatography-negative chemical ionization mass spectrometry in selective ion monitoring mode. J Chromatogr A 1251:16–26
8. Gonzalez-Gago A, Pröfrock D, Prange A (2015) Comparison of GC–NCI MS, GC–ICP-MS, and GC–EI MS–MS for the determination of PBDEs in water samples according to the water framework directive. Anal Bioanal Chem 407:8009–8018
9. Ayala-Cabrera JF, Moyano E, Santos FJ (2020) Gas chromatography and liquid chromatography coupled to mass spectrometry for the determination of fluorotelomer olefins, fluorotelomer alcohols, perfluoroalkyl sulfonamides and sulfonamido-ethanols in water. J Chromatogr A 1609
10. Fang J, Zhao HZ, Zhang YH, Lu MH, Cai ZW (2020) Atmospheric pressure chemical ionization in gas chromatography-mass spectrometry for the analysis of persistent organic pollutants. Trends Environ Anal 25
11. Niu YM, Liu JF, Yang RH, Zhang J, Shao B (2020) Atmospheric pressure chemical ionization source as an advantageous technique for gas chromatography-tandem mass spectrometry. Trac-Trend Anal Chem 132
12. Li DX, Gan L, Bronja A, Schmitz OJ (2015) Gas chromatography coupled to atmospheric pressure ionization mass spectrometry (GC-API-MS): review. Anal Chim Acta 891:43–61
13. Cherta L, Portoles T, Beltran J, Pitarch E, Mol JGJ, Hernandez F (2013) Application of gas chromatography-(triple quadrupole) mass spectrometry with atmospheric pressure chemical ionization for the determination of multiclass pesticides in fruits and vegetables. J Chromatogr A 1314:224–240
14. Portoles T, Mol JGJ, Sancho JV, Hernandez F (2012) Advantages of atmospheric pressure chemical ionization in gas chromatography tandem mass spectrometry: pyrethroid insecticides as a case study. Anal Chem 84:9802–9810
15. Cherta L, Portoles T, Pitarch E, Beltran J, Lopez FJ, Calatayud C, Company B, Hernandez F (2015) Analytical strategy based on the combination of gas chromatography coupled to time-of-flight and hybrid quadrupole time-of-flight mass analyzers for non-target analysis in food packaging. Food Chem 188:301–308

16. Kauppila TJ, Syage JA, Benter T (2017) Recent developments in atmospheric pressure photoionization-mass spectrometry. Mass Spectrom Rev 36:423–449
17. Mirabelli MF, Wolf JC, Zenobi R (2017) Atmospheric pressure soft ionization for gas chromatography with dielectric barrier discharge ionization-mass spectrometry (GC-DBDI-MS). Analyst 142:1909–1915
18. Amirav A, Keshet U, Danon A (2015) Soft cold EI – approaching molecular ion only with electron ionization. Rapid Commun Mass Spectrom 29:1954–1960
19. Zoccali M, Tranchida PQ, Mondello L (2019) Fast gas chromatography-mass spectrometry: a review of the last decade. Trac-Trend Anal Chem 118:444–452
20. Pico Y, Alfarhan AH, Barcelo D (2020) How recent innovations in gas chromatography-mass spectrometry have improved pesticide residue determination: an alternative technique to be in your radar. Trac-Trend Anal Chem 122
21. Mastovska K, Lehotay SJ (2003) Practical approaches to fast gas chromatography-mass spectrometry. J Chromatogr A 1000:153–180
22. Lee J, Shin Y, Lee J, Lee J, Kim E, Kim JH (2017) Sensitivity enhancement using a microbore column and pulsed pressure injection in the simultaneous analysis of 356 pesticide multiresidues by gas chromatography-tandem mass spectrometry. Appl Biol Chem 60:385–390
23. Giocastro B, Piparo M, Tranchida PQ, Mondello L (2018) Cryogenic modulation fast GC x GC-MS using a 10 m microbore column combination: concept, method optimization, and application. J Sep Sci 41:1112–1117
24. Cruz R, Marques A, Casal S, Cunha SC (2019) Fast and environmental-friendly methods for the determination of polybrominated diphenyl ethers and their metabolites in fish tissues and feed. Sci Total Environ 646:1503–1515
25. Tienstra M, Portolés T, Hernández F, Mol JGJ (2015) Fast gas chromatographic residue analysis in animal feed using split injection and atmospheric pressure chemical ionisation tandem mass spectrometry. J Chromatogr A 1422:289–298
26. Sapozhnikova Y, Lehotay SJ (2015) Review of recent developments and applications in low-pressure (vacuum outlet) gas chromatography. Anal Chim Acta 899:13–22
27. Lehotay SJ, de Zeeuw J, Sapozhnikova Y, Michlig N, Rousova-Hepner J, Konschnik JD (2020) There is no time to waste: low-pressure gas chromatography–mass spectrometry is a proven solution for fast, sensitive, and robust GC–MS analysis. LCGC N Am 38(8):457–466
28. Khan ZS, Girame R, Utture SC, Ghosh RK, Banerjee K (2015) Rapid and sensitive multiresidue analysis of pesticides in tobacco using low pressure and traditional gas chromatography tandem mass spectrometry. J Chromatogr A 1418:228–232
29. Mastovska K, Wylie PL (2012) Evaluation of a new column backflushing set-up in the gas chromatographic-tandem mass spectrometric analysis of pesticide residues in dietary supplements. J Chromatogr A 1265:155–164
30. Lehotay SJ, Sapozhnikova Y, Han L, Johnston JJ (2015) Analysis of nitrosamines in cooked bacon by QuEChERS sample preparation and gas chromatography-tandem mass spectrometry with Backflushing. J Agric Food Chem 63:10341–10351
31. Muscalu AM, Górecki T (2018) Comprehensive two-dimensional gas chromatography in environmental analysis. TrAC Trends Anal Chem 106:225–245
32. Wooding M, Rohwer ER, Naudé Y (2017) Determination of endocrine disrupting chemicals and antiretroviral compounds in surface water: a disposable sorptive sampler with comprehensive gas chromatography – time-of-flight mass spectrometry and large volume injection with ultra-high performance liquid chromatography–tandem mass spectrometry. J Chromatogr A 1496:122–132
33. Shaul NJ, Dodder NG, Aluwihare LI, Mackintosh SA, Maruya KA, Chivers SJ, Danil K, Weller DW, Hoh E (2015) Nontargeted biomonitoring of halogenated organic compounds in two ecotypes of bottlenose dolphins (Tursiops truncatus) from the Southern California Bight. Environ Sci Technol 49:1328–1338

34. Sapozhnikova Y, Hoh E (2019) Suspect screening of chemicals in food packaging plastic film by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry. LCGC N Am 37:52–65
35. Keshet U, Goldshlag P, Amirav A (2018) Pesticide analysis by pulsed flow modulation GCxGC-MS with cold EI – an alternative to GC-MS-MS. Anal Bioanal Chem 410:5507–5519
36. Tranchida PQ, Aloisi I, Giocastro B, Zoccali M, Mondello L (2019) Comprehensive two-dimensional gas chromatography-mass spectrometry using milder electron ionization conditions: a preliminary evaluation. J Chromatogr A 1589:134–140
37. Ballesteros-Gomez A, de Boer J, Leonards PEG (2013) Novel analytical methods for flame retardants and plasticizers based on gas chromatography, comprehensive two-dimensional gas chromatography, and direct probe coupled to atmospheric pressure chemical ionization-high resolution time-of-flight-mass spectrometry. Anal Chem 85:9572–9580
38. Cech NB, Enke CG (2001) Practical implications of some recent studies in electrospray ionization fundamentals. Mass Spectrom Rev 20:362–387
39. Liigand P, Kaupmees K, Haav K, Liigand J, Leito I, Girod M, Antoine R, Kruve A (2017) Think negative: finding the best electrospray ionization/MS mode for your analyte. Anal Chem 89:5665–5668
40. Hu Z, Qi P, Wang N, Zhou Q-Q, Lin Z-H, Chen Y-Z, Mao X-W, Jiang J-J, Li C (2020) Simultaneous determination of multiclass illegal dyes with different acidic–basic properties in foodstuffs by LC-MS/MS via polarity switching mode. Food Chem 309:125745
41. Ninga E, Sapozhnikova Y, Lehotay SJ, Lightfield AR, Monteiro SH (2021) High-throughput mega-method for the analysis of pesticides, veterinary drugs, and environmental contaminants by ultra-high-performance liquid chromatography–tandem mass spectrometry and robotic mini-solid-phase extraction cleanup + low-pressure gas chromatography–tandem mass spectrometry, part 2: catfish. J Agric Food Chem 69:1169–1174
42. Laaniste A, Leito I, Kruve A (2019) ESI outcompetes other ion sources in LC/MS trace analysis. Anal Bioanal Chem 411:3533–3542
43. Garcia-Ac A, Segura PA, Viglino L, Gagnon C, Sauvé S (2011) Comparison of APPI, APCI and ESI for the LC-MS/MS analysis of bezafibrate, cyclophosphamide, enalapril, methotrexate and orlistat in municipal wastewater. J Mass Spectrom 46:383–390
44. Tokumura M, Miyake Y, Wang Q, Nakayama H, Amagai T, Ogo S, Kume K, Kobayashi T, Takasu S, Ogawa K (2018) Methods for the analysis of organophosphorus flame retardants – comparison of GC-EI-MS, GC-NCI-MS, LC-ESI-MS/MS, and LC-APCI-MS/MS. J Environ Sci Health A 53:475–481
45. Silva RD, De Menezes MGG, De Castro RC, Nobre CDA, Milhome MAL, Do Nascimento RF (2019) Efficiency of ESI and APCI ionization sources in LC-MS/MS systems for analysis of 22 pesticide residues in food matrix. Food Chem 297
46. Ramirez CE, Wang C, Gardinali PR (2014) Fully automated trace level determination of parent and alkylated PAHs in environmental waters by online SPE-LC-APPI-MS/MS. Anal Bioanal Chem 406:329–344
47. Brecht D, Uteschil F, Schmitz OJ (2020) Development of a fast-switching dual (ESI/APCI) ionization source for liquid chromatography/mass spectrometry. Rapid Commun Mass Sp 34
48. Galani JHY, Houbraken M, Van Hulle M, Spanoghe P (2019) Comparison of electrospray and UniSpray, a novel atmospheric pressure ionization interface, for LC-MS/MS analysis of 81 pesticide residues in food and water matrices. Anal Bioanal Chem 411:5099–5113
49. Guillarme D, Veuthey J-L (2017) Chapter 1 – theory and practice of UHPLC and UHPLC–MS. In: Holčapek M, Byrdwell WC (eds) Handbook of advanced chromatography/mass spectrometry techniques. AOCS Press, pp 1–38
50. Kachhawaha AS, Nagarnaik PM, Labhasetwar P, Banerjee K (2020) A review of recently developed LC–MS/MS methods for the analysis of pharmaceuticals and personal care products in water. J AOAC Int 103:9–22

51. Malysheva SV, Goscinny S, Malarvannan G, Poma G, Andjelkovic M, Voorspoels S, Covaci A, Van Loco J (2018) Development and validation of a quantitative UHPLC-MS/MS method for selected brominated flame retardants in food. Food Addit Contam A 35:292–304
52. Castro V, Montes R, Quintana JB, Rodil R, Cela R (2020) Determination of 18 organophosphorus flame retardants/plasticizers in mussel samples by matrix solid-phase dispersion combined to liquid chromatography-tandem mass spectrometry. Talanta 208:120470
53. Malachova A, Sulyok M, Beltran E, Berthiller F, Krska R (2014) Optimization and validation of a quantitative liquid chromatography-tandem mass spectrometric method covering 295 bacterial and fungal metabolites including all regulated mycotoxins in four model food matrices. J Chromatogr A 1362:145–156
54. Nanita SC, Kaldon LG (2016) Emerging flow injection mass spectrometry methods for high-throughput quantitative analysis. Anal Bioanal Chem 408:23–33
55. Mol HG, van Dam RC (2014) Rapid detection of pesticides not amenable to multi-residue methods by flow injection-tandem mass spectrometry. Anal Bioanal Chem 406:6817–6825
56. Sapozhnikova Y, Zomer P, Gerssen A, Nuñez A, Mol HGJ (2020) Evaluation of flow injection mass spectrometry approach for rapid screening of selected pesticides and mycotoxins in grain and animal feed samples. Food Control 116:107323
57. Han L, Sapozhnikova Y, Nuñez A (2019) Analysis and occurrence of organophosphate esters in meats and fish consumed in the United States. J Agric Food Chem 67:12652–12662
58. Pirok BWJ, Stoll DR, Schoenmakers PJ (2019) Recent developments in two-dimensional liquid chromatography: fundamental improvements for practical applications. Anal Chem 91:240–263
59. Sarrut M, Cretier G, Heinisch S (2014) Theoretical and practical interest in UHPLC technology for 2D-LC. Trac-Trend Anal Chem 63:104–112
60. Cacciola F, Donato P, Sciarrone D, Dugo P, Mondello L (2017) Comprehensive liquid chromatography and other liquid-based comprehensive techniques coupled to mass spectrometry in food analysis. Anal Chem 89:414–429
61. Michlig N, Lehotay SJ, Lightfield AR, Beldoménico H, Repetti MR (2021) Validation of a high-throughput method for analysis of pesticide residues in hemp and hemp products. J Chromatogr A 1645:462097
62. D'Atri V, Causon T, Hernandez-Alba O, Mutabazi A, Veuthey J-L, Cianferani S, Guillarme D (2018) Adding a new separation dimension to MS and LC–MS: what is the utility of ion mobility spectrometry? J Sep Sci 41:20–67
63. Marquez-Sillero I, Aguilera-Herrador E, Cardenas S, Valcarcel M (2011) Ion-mobility spectrometry for environmental analysis. Trac-Trend Anal Chem 30:677–690
64. Regueiro J, Negreira N, Berntssen MHG (2016) Ion-mobility-derived collision cross section as an additional identification point for multiresidue screening of pesticides in fish feed. Anal Chem 88:11169–11177
65. Goscinny S, Joly L, De Pauw E, Hanot V, Eppe G (2015) Travelling-wave ion mobility time-of-flight mass spectrometry as an alternative strategy for screening of multi-class pesticides in fruits and vegetables. J Chromatogr A 1405:85–93
66. Lehotay SJ, Sapozhnikova Y, Mol HGJ (2015) Current issues involving screening and identification of chemical contaminants in foods by mass spectrometry. Trends Anal Chem 69:62–75
67. Milman BL (2011) Target identification in methods. In: Chemical identification and its quality assurance
68. ISO 22892:2006 (2006) Soil quality – guidelines for the identification of target compounds by gas chromatography and mass spectrometry. www.iso.org/iso/catalogue_detail.htm?csnumber=35198
69. Angeles LF, Aga DS (2018) Establishing analytical performance criteria for the global reconnaissance of antibiotics and other pharmaceutical residues in the aquatic environment using liquid chromatography-tandem mass spectrometry. J Anal Methods Chem 2018:7019204–7019204
70. United States Enviromental Protection Agency (2018) Method 8270E. Semivolatile organic compounds by gas chromatography/mass spectrometry

71. United States Environmental Protection Agency (2018) Method 8260D. Volatile organic compounds by gas chromatography/mass spectrometry
72. EU SANTE/12682/2019 (2019) Analytical quality control and method validation procedures for pesticide residues analysis in food and feed
73. US Food and Drug Administration (FDA) (2020) Office of Regulatory Affairs (ORA) Laboratory manual vol II: methods, method verification and validation. ORA-LAB545
74. FDA (2003) Guidance for industry 118 confirmation of identity of animal drug residues. www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm052658.pdf
75. United States Department of Agriculture Agricultural Marketing Service, Science & Technology Pesticide Data Program. Data and Instrumentation. Revision 8, 2021
76. Schürmann A, Dvorak V, Crüzer C, Butcher P, Kaufmann A (2009) False-positive liquid chromatography/tandem mass spectrometric confirmation of sebuthylazine residues using the identification points system according to EU directive 2002/657/EC due to a biogenic insecticide in tarragon. Rapid Commun Mass Spectrom 23:1196–1200
77. Kaufmann A, Butcher P, Maden K, Walker S, Widmer M (2010) Comprehensive comparison of liquid chromatography selectivity as provided by two types of liquid chromatography detectors (high resolution mass spectrometry and tandem mass spectrometry): "Where is the crossover point?". Anal Chim Acta 673:60–72

Chromatography High-Resolution Mass Spectrometry in Food and Environmental Chemistry

Yong-Lai Feng

Contents

Y.-L. Feng (✉)
Exposure and Biomonitoring Division, Environmental Health Science and Research Bureau, Environmental and Radiation Health Sciences Directorate, Healthy Environments and Consumer Safety Branch, Health Canada, AL: 2203 B, Ottawa, ON, Canada
e-mail: yong-lai.feng@hc-sc.gc.ca

Yolanda Picó and Julian Campo (eds.), *Mass Spectrometry in Food and Environmental Chemistry*, Hdb Env Chem (2023) 119: 149–186, DOI 10.1007/698_2022_892,

Published online: 18 October 2022

Abstract In the past 20 years, the evolution of mass spectrometry has undergone tremendous technological advances to improve mass resolution, accuracy, and sensitivity, and brought commercially practical high-resolution mass spectrometers that can measure a mass-to-charge ratio (m/z) at the fourth or fifth decimal place (exact mass). Advanced high-resolution mass spectrometry (HRMS) instruments now feature fast scan speeds, sufficient dynamic range, and MS/MS capability in hybrid instruments, which facilitate the exploration of a large number of known and unknown chemicals in modern environmental analysis and food analysis. This chapter introduces the basic concepts of resolution, resolving power, exact mass, accurate mass, mass error, and mass measurement precision. In this chapter, two main modern high-resolution mass spectrometers, namely quadrupole time-of-flight mass spectrometer (Q-TOF) and quadrupole Orbitrap (Q-Orbitrap) mass spectrometer are introduced. The advantages of HRMS combined with gas chromatography and liquid chromatography in modern environmental and food analysis are discussed. This chapter also provides an overview of recent developments in extensive screening and non-targeted analysis (NTA) using state-of-the-art HRMS in food and environmental analysis. The NTA data acquisition modes and data analysis process and applications of HRMS-based non-targeted analysis approaches in the analysis of organic contaminants in foods for food quality and safety, are introduced, including lipidomics, proteomics, and metabolomics, as well as environmental samples for environmental safety.

Keywords Chromatography, Environmental analysis, Food analysis, High-resolution mass spectrometry, Non-targeted analysis

1 Introduction

Mass spectrometry (MS) is an analytical technique for measuring the mass-to-charge ratio of ions. Finnigan introduced the first commercial quadrupole mass spectrometer to the market in 1968. Although there has been a lumber of developments in quadrupole mass spectrometry technology, the resolution of quadrupole mass spectrometers is still less than 5,000. Therefore, the quadrupole mass spectrometers are generally considered low-resolution mass spectrometers (LRMS). However, LRMS is limited to measuring single-digit mass units (integer mass), and cannot separate the isobars that have the same integer mass. It is also unable to separate a large number of chemical mixtures in environmental and food samples, which lags far behind the requirements for foodomics and metabolomics analysis and non-targeted analysis (NTA) of environmental and food samples. Although tandem mass spectrometer working in selected reaction-monitoring (SRM) mode improves the mass selectivity and sensitivity and is the most widespread technique in both environmental and food analysis, the use of SRM mode on tandem MS is limited to the number of compounds monitored per analysis, and it cannot screen for unknowns, and needs reference standards.

In the past 20 years, the evolution of mass spectrometry has undergone tremendous technological advances to improve mass resolution, accuracy, and sensitivity, and brought commercially practical high-resolution mass spectrometers that can measure a mass-to-charge ratio (*m/z*) at the fourth or fifth decimal place (exact mass). Advanced high-resolution mass spectrometry (HRMS) instruments now have fast scan speeds, sufficient dynamic range, and the capability of MS/MS in hybrid instruments. The main advantage of HRMS instruments is that they can record a theoretically unlimited number of compounds in full scan mode, which allows the development of screening strategies based on accurate mass database searches to identify non-targeted compounds through retrospective data analysis. Hybrid HRMS instruments (MS/MS) can provide structural information and can detect ultra-trace levels of thousands of chemicals in the NTA approach. The advantages of these aspects combined with time dimension separation (chromatography) greatly benefit the modern environmental and food analysis. This chapter will introduce the basic concepts of resolution, resolving power, exact mass, accurate mass, mass error, and mass measurement precision. This chapter mainly introduces two main modern high-resolution mass spectrometers, namely quadrupole time-of-flight mass spectrometer (Q-TOF) and quadrupole Orbitrap (Q-Orbitrap) mass spectrometer. The possibility of using HRMS for extensive screening and NTA in food and environmental analysis will be outlined, and the NTA data acquisition modes and data analysis process are introduced. This chapter will also introduce some applications of HRMS-based non-targeted analysis approaches in the analysis of organic contaminants and toxins in foods for food quality and safety, including lipidomics, proteomics and metabolomics, as well as environmental samples for environmental safety.

2 High-Resolution Mass Spectrometry (HRMS)

In mass spectrometry, the nominal mass is defined as a mass-to-charge ratio (*m/z*) measured or calculated only as an integer. Before modern HRMS appeared on the market, the quadrupole mass spectrometer was considered a nominal mass spectrometer. Due to its low resolution in mass separation, it can only provide information about the nominal mass of molecules or fragments. In contrast, HRMS is a type of mass spectrometry in which the mass-to-charge ratio (*m/z*) of each ion is measured to several decimal places, and the accurate mass is measured instead of the nominal mass on a conventional low-resolution mass spectrometer (LRMS). HRMS is particularly useful for distinguishing molecular formulas with the same nominal mass and is able to provide the more probable empirical formulas.

2.1 Mass Resolution and Mass Resolving Power

In mass spectrometry, resolution is a measurement of the ability to distinguish two ions with slightly different *m/z* ratios as shown in Fig. 1a. If the valley between two mass spectral peaks with the same height and width is a specified fraction of the height of the smaller peak in a mass spectrum, 10% can obtain the maximum separation, and 50% can get the minimum separation. The IUPAC definition for resolution (R) in mass spectrometry is calculated by the ratio of the mass of the second peak (m_2) to the difference of two adjacent masses (m_2 - m_1 or ΔM) (Fig. 1a). The mass difference of two adjacent masses (m_2 - m_1 or ΔM) is called the resolving power [1, 2]. However, the mass resolving power at a certain *m/z* value is also commonly defined as the ratio of $m_2/(m_2 - m_1)$ [3, 4]. For two peaks of the same height, $m_2 - m_1 \approx \Delta m50\%$, where $\Delta m50\%$ is the full width at half maximum (FWHM) of a single peak, and the mass resolving power can be calculated by $m_2/\Delta m50\%$. For an isolated mass peak m, the mass resolving power is m/FWHM as shown in Fig. 1b. Sometimes, the resolving power (R) is simply defined as the value of FWHM at a specific *m/z* (usually at *m/z* 400). Therefore, when reporting values, it is important to clarify the method that is used to determine mass resolution. A higher value for resolution usually indicates a better separation of peaks in mass spectrum. In general, a mass spectrometer with resolving power over 10,000 at *m/z* 400 and a mass accuracy less than 5 ppm is defined as a high-resolution mass spectrometer. These quadrupole mass filters, triple quadrupole, and quadrupole ion trap mass analyzers, are generally not classified as high-resolution mass analyzers. Therefore, the HRMS is more selective than LRMS.

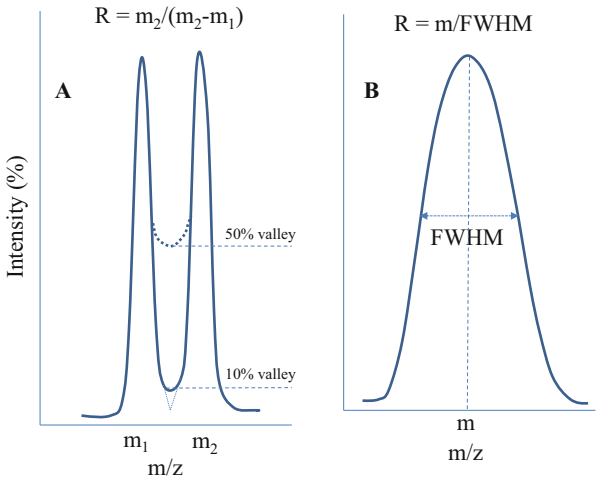


Fig. 1 The schematic diagram of mass resolution, (a) The resolution between two adjacent ion m/z peaks, (b) The resolution for an isolated ion m/z peak

2.2 Exact Mass, Accurate Mass, Mass Accuracy, and Mass Measurement Precision

The exact mass, also known as the theoretical *m/z* value, is the mass calculated from the molecular formula using the known mass of a specific isotope (usually the most abundant), giving the monoisotopic peak, with the appropriate number of decimal places. For example, the weights of ^{12}C, ^{16}O, ^{14}N, and ^{1}H are 12.00000 amu, 15.9949 amu, 14.0031 amu, and 1.00783 amu, respectively. As a result, the exact mass or the theoretical mass of 2-octanone, $C_8H_{16}O$ is 128.12018, and the exact mass or the theoretical mass of naphthalene, $C_{10}H_8$ is 128.06264. In contrast, accurate mass is a measurement of ion mass within a specified error, and mass accuracy is a measurement of mass error. The mass error is defined as the ratio of the difference between the measured and the theoretical *m/z* to the theoretical *m/z* value, expressing in parts per million (ppm). When measuring the mass of an ion, mass measurement precision is to define the repeatability of the measurement, calculating as $m/\delta m$, in which δm is the root-mean-square error from a large number of repeated measurements [5]. In the absence of systematic error, mass accuracy is the same as mass precision.

The accurate mass measured by HRMS is useful for the assignment of candidate molecular formulas of compounds with elemental compositions that are measured as an identical mass by LRMS. For example, on HRMS the accurate masses of the protonated 2-octanone, $C_8H_{16}O$, and the protonated naphthalene, $C_{10}H_8$, are measured as *m/z* 129.1274, and *m/z* 129.0699 with a mass error of 5 ppm, respectively. However, the measured masses are the same as *m/z* 129.1 on a regular low-resolution instrument. Therefore, the accurate mass can provide an exact molecular formula assignment. As the number of atoms in a compound increases, the number of possible elemental compositions in a given experimental mass measurement also increases. As a result, higher resolving power is required to resolve closely spaced mass doublets in complex mixtures for accurate formula assignment, as the resolving power influences the measurement of accurate mass. If two ion peaks are not (or incompletely) resolved, the resulting measured mass profile is the sum of the two individual mass profiles, giving the top of the combined profile locating at somewhere between the exact masses of the two individual peaks thereby impacting the accuracy on the assignment of each mass and thereby impacting the accuracy of formula assignment. In real sample analysis, the sample matrices also affect the accurate mass measurement. Generally, a minimum resolving power of 50,000 and 2 ppm mass accuracy are usually required to provide a consistent and reliable molecular mass assignment of the analyte in complex matrices, but it depends on the complexity of the sample and the concentration levels of analytes and interferences in the sample [6]. It is also worth noting that measuring exact mass can only distinguish molecules with different molecular formulas but the same nominal mass. HRMS alone cannot be used to distinguish isomers.

3 Types of High-Resolution Mass Spectrometer

The classic high-resolution mass spectrometers include double-focus sector or Fourier-transform ion cyclotron resonance (FT-ICR), which can provide a high resolution of over 500,000 to 1,000,000. However, disadvantages include slow scanning speed, complex processing, and high purchase and maintenance costs. With the advancement of mass spectrometry technology, new modern high-resolution mass spectrometers including time-of-flight (TOF) MS and Orbitrap MS have become more and more common in laboratories. The advantages of these modern mass spectrometers include fast scanning speed, sufficient dynamic mass range, and the possibility of MS/MS when part of a hybrid instrument, as well as greatly improved mass resolution, accuracy and sensitivity, and cost affordable in most laboratories. Meanwhile, because these modern MSs have a better signal-to-noise ratio in scanning mode, they are becoming increasingly popular.

3.1 Quadrupole Time-of-Flight Mass Analyzers

Time-of-flight mass spectrometry (TOF-MS) was first proposed by Stephan in 1946 [7]. This is a method of mass spectrometry in which the *m/z* of ions is determined by the measurement of their flight times, which is performed by accelerating the ions with an electric field of known strength. In the TOF-MS shown in Fig. 2a, ions having the same charge are given the same kinetic energy. Under the same kinetic energy, the velocity of the ions will depend on the *m/z*. Heavier ions with the same charge (larger m/z) fly at a lower speed, and lighter ions with the same charge (smaller m/z) fly at a higher speed. Since the flight time depends on the velocity of the ion within the same flight distance (d), the time required for the ion to reach the detector is measured as its *m/z*, as shown in Fig. 2a. In this type of mass spectrometer, masses are separated by the flight time of ions to the detector. Ions exhibiting a lighter mass will have a shorter flight time, while heavier ions will take longer to cross the flight path to the detector. The resolving power of TOF mass analyzer is $m/\Delta m50\% = (T/2\Delta t)$, where T is the total flight time of the ion and Δt is the mass spectral peak width. Modern time-of-flight analyzers also utilize a reflectron device introduced by Mamyrin in 1973 [8], which serves to correct for kinetic energy dispersion and spatial spread of ions with the same *m/z* but have varying velocities. In the reflectron system, faster ions penetrate farther into the reflectron than slower ions and travel more distance to reach the detector (Fig. 2a). This reflectron correction allows ions of the same *m/z* to reach the detector at the same time. The reflectron device increases the length of the ion flight path without defocusing due to the spread of the initial velocity, and thereby mass resolving power. Recent advances in hybrid mass spectrometry systems combine quadrupole technology (for mass selection and/or ion fragmentation) with a time-of-flight mass analyzer for high-resolution mass measurement and further increase the selectivity, improving the applications of TOF mass analyzer. Figure 2b shows that the Q-TOF-MS instrumentation resembles

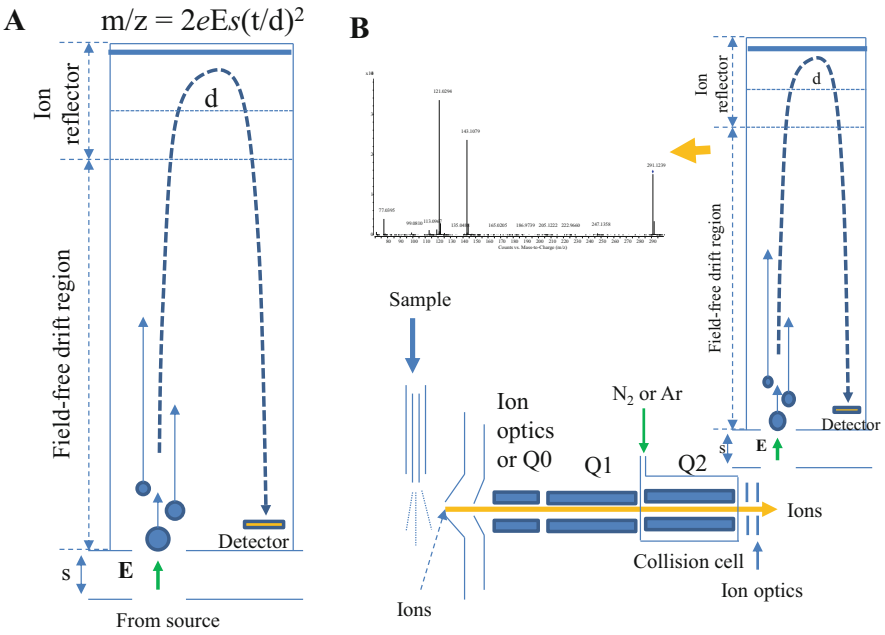


Fig. 2 Schematic diagram of time-of-flight mass spectrometer (**a**) and hybrid quadrupole time-of-flight mass spectrometer (**b**): E, electric filed strength; d, distance of flight; t, time of flight; *s*, the length of the source region; m, mass; z, number of electron charges; *e*, charge of electron; Q0, quadrupole zero, Q1, quadrupole 1; Q2, quadrupole 2; N_2, nitrogen gas; Ar, argon gas

two quadrupoles and a time-of-flight tube. The first quadrupole (Q1) can function as a mass filter to select specific ions based on their *m/z*. In radio frequency (RF) only mode, the Q1 quadrupole let all ions be transmitted through the quadrupole. The second quadrupole (Q2) acts as a collision cell, where neutral gas molecules such as nitrogen or argon bombard the ions from Q1 to fragment the ions to produce product ions by collision-induced dissociation (CID). In the RF-only mode, all ions from the Q1 pass through the Q2 quadrupole without subsequent fragmentation of the ions. After leaving the Q2, the ions are subjected to an electric field pulse and accelerated orthogonally to their original direction into the ion modulator region of the TOF analyzer for separation and measurement, as shown in Fig. 2b. The resolution of modern hybrid Q-TOF mass analyzers has been significantly improved. For example, the Bruker maXis II™ and maXis II™ ETD QTOF systems can provide a Full-Sensitivity Resolution (FSR) of 80 K, while the Bruker Impact II can provide an FSR of 50 K; Agilent 6546 LC/Q-TOF can provide a resolution of 60 K (for high masses ($\geq$1,000 Da)) and a resolution of over 30 K (for low masses). The advantages of TOF mass analyzers include no upper m/z limit in theory (or larger dynamic range) and faster response/scan rate than FTICR mass analyzers. The former makes it be useful for identifying singly charged ions of high molecular weight, and the later can be used in applications that require shorter acquisition times, such as in combination

with fast chromatographic separation techniques, for example, ultra-high performance liquid chromatography (UHPLC) or gas chromatography (GC).

3.2 Quadrupole Orbitrap Mass Analyzers

Orbitrap is a type of Fourier transform mass analyzers, which was invented in 1999 [9] and has been widely distributed since it was put into commercial sales in 2004 [10]. Compared with TOF mass analyzers, Orbitrap has much higher mass resolving power and mass measurement accuracy. In terms of working principle, the Orbitrap is an ion trap mass analyzer, which is different from the traditional Fourier transform ion cyclotron resonance (FTICR). The Orbitrap mass analyzer consists of an outer barrel-like electrode and a coaxial inner spindle-like electrode. The latter traps ions in an orbital motion around the spindle and acts as an analyzer and detector at the same time [9, 10]. Since the electrostatic attraction of the ions to the inner electrode is balanced by their inertia, the ions are trapped and circulate around the inner electrode on elliptical trajectory, and move back and forth along the axis of the central electrode, so their trajectories in space resemble helices. Using the Fourier transform of the oscillation frequency signals, the image current from the ions trapped on the outer electrodes is detected and converted into a mass spectrum. With the introduction of the C-trap, different operation modes of Orbitrap have been developed. The C-trap can be used to accept multiple fills and allow calibrant ions and analyte ions to be injected into the Orbitrap analyzer at the same time, and measured in a single spectrum to achieve an internal calibration of each spectrum, which is significantly different from the TOF mass analyzer. In addition, the C-trap also allows multiple injections of ions that are fragmented or selected under different conditions to be stored together and acquired in a single Orbitrap spectrum. The advantages of the C-trap allow the use of higher-energy collision dissociation (HCD) and quadrupole mass filters in combination. Based on this working mechanism, several hybrid Orbitrap mass spectrometers have been developed, including Q Exactive, Orbitrap Fusion, and Orbitrap ID-X Tribrid mass spectrometers. The development of modern hybrid Orbitrap mass spectrometers and their working principles have been comprehensively summarized by Hecht et al. [11]. Figure 3 shows a typical example of a quadrupole Orbitrap hybrid mass spectrometer, Q Exactive MS, which is a combination of a quadrupole mass filter and an Orbitrap analyzer. The Q Exactive instrument incorporates the development of new technologies such as S lens, eFT, extended mass range, and fast polarity switching. In addition to improving selectivity, the quadrupole mass filter also enables multiple scan modes such as a multiple single ion monitoring (SIM) mode to rapidly switch between different narrow mass ranges, which allows ions of selected masses to enter the C-trap and to sequentially accumulate them up to a mixed population of the desired ions, and achieving a joint analysis of ions in a single Orbitrap detection. Similarly, Q Exactive MS also allows fragmentation of different precursor *m/z* in rapid succession while capturing and retaining the resulting fragment populations all

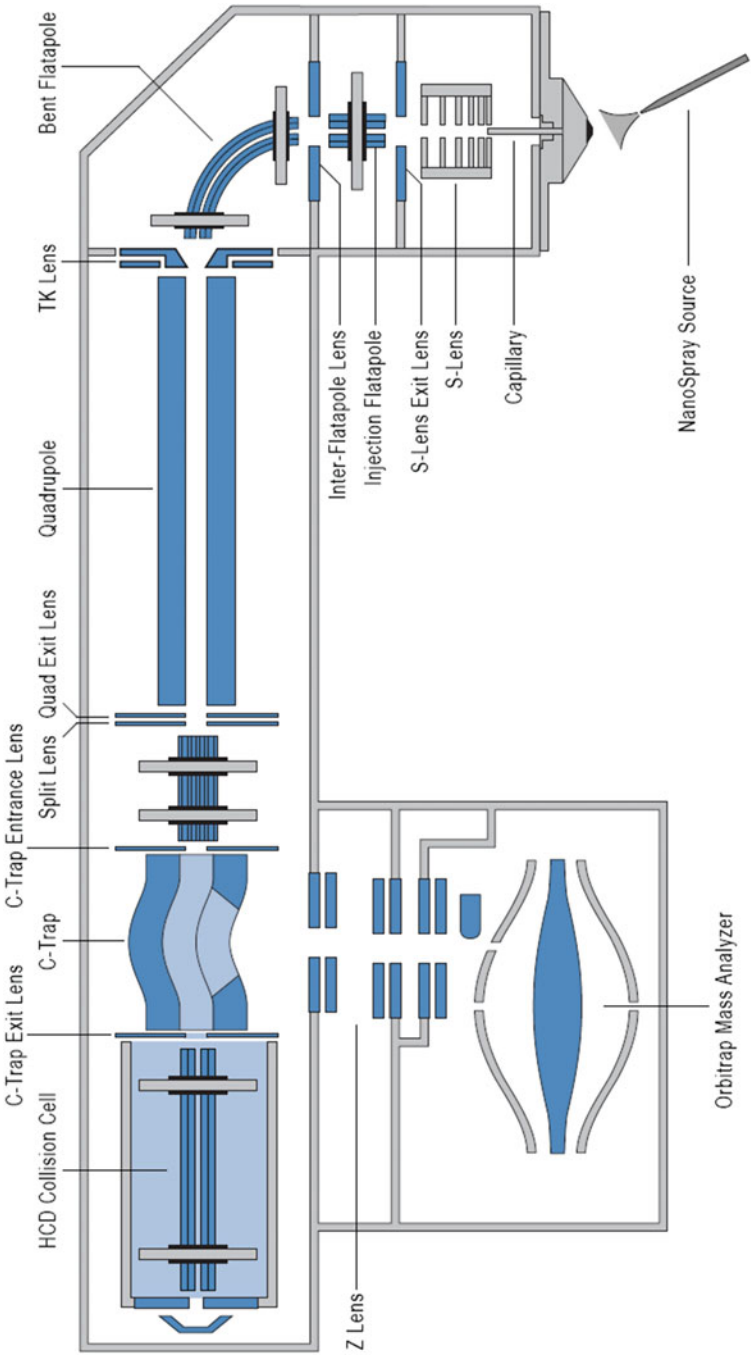


Fig. 3 Schematic layout of quadrupole-Orbitrap hybrid mass spectrometer, the Q Exactive (Reproduced with permission of Thermo Fisher Scientific)

together in the HCD collision cell, followed by their joint analysis in the Orbitrap analyzer. Compared with QTOF, Orbitrap can simultaneously detect ions in a wide *m/z* range, and provide higher resolution and more accurate mass measurement without reducing sensitivity when using higher resolutions. The new Orbitrap ID XTM TribridTM MS system can provide a resolution up to 500 K, which can provide the fine structure of isotopes and unravel molecular formulas of true unknowns, and completely separate nominally isobaric compounds. In addition to improving mass resolution, the new Orbitrap design can also perform deep scans to automatically generate inclusion and exclusion lists for further MS/MS fragment scan, which is very useful for non-targeted screening analyses and identification of unknowns in metabolomics study. Another advantage of Orbitrap is the capability of reliable quantitative purposes. However, compared with the TOF mass analyzer, the resolution of Orbitrap is proportional to the measurement time, which may impact the data points when using ultrahigh performance liquid chromatography (UHPLC) equipment or attempting polarity-switching experiments.

4 Chromatography-High-Resolution Mass Spectrometry for Screening and Non-target Analysis

4.1 Chromatography-High-Resolution Mass Spectrometry

One of the main advantages of HRMS is that it can provide accurate mass measurement and high resolution for separating two adjacent ions with slightly different *m/z* ratios, such as nominal isobaric compounds in a mass spectrum. The accurate mass can better predict elemental compositions in the molecular formula assignment. However, the high resolving power cannot separate molecular isomers or polyatomic ions with the same molecular formula, including constitutional isomers, in which bonds between the atoms differ, and stereoisomers, in which the bonds are the same but the relative positions of the atoms differ. As these isomers have the same number of atoms of each element, their accurate masses are the same in mass spectrum. Although they have the same formula, these isomers may not share similar chemical or physical properties. Therefore, in addition to the *m/z* dimension separation, retention time dimensional separation can increase the power to separate isomers. Chromatographic techniques can perform time-dimensional separation to separate these isomers prior to HRMS analysis. Gas chromatography (GC) and liquid chromatography (LC) are the two main time-dimensional separation techniques used in conjunction to HRMS.

Gas chromatography (GC) is a type of chromatography used to separate and analyze compounds that can evaporate without being decomposed. It is also sometimes known as vapor-phase chromatography (VPC), or gas–liquid partition chromatography (GLPC). In gas chromatography, when the compound mixture passes through the stationary phase, the separation is performed by the compound mixture

being distributed on the gas chromatographic column with a gradient temperature between the mobile phase (carrier gas) and stationary phase which is a microscopic layer of viscous liquid on a surface of solid particles. GC is usually applicable to the separation of volatile and semi-volatile small molecular compounds.

Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid, where sample ions or molecules are dissolved. The sample with the mobile liquid will pass through the column packing with a stationary phase composed of irregularly or spherically shaped particles. LC, namely high-performance liquid chromatography (HPLC) or ultra-high performance liquid chromatography (UHPLC), is the most common chromatographic technique hyphenated to HRMS to be used in environmental and food analysis due to its applicable to a more wide range of chemicals from small to large molecules. Capillary electrophoresis (CE) is another liquid separation technique. In CE, compounds are separated according to their ion mobility and/or partitioning into another phase through non-covalent interactions. Compared to LC, CE theoretically has higher separation ability. However, in the past, it was not commonly hyphenated to HRMS.

Chromatographic technique can not only help separate isomers but also help separate high similar structures or isobars, thereby increasing the separation of analytes in HRMS measurement, thereby achieving accurate molecular assignment. In addition, chromatographic technique can also reduce the influence of matrices in sample on ionization efficiency, especially in electrospray ionization (ESI) mode which is commonly used in LC-HRMS systems for non-targeted analysis.

4.2 Non-targeted Analysis with High-Resolution Mass Spectrometry

The widespread use of synthetic chemicals in industrial applications has led to food and environmental pollution, as well as public exposure to those chemicals from various sources through foods and the environment. Monitoring the targeted contaminants in food, environmental, and human samples is often used as an approach to assess food and environmental pollution and human exposure to these chemicals. Although the use of targeted monitoring methods can achieve high sensitivity, targeted monitoring only evaluates human exposure to a small portion of the contaminants in food and the environment. In addition to legacy contaminants, hundreds of new chemicals are produced in industry every year. These substances enter the environment where they coexist with hundreds of their transformation products as well as with those of legacy contaminants. This high number of potential compounds pose a challenge to the targeted analysis. Therefore, it is emerging to use non-targeted analysis approaches to explore and elucidate the structures of these unknown chemicals in food and the environment for pollution assessment and human exposure assessment [12, 13].

Compared with conventional low-resolution quadruple mass spectrometry, HRMS can provide high resolution and accurate mass that can help identify targeted species, narrow down possible chemical formulas, and elucidate unknown structures, which makes it a power tool for non-target screening analysis. A particular advantage of HRMS is the ability to acquire accurate mass spectrometry data of all precursor and fragment ions in the sample in data-independent acquisition (DIA) mode. This enables confident identification of "expected components" through comparison with library data and retrospective investigation of DIA data to detect and identify new unknown compounds that standards are not commercially available or are difficult to obtain. Generally, measurement of the intact exact mass of molecular ions can be obtained from a full scan without collision energy. The full scan with collision energies can be used as the complimentary information of exact mass to prioritize and identify molecular ions through the intensity decrease trend of the candidate ion and intensity increase trend of potential corresponding product ions with increasing of collision energy. A targeted MS/MS scan is usually performed on each prioritized candidate ion to elucidate their structures [14]. Another strength is to conduct semi-targeted analysis through combination of a list of targeted compounds in non-targeted analysis. Non-targeted analysis presents the best coverage of compounds in sample but low reproducibility and substantial data processing. Semi-targeted analysis can represent a mid-way strategy enabling a good compromise between compound coverage, reliability, effort in method construction and data treatments. Although the use of HRMS-based non-targeted analysis (NTA) approaches to identify unknown xenobiotics in food and environmental samples is still a new analytical frontier, the workflow usually includes sample collection, sample preparation, data acquisition, data mining/processing, and reporting. Due to the scope of this chapter, data acquisition, data mining, formula assignment, and unknown identification will be introduced.

4.2.1 Data Acquisition

There are two data acquisition modes in NTA: data-independent acquisition (DIA) and data-dependent acquisition (DDA).

The data-independent acquisition (DIA) is a full-scan MS^2 acquisition mode of molecular structure determination. In DIA mode, all ions within a selected *m/z* range are fragmented and analyzed in a second stage of tandem mass spectrometry. There are two common DIA modes: all-ion MS/MS (MS^{All} or MS^{E}) scan mode and sequential window acquisition of all theoretical fragment-ion spectra (SWATH) mode. In the former mode, all ions in the scanned mass window are subjected to fragmentation under various collision energies. The DIA data under zero collision energy provides the intact exact mass information. The change trend of candidate ion intensity with collision energy also helps to identify candidate molecular ions. DIA is non-targeted, does not require any prior knowledge of chemicals, and can be used for truly unknown analysis. Due to non-specific manner without prior knowledge, DIA MS^{All} MS^2 acquisition has higher hit rate than data dependant acquisition

(DDA) mode. However, since all ions in the mass range are fragmented, the product ions from other co-eluting components will reduce the quality of MS^2 spectra. This makes the structure elucidation and assignment challenging. In order to improve the quality of MS^2 spectra in DIA MS^{All} mode, the new DIA mode SWATH was introduced for the first time in non-targeted analysis in 2012 [15]. In this mode, a small mass window, for example, a 20 Da window, is used to reduce the product ions from co-eluting components and matrix ions. In this way, the DIA SWATH mode can produce higher quality of MS^2 spectra than the DIA MS^{All} mode, while maintaining the same MS^2 acquisition hit rate [16], which is more suitable for a metabolite identification study [17]. The MS^2 spectra in SWATH mode can also perform library search on any precursor and structural analog search based on product fragment by using the chromatographic profile as well. Compared to DDA, this way can identify more compounds at lower concentration levels [18]. The richness of SWATH data allows the use of a variety of data analysis methods, including detection of metabolites through prediction and mass defect filtering, and quantification from high-resolution MS precursor ion chromatograms or fragment chromatograms [19]. However, more effort is needed to define standard operating procedures (SOPs) for DIA MS^{All} and DIA SWATH acquisition methods, which can be routinely applied to the identification of unknown compounds in NTA [20, 21].

In contrast, the data-dependent acquisition (DDA) is a product ion scan mode. In this mode, the mass spectrometer automatically selects the precursor ions that meet the predefined peak intensity or ion abundance in the given *m/z* range in the first stage of tandem MS (MS1) and then performs MS/MS scan for the selected precursor ions in the second stage of tandem MS (MS2). In DDA mode, data is acquired in a manner of automatically switching between MS full scan and MS/MS product ion scan. Since only these precursor ions with a higher intensity than the predefined intensity are selected, the number of fragmented precursor ions is smaller, which reduces the MS/MS (MS^2) background and improves the quality of MS^2 spectrum. However, in the analysis of complex mixtures, the ionization suppression of co-eluting endogenous materials and competition with a large number of matrix peaks can interfere with the precursor ions that trigger MS^2 acquisition, resulting in a low MS^2 spectrum hit rate. Therefore, a two-injection workflow is often used to improve the MS^2 acquisition hit rate. In this way, the first injection is used to determine potential compounds, and the second injection is used to conduct targeted MS^2 acquisition to obtain the MS^2 spectra of all these candidates. The advantage of this strategy is that it can increase the MS^2 acquisition hit rate to 100% while maintaining high quality of MS^2 spectra. The disadvantage of this strategy is that the second injection requires a separate method, which includes the target list from the result of first injection, so it is time-consuming. Another disadvantage of DDA is that the stochastic peak selection based on the peak intensity is not reproducible in precursor selection process, which results in quantitation from precursor ions less specific than using fragment ions [22]. Therefore, more effort is needed to prevent the fragmented ions from being reselected into different scans to increase the number of precursors selected for fragmentation and reduce concentration deviations.

4.2.2 Data Mining/Processing

After data acquisition, data analysis and data mining usually start with molecular feature extraction to prioritize peaks of interest. This process includes peak detection, peak alignment, and peak selection.

Structure-based information such as specific fragment ions, neutral losses, isotopes, and mass differences can also be used to prioritize molecular ion peaks, identify candidate ions, and assign molecular formulas to certain chemical groups. Generally, specific fragment ions related to the structure of certain chemical groups are first often used to perform extracted ion chromatogram (EIC) or extracted mass chromatogram (EMC) to prioritize molecular ion peaks of the chemical groups. Other molecular features, such as mass defects, isotope patterns, mass accuracy, neutral losses, and ion intensities are usually used to further filter false positive candidates in the first step of EIC or EMC extraction.

The adduct ions, such as $[M + H]^+$, $[M + Na]^+$, $[M + NH_4]^+$ in a positive ESI mode, and $[M - H]^-$ and $[M + HCOOH - H]^-$ in a negative ESI mode, are useful filters for assigning candidate molecular ions. When using adduct ions to assign candidate ions, spectral annotation tools, such as findMAIN, is usually used [23]. Mass defect filtering (MDF) is an effective approach for interpreting and extracting chemical information from large mass spectral data sets. It is a useful tool in non-targeted screening analysis, especially in the screening of unknown compounds in homologs [24, 25]. A defined mass defect window centered at the mass defect value of the precursor compound ions can be used to detect common and uncommon metabolites.

In addition, it is necessary to perform peak alignment in different samples to adjust for minor variations in mass and retention times and to improve identification confidence for tentative candidate ions. If the retention time information in databases is not available, the retention time can be compared with the retention times predicted by quantitative structure-retention relationship (QSRR) based or logP-based models. In case-control studies, the change or difference in mass profile between the control group and treatment group is a very useful criterion for determining the priority of ion peaks of interest for further study [26].

4.2.3 Molecular Formula Assignment and Structure Annotation, and Identification Confidence Levels

In data analysis, the formula assignment is considered to be the identification confidence Level 4 according to Schymanski's definition [27]. The high resolution can improve the separation of isotopic fine structures, thereby improving the reliability of elemental composition assignment in assigning formulas. However, it is difficult to answer how much mass resolving power is enough for an accurate formula assignment. When the molecular mass is less than 300 Da and the molecular formula contains only C, H, N, and O, an accurate mass with a resolution of 10 K can

usually provide a clear assignment of elemental composition [28]. However, when the molecular mass exceeds 300 Da, even with a mass error of 0.1 ppm, the assignment of elemental composition becomes more and more difficult [28]. When the number of elements in the composition increases, such as C, H, N, S, O, P, and potentially F, Cl, Br, and Si, more than 1.6 million molecular formulas in the range 0–500 Da can be possibly generated for one accurate mass. Therefore, the high mass accuracy alone is not enough to exclude enough formula candidates in assigning formulas. In general, molecular formula assignment is not a simple process in non-targeted screening analysis. This step usually uses a combination of heuristic rules including the Seven Golden Rules [29], MS^n fragment interpretation, neutral loss analysis, isotope pattern matching, and rings plus double bonds equivalent (RDBE) for a more accurate assignment [30].

Tandem MS (MS/MS and MS^n) fragmentation is usually used to elucidate the elemental structure information after formula assignment for possible structure(s). In this step, the MS/MS spectra of the candidates are compared with spectra from databases such as MetFrag or predicted spectra using prediction tools to achieve high match score with the library spectrum to enhance the identification confidence. The retention index that is to best fit the experimental information can also enhance the identification confidence. The probable structures are usually those that have not yet been confirmed by standard or available literature information, but they are named as the best candidate structures using library spectrum matching or predicted spectrum matching. To confirm the probable structures, reference standards are needed to compare MS, MS/MS, and retention time matching with the probable structures. In the absence of an authentic reference standard, the comparison of the exact masses with online structural information can be the alternative way for confirmation.

5 Application of High-Resolution Mass Spectrometry in Food Analysis

Thousands of chemicals, their metabolites, and transformation product from food processing have the potential to contaminate our food. Therefore, monitoring those contaminants in food is important to maintain food safety and protect our health.

5.1 Food Lipidomics

Foodomics is a combination of advanced analytical techniques and bioinformatics to address different questions in food science and nutrition, and improve the well-being, health, and confidence of consumers, including all omics in food analysis (genomics, proteomics, lipidomics, glycomics, peptidomics, and metabolomics) [31]. It requires reliable qualitative and quantitative information about food

contamination and food proteome components to extract new integrative information from the complex multivariable space of omics. This new information will improve understanding of processes in food science and technology, consequently new functions of food, markers of food quality and safety, and completely transform the concept of food safety. The use of high-resolution mass spectrometry (HRMS) can greatly improve analytical performance and provide a good combination of selectivity and sensitivity, which can trace the chemical fingerprint of food and define molecular parameters for product qualification, authentication, and safety. HRMS-based analytical methods can ensure the origin of food in terms of food traceability, and discover biomarkers to identify potential food safety issues in food quality.

Lipids are very important for human health. Lipidomics analysis in food science research includes composition analysis of lipids from different biological resources, revealing the mechanism and optimization of reaction conditions for modification of structured lipids, characterizing food safety and quality assurance, and evaluating bio-function and nutrition levels of lipids in living organisms. For example, despite uncontrolled environmental variability and non-standardized diets, the lipidomics analytical method using LC–HRMS was able to successfully profile the difference between canine breeds [32]. Non-targeted lipidomics anlaysis using an LC-HRMS system in data dependant acquisition mode was used to elucidate the global lipidome of 155 agricultural plant products of 58 species from 23 plant families, including grains, beans, nuts, vegetables, fruits, and tuber. This way was able to comprehensively elucidate the existing lipid structures for each agricultural product. In addition to facilitating quality control of food products, the study has also advanced the understanding of the physiological functions of food on human health [33]. Milk is a rich dietary source of lipids, including many lipids that have important functions in living organisms. Therefore, milk lipidomics analysis can not only provide important information when estimating the nutritional value of milk but also serve as a marker for the characterization of milk types, thereby providing a tool for detecting and preventing milk fraud. In order to provide nutritionists with more comprehensive information about the lipid distribution in different milks, the HRMS-based non-targeted analysis approach was used to profile different milk samples and identify biomarkers for milk fraud identification [34]. Lipidomics can also reveal the global profile of the intact lipid molecules in biological systems and provide qualitative and quantitative information. In this field, the lipidomics analysis using HRMS can also be used to reveal the influence of nitrogen sources on lipid biosynthesis of *Mortierella alpine*. This information provides a basis for further technological developments and the genetic modification of industrial microorganisms to improve the production yield of high-value-added polyunsaturated fatty acids [35]. Overall, HRMS can facilitate lipidomics analysis for many applications in foodomics. There are some reviews on mass spectrometry-based lipidomics to provide more details in this field [36, 37].

From the genome loses its active influence, proteomics is particularly important for when food proteins are subjected to different factors and food processing conditions that may not be genetically regulated. Food allergens are proteins or

peptides that trigger immune-mediated reactions in susceptible people. In recent years, the number of sensitive consumers, especially children with food allergies, has increased. There are some commercially available methods that are mainly based on immunological methods for specific proteins, such as enzyme-linked immunosorbent assays (ELISA) and polymerase chain reactions (PCRs) for specific DNA molecules for allergen detection. However, they have the limitation of distinguishing capability and selectivity. Therefore, there is a need to use MS-based proteomics approaches to explore allergenic ingredients that are not reported on the labels of food commodities. Due to the advantages of high selectivity and sensitivity from accurate mass measurement and resolution of complex mixtures, the HRMS-based NTA method allows identifying and quantifying the undeclared addition of allergenic milk proteins in samples of chicken and turkey sausages labeled as milk-free, thereby protecting consumers with a serious health problem from these food allergens [38]. In order to perform an in-depth comparison of the Whey proteome of different species, the HRMS-based proteomics approach was also applied to explore the milk Whey proteome of Indian *Zebu* cattle. This proteomic information was used as a comprehensive reference database for Indian cattle genetic resources for the production of functional foods and infant products. This assay led to the identification of 6,210 proteins from *in-gel* digestion and *in-sol* digestion [39]. Egg allergy is one of the most common food allergies, especially in children. In order to identify any egg allergenic ingredient, a UHPLC-HRMS based non-targeted analysis approach was developed to identify potential peptide biomarkers to detect and to quantify egg allergens in processed food products. This HRMS-based method is specificity, trueness, and robustness and can also be used as a targeted analysis method to quantify food allergens [40]. Some recent review papers comprehensively reviewed HRMS-based non-targeted analysis approaches used for the characterization and detection of food allergens [41, 42]. Food proteomics analysis can also be applied to adulteration detection. In a recent study, a novel analytical strategy using a state-of-the-art high-resolution Orbitrap mass spectrometer to perform pork gelatin speciation and detect adulteration was developed. In this method, HRMS was used to detect and identify targeted proteotypic peptides using proteomic bottom-up approaches in combination with in silico characterization of collagen type I, collagen type II and collagen type III. The MS^2 spectra of targeted proteotypic peptides at high resolution were used to compare with the amino acid sequence of each collagen type targeted proteotypic peptide for identification. The specific fragment ions were used to identify precursor peptide-to-fragment ion transitions for targeted gelatin species determination and adulteration detection [43]. In another recent work, a novel food proteomic approach was developed using HRMS to detect meat adulteration through proteogenomic annotation and carefully selected surrogate tryptic peptides. In this method, four proteotypic peptides (i.e., peptides from myoglobin, myosin-1, myosin-2, and β-hemoglobin) were successfully used for meat species determination and adulteration [44]. HRMS-based food proteomics in methodology and other applications have also be comprehensively reviewed previously [45–48].

Genomics, with its global perspective, is now reshaping the future of human metabolic health. Identification of the genetic components in food can be used as

traceability and to detect the presence of both genetically modified organisms and allergens. Genomics is also widely used in food authentication. DNA modification by exogenous chemicals and endogenous metabolites, also termed as adductomics, can be used as markers of food quality. As the successor of genomics and proteomics, metabolomics shifts the view of biology from genes to proteins to metabolites, which provides the information on processes in cells, tissues, and organisms to each other. Study on biological systems at the level of metabolites can provide in-depth insights into the biochemistry of diets, toxicity, medicine, physiology, and pathology, and therefore ensure food quality and safety. For example, an HRMS-based comparative non-targeted metabolomics analysis approach was utilized to identify marker candidates in microbially inoculated chicken eggs [49]. The metabolite markers discovered in this study may contribute to further industrial implementation of these newer spoilage markers, and ultimately benefit quality assurance and food safety in poultry egg production. In another study, a non-targeted analysis approach using UHPLC-Q-Orbitrap system to profile and identify metabolites in tea to evaluate health benefits of the tea [50]. The assay provided detailed information on the chemical composition of the tea during the fermentation process and provides experimental evidence for quality control and evaluation by observing the occurrence and dynamic changes of metabolites and lipids. Overall, HRMS has become a powerful tool in foodomics analysis due to its high-resolving power in the separation of *m/z*. HRMS-based foodomics approaches have been applied to screen and identify metabolites, DNA adducts, lipids, etc. in various food samples to ensure food quality and safety [51–54]. More detailed information about HRMS-based foodomics such as metabolomics and genomics, their applications, and future perspectives have been comprehensively reviewed previously [55–60].

5.2 Food Contaminants and Natural Toxins

The globalization of the market increases the importance of food quality and safety for consumers. Illegal use of additives and chemical contaminants such as agrochemicals, environmental contaminants, veterinary drugs, allergens, and toxins, is a prominent problem in food safety. Therefore, legislation in the different countries worldwide drives the monitoring of residues of pesticides and veterinary drugs and contaminants in foods and the products traceability to ensure control and management through all stages of food production, processing, and distribution. LC-MS/MS and GC-MS/MS-based methods are commonly applied to such analysis. However, in current monitoring programs, low-resolution mass spectrometry is traditionally used to analyze a limited number of targeted well-known contaminants with MS/MS mode, which is not enough to ensure food safety. Although high sensitivity and the ability to simultaneously monitor multiple contaminants can help meet some urgent requests for measuring low-concentration contaminants in samples, the major requirement for the effective application of such targeted approaches is to know the identity of the compound before it can be measured. In particular, the presence of

undesired molecules either of natural or of synthetic origin is of paramount importance for the assessment of food safety, starting from raw materials up to the final products. Therefore, the development of novel and complementary non-targeted analytical methodologies is highly advisable to screen and identify unknown compounds in foods. Due to the advantages of HRMS, the LC-HRMS-based non-targeted analysis approach has been increasingly used in food residue and contamination analysis. With the development of more data processing tools, LC-HRMS-based non-targeted analysis can now quickly characterize hundreds of never-before-studied chemicals in food.

Compared with traditional LC-MS/MS, Orbitrap and Q-TOF mass spectrometers provide better signal specificity and sensitivity in quantitative multi-residue analyses. The higher mass resolving power of HRMS reduces the error in the assignment of the precursor ion mass of a candidate compound that has the same nominal mass from the co-eluting analytes [55]. Therefore, by monitoring animal urine instead of testing animal products, LC-HRMS has been applied to detect the illegal use of synthetic hormone growth promoters and the illegal use of β-agonists [61, 62]. The combination of liquid chromatography and HRMS was also applied to the comprehensive characterization of contaminants in food, such as polyphenols in beer to improve food safety for consumers [63], abuse of synthetic pesticides in farm and agriculture products [64–66], and non-targeted screening organic trace substances in the resources used for drinking water treatment, and their presence in drinking water for drinking water quality and safety [67, 68].

Veterinary drugs are widely used in the livestock industry, which makes parent drugs as residues, in addition to drug metabolites or transformation products, be present in food products. These drug residues and their metabolites or transformation products are regulated either as maximum limits or with an analytical threshold (minimum required performance limit) for banned substances. Compared with traditional low-resolution mass spectrometry, the application of high-resolution mass spectrometry in residue analysis significantly improves the ability of multi-residue analysis.

Pesticides are used to protect crops from pests, thereby increasing crop yields. However, these chemicals are potentially harmful to human health. Therefore, they are globally regulated by establishing tolerances or maximum residue limits (MRLs) to ensure that they remain at a safe level in foods. Approximately 1,000 pesticides have been produced worldwide for use in crops. Therefore, it is a challenge to analyze the presence of these chemicals, their metabolites, and degradation products when precise knowledge of pesticide application or abuse is lacking. The current common approach is to use GC and LC coupled with tandem mass spectrometry (mostly triple quadrupole with low resolution) to analyze target multi-residue pesticides in agriculture products [69], which can provide high sensitivity and selectivity in quantitation. However, these approaches are limited to analyzing too many potential pesticide targets in one method, thereby reducing selectivity and sensitivity, and they cannot detect the presence of pesticides that are not on the target list. In contrast, hybrid high-resolution mass spectrometers such as Q-TOF and Q-Orbitrap can provide accurate mass measurements and the MS/MS confirmation at the same

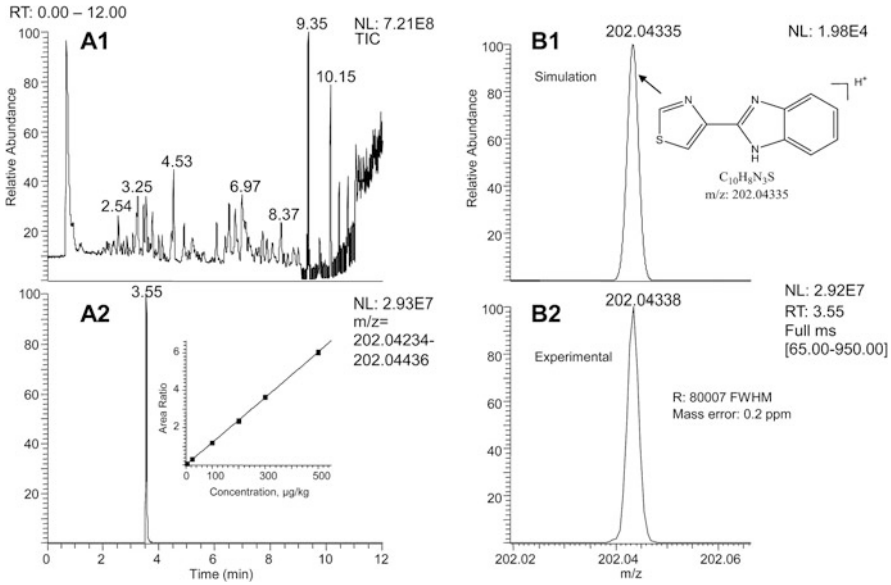


Fig. 4 UHPLC/ESI Q-Orbitrap MS chromatograms and spectra from a full MS/dd-MS^2 experiment: (A1) extracted ion chromatogram (displayed as a stick per scan) of carbendazim $[M + H]^+$ *m/z* 192.07675 with a mass tolerance of 5 ppm; (A2) dd-MS^2 total ion chromatogram of carbendazim $[M + H]^+$ *m/z* 192.07675; (B1) mass spectrum (full MS scan) from chromatogram A1 at 3.25 min; (B2) dd-MS^2 or a product-ion spectrum of carbendazim $[M + H]^+$ *m/z* 92.07675 from chromatogram A2 at 3.25 min. Pesticide spike concentration level (a total of 166 pesticides): 90 µg/kg in a blank potato sample. (Reproduced with permission of J. Agric. Food Chem.) [72]

time with either targeted analysis for quantitation or non-targeted analysis for exploring more pesticides and new chemicals in crop products [70], vegetables [71], fruits [72–74] and tea [75–77]. As the sensitivity and resolution of modern Q-Orbitrap mass spectrometer have been significantly improved, the LC-Q-Orbitrap mass spectrometer has shown acceptable performance in quantifying pesticide residues in fruits [72]. Fig. 4 shows the extracted ion chromatogram (Fig. 4A1) and dd-MS^2 total ion chromatogram (Fig. 4A2) of carbendazim, respectively. In this NTA method, the DIA data were acquired using Full MS-SIM mode and the DDA data were acquired using Full MS/dd-MS^2 mode. In the later mode, when the ion intensity is higher than the set value, the product-ion spectrum with accurate mass measurement is automatically obtained. In the identification of pesticides, the dd-MS^2 product-ion spectrum (Fig. 4B2) was used to confirm the identity of a pesticide and characterize the structures of unknown pesticides in addition to the full MS spectrum (Fig. 4B1) for online and in-house library accurate mass matching search. In MS/MS mode, the background caused by the presence of isobaric compounds is significantly reduced (Fig. 4A2). Therefore, the use of HRMS for multi-residue pesticide analysis can quantify and identify hundreds of pesticides in one injection [72]. Although HRMS databases containing accurate mass,

chromatographic retention times, and high-resolution mass spectra are more useful in the assignment of unknown compounds, they are different from low-resolution mass databases and libraries. There are still not enough effective HRMS databases for the assignment of unknown compounds using HRMS data. Although HRMS has the advantage of mass-resolving power, compared with triple quadrupole mass spectrometry, it requires different concepts, experiments, and guidance to screen, identify, and quantify pesticides in complex food matrices. New data processing tools need to be developed to create an effective and optimized screening approach to speed up the analysis and identification of compounds, reduce the need for chemical standards, and harmonize analytical procedures. As pollutants are ubiquitous in the environment, it is always a difficult task to analyze organic pollutants from the environment in food and drinking water. It has an extensive history of using GC as the separation technique in food analysis. With the development of HRMS in resolving power and sensitivity to tandem quadrupole mass spectrometry and sufficient full-scan sensitivity, the application of advanced hybrid HRMS in a combination of UHPLC is increasingly used to monitor unexpected contaminants from various sources at relevant concentrations and analysis of chemical spaces [78]. In addition, due to the lack of commercial availability of reference substances and of prior information of unknown degradation products and metabolites, the instrument is required to provide semi-targeted or non-targeted screening analysis, not only detecting suspected peaks but also identifying and confirming underlying chemical structures or any unknown exogenous compounds in a given sample. The liquid chromatography high-resolution mass spectrometry for pesticide screening in foods has been recently reviewed [79].

Natural toxins can be either plant toxins, fungal toxins (mycotoxins) or marine biotoxins (phycotoxins). Since they are naturally generated and commonly contain a number of isomeric analogs, it is difficult to accurately analyze these toxins, which leads to a large number of false-positive and false-negative findings in low-resolution LC–MS/MS and results in the pressure to switch to alternative detection approaches. For example, many mycotoxins have to be detected at a very low level, requiring a good cleanup and high-sensitivity detection. Due to the high-resolution and sensitivity of modern HRMS, many mycotoxins have been identified in green tea [80], nuts [81], feedstuffs [82], beer [83], cereal [84], edible insects [85], and human breast milk [86], indicating the widespread presence in all types of food. Their metabolites identified in breast milk also indicate that these mycotoxins may have potential health effects [86]. Despite the number of regulated mycotoxins is limited, there is still a strong interest in more extensive screening to understand the broader prospects, because there is evidence that a variety of fungal metabolites and conjugates such as glycosides are naturally present in food [87, 88]. The situation of marine biotoxins is similar to that of mycotoxins, and a number of specific toxins have been regulated. In food analysis, there also is interest in monitoring exercises to study the incidence of contamination of such toxins from different structural classes. Compared with conventional LC-MS/MS methods, HRMS can facilitate the monitoring of a broad space of mycotoxins and has become

a preferred technique for analyzing natural toxins without the need of reference materials [55].

6 Application of High-Resolution Mass Spectrometry in Environmental Analysis

Although the modern industry has benefited our lives with more artificial products, the environment has been heavily polluted by various chemicals produced by industry and anthropogenic activity. Due to the diversity of environmental samples, it is always a challenge to analyze contaminants sensitively and reliably with conventional LC-MS/MS methods.

6.1 Analysis of Emerging Contaminants in Environmental Exposure

In the environmental exposure, organic contaminants in environmental samples are very diverse with different physicochemical properties, which give challenges to analysis of them. Meanwhile, the number of organic pollutants reported in the literature is increasing year by year. Those new pollutants, the so-called "contaminants of emerging concern," are frequently present in the environment at low concentrations, which makes the analysis of environmental samples even more challenging in terms of sensitivity and identification by using the conventional LRMS. Since HRMS can provide both selectivity and sensitivity, with the recent development of Orbitrap-MS-based instruments and the improvements to TOFMS systems, advanced hybrid HRMS is increasingly used by research laboratories and routine analytical laboratories.

The emerging contaminants in waters raise much more concerns recently, because new and legacy synthetic compounds in industrial and consumer products can enter natural waters via wastewater treatment plant effluents, urban and industrial sewage, erosional runoff, and leaching from agricultural areas. Therefore, the use of LC-HRMS-based methods has been developed to characterize these new and legacy compounds with highly lipophilic characteristics such as polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAH), and pharmaceuticals as emerging contaminants in waters. HRMS can help characterize much more contaminants in sample than LRMS. For example, more than 180 contaminants including pharmaceuticals, personal care products, pesticides, biocides, additives, corrosion inhibitors, musk fragrances, UV light stabilizers, and industrial chemicals in sediments were screened and characterized by an LC-HRMS method [89, 90]. HRMS can also be utilized to monitor and track emerging chemicals and pollution trends in water sources in order to achieve a more holistic water quality assessment, thereby

raising awareness of the multiple potentials for improving water pollution elimination and management efficiency [91]. Compared with tracking source contaminants, the behavior and transformation of organic contaminants in waters is more challenging in analyzing emerging contaminants and protecting waters. Therefore, the information about organic contaminants and their fate and their transformation products (TPs) in the water cycle as well as their loads and input pathways is very important for understanding water quality and contamination. To achieve this goal, the state-of-the-art of LC-HRMS/MS has the advantage of detecting known targets, suspected compounds like TPs, and unknown emerging compounds [92]. It is also important to study the presence of transformation products (TPs) of emerging contaminants that can be potentially found in the environment after biological or chemical degradation. For this purpose, the advanced LC-HRMS systems are perfect and powerful tools for evaluating molecular changes and identifying unknown molecules. In addition, in environmental and exposure science, it is also important to establish experimental evidence of the role of chemical exposure in humans and environmental systems. Therefore, using HRMS data, the global emerging contaminant early warning network can be used to rapidly assess the spatial and temporal distribution of emerging contaminants of concern in environmental samples [93]. The applications of HRMS in a non-targeted analysis of emerging contaminants in the environment have been reviewed previously [94, 95].

6.2 Screening of Halogenated Compounds

Halogenated compounds are persistent in the environment, including polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs), and per- and poly-fluoroalkyl substances (PFASs). PCBs were studied and banned earlier than BFRs and PFASs. BFRs have been widely used in a variety of commercial products including children's sleepwear, foam cushions in chairs, computers, plastics, and electronics to reduce fire-related deaths, injuries, and property damage. Before it was banned, polybrominated diphenyl ethers (PBDEs) have been a popular ingredient in flame retardants. The widespread use of BFRs has led to their widespread presence in the environment and human and wildlife samples, as well as in places far away from where they were produced or used, which has caused the greatest health concern. BFRs are environmentally persistent, lipophilic, and bioaccumulative in animals and humans, and have many congeners. They can also degrade and transform into various transformation products under environmental conditions, which poses a challenge for screening them in samples. PFASs include fluorotelomer acids, alcohols, and sulfonates. So far, more than 4,000 PFASs have been registered. They have been widely used in making stain repellents (such as polytetrafluoroethylene and Teflon) that are widely applied to fabrics and carpets. PFASs are also used in the manufacture of paints, adhesives, waxes, polishes, metals, electronics, and caulks, as well as grease-proof coatings for food packaging. They are also persistent in the environment and in humans. The long-chain

perfluoroalkyl acids (PFAAs) are of particular concern due to the combination of environmental persistence, high bioaccumulation potentials, and more toxicity. Due to the slow development of global regulatory restrictions or bans on long-chain PFASs, they are being replaced by alternative short-chain PFASs. This increases more unknown PFASs, including new alternatives and legacy substances, and their transformation products in the environment, providing a great source of uncertainty for ascertaining environmental and human health risks. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are two types of PFASs. They are mostly monitored and studied for their presence in the environment and humans due to concerns about widespread global distribution in humans and in wildlife. Mass interferences from different halogenated congeners represent an additional challenge for LRMS-based methods to screen and identify halogenated compounds in the environment and humans to deal with environmental and human exposure. Therefore, HRMS has been becoming a powerful tool for screening a broad range halogenated compounds including organohalogenated compounds in various matrix extract [96–98]. The use of HRMS for the screening and characterization of PFASs in environmental and human samples has been recently summarized [99].

6.3 Identification of Contamination Source

In the environment, diffuse pollution sources can impact waters to degrade water quality and adversely affect the health of human and ecosystem. This is especially true for the urban receiving waters, rivers, small streams, and nearshore marine waters. Therefore, identifying pollution sources to reduce pollution and exposure risks is very important in environmental monitoring. However, this is challenging and limits the effectiveness of management actions intended to reduce such risk due to the diversity of pollution sources and the lack of fingerprinting of a broad of contaminants in samples. Accurate identification of potential sources of contaminants is essential for effective water quality management. However, the conventional LC-MS/MS methods can only track a small number of target known indicator compounds of chemical sources, so it is not enough to profile source specificity and characterize identity of the pollution source. Compared with LRMS, high-resolution mass spectrometry combined with LC or GC can detect hundreds to thousands of chemicals easily (both known and unidentified). The use of HRMS-based non-targeted analysis methods can establish unique chemical fingerprints and provide more statistical power for source characterization without relying on pre-existing knowledge or assumptions about chemical composition to distinguish and resolve the contributions of anthropogenic contaminant sources to receiving waters. Recently, a non-targeted analysis method based on LC-HRMS as a proof-of-concept has been successfully used to differentiate the chemical fingerprints of municipal wastewater influent, roadway runoff, and baseflow in urban storm drains [100].

Microplastics (MPs) are defined as synthetic polymers with dimensions less than 5 mm. Once microorganisms are generally unable to mineralize and assimilate them, they will show accumulation and persistence in the environment. Because the ingestion of these substances can cause toxicity to both humans and other living organisms, the presence of MPs in the aquatic environment attracts more attention. As they possess different physicochemical properties from chemicals, it is a challenge to characterize these MPs, especially to track their sources to reduce the exposure risk [101]. The traditional method is to use mass spectrometry to measure the total ion chromatogram to reflect the thermal desorption or pyrolysis degradation products of the polymer, as well as additives and leachable substances, which constitute the "chemical fingerprint" of environmental microplastics [102–104]. This approach using LRMS limits the throughput of analysis and reduces the number of high molecular weight compounds that can be included in the chemical fingerprint. In contrast, HRMS can accurately determine thousands of unique elemental compositions to represent more detailed fingerprints, so that it can distinguish subgroups of microplastics, even for the same polymer type, and rapidly identify the source [105].

6.4 *Transformation and Metabolism of Contaminants in the Environment and Human*

In the aquatic environments, organic contaminants, especially compounds with complex structures (such as pesticides, biocides, and pharmaceuticals) that are currently concerned, can be degraded, metabolized, and transformed to form metabolites or transformation products (TPs), which contribute to the overall chemical burden in the environment. Although the TPs have been found in concentrations that are of concern, they are still rarely considered in water and air quality as well as in chemical risk assessments. Due to insufficient understanding of the overall contribution of TPs to the total chemical substances in the environment, there is a gap in knowledge about TPs and risk assessment. Another challenge is the lack of analytical reference standards for most potential TPs, which makes it difficult for researchers to use traditional LRMS, GC-MS/MS, and LC-MS/MS to quantitatively measure TPs in the environment. In addition, the results of laboratory degradation studies may not be representative of actual environmental conditions. In contrast, HRMS-based method has been successfully used to perform overall screening for the presence of potential TPs of 52 highly used and structurally diverse pesticides, biocides, and pharmaceuticals in representative surface water samples from various locations [106]. Figure 5 shows the retention time of the exact mass ($C_{21}H_{15}N_3O_5$, $[M + H]^+$) at m/z 390.1084 for a TP of the fungicide azoxystrobin (Fig. 5a) and the retention time of the parent compound azoxystrobin (Fig. 5b) in the sample from *Le Boiron*. In this study, the identification of the TPs was performed by a combination of fragmentation patterns (Fig. 5c, d for the TP and the parent compound,

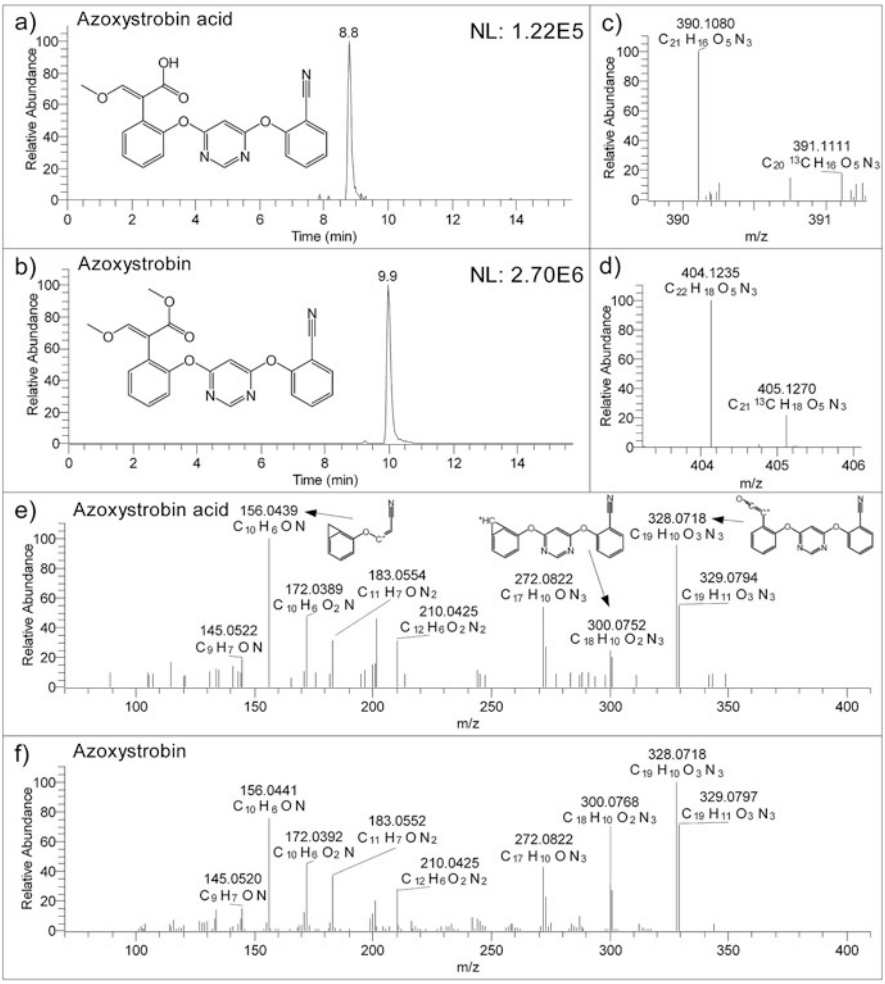


Fig. 5 Structure of the TP azoxystrobin acid and its parent compound, the fungicide azoxystrobin, with the corresponding chromatograms (**a**, **b**), HR-MS spectra of the molecular ions, including isotopic pattern (**c**, **d**), and the HR-MS/MS spectra (**e**, **f**). The drawn fragment structures are those proposed by MassFrontier. All spectra are shown for the sample from *Le Boiron* (Reproduced with permission of Environ. Sci. Technol.) [106]

respectively) and high-resolution MS/MS spectrum (Fig. 5e, f for the TP and the parent compound, respectively). With the aid of MassFrontier fragmentation prediction, the product of ester hydrolysis, azoxystrobin acid, was finally confirmed as a major TP present in the aquatic environment.

Because pharmaceuticals are high-consumption products in the world, they are ubiquitous in the environment. Because of their potential adverse effects on human health, pharmaceuticals as environmental pollutants are receiving more and more attention. Compared with the parent pharmaceuticals, so far, there is little

information about their transformation pathways and their transformation products in the environment. Due to the lack of analytical approaches to monitor these transformation products and metabolites, there is also a lack of information about human occurrence and exposure. Metabolism and transformation are frequently associated with the detoxification of contaminants. However, the transformation products and metabolites can also be toxic or even more toxic than their parent compounds, which can cause health problems by exposure to these transformation products and metabolites. Therefore, it is emerging to screen and identify these pharmaceutical transformation products and metabolites in the environment. Advanced hybrid HRMS (time-of-flight (ToF) or Orbitrap) based non-targeted analysis approaches can provide accurate mass data at satisfactory sensitivity and open up for comprehensive non-targeted screening for pharmaceutical transformation products in environmental samples such as river sediment [107, 108].

7 Future Perspectives

In the past decade, high-resolution mass spectrometers have made dramatic advancements in resolution, scan speed, sensitivity, and hybrid capability. Due to the advancement of HRMS, the use of HRMS for food and environmental analysis has become more and more popular. HRMS-based NTA approaches have already shown the feasibility of detecting and identifying emerging contaminants and unknown compounds in food and environmental samples, which can subsequently trigger future target analysis, exposure-mitigating measures, and new priorities for food and environmental safety. However, up to now, HRMS instrument is still generally considered a research tool, and the NTA based on HRMS is still the frontier research of analytical chemistry. Although applications of HRMS in the monitoring of the wide range of potential contaminants and residues in food and environmental samples, including pesticides, veterinary drugs, and natural toxins, are steadily increasing, there is still a gap between the current methodology and routine food and environmental analysis.

In order to support food and environmental chemicals legislation, any information from HRMS-based non-targeted analysis (NTA) needs to be accompanied by clear statements about the quality of NTA data when it enters the regulatory process. Therefore, a harmonized quality assurance and quality control (QA/QC) protocol from sample collection and preparation to data analysis in the NTA process and minimum quality requirements are needed to provide high-quality data for regulatory processes. Given the unknown nature of the unknown chemicals, these same parameters in the targeted analysis might not be directly applied to NTA, which may affect the NTA's performance in terms of data reliability and reproducibility. Therefore, the confidence level for identifying compounds should be defined and standardized to allow effective communication between users through literature and databases. Chemical space is another issue that needs to be well defined and standardized in the NTA protocol. In general, there is no argument to keep as

much content of potential environmental contaminants and their metabolites as possible in the final sample prior to NTA. However, different definitions of chemical spaces will affect sample preparation process and consequently NTA results in communication with data users. Therefore, when applying NTA, it is necessary to harmonize the definition of chemical spaces and corresponding sample preparation in future food and environmental analysis. In addition, different sample matrices may also affect the NTA results in the same defined chemical space, and sample preparation will directly affect the signal-to-noise ratios and the quality of the MS^2 spectrum. However, there is a consensus that the general sample preparation method always starts from targeting certain chemical substances covering the defined chemical spaces, and these related chemical substances will be measured using NTA for method validation. Meanwhile, it is necessary to develop reference standard mixtures and reference matrix materials for HRMS-based NTA method validation to improve the comparability and reproducibility of NTA data. Of course, the number of standard compounds might not be enough to cover the diversity of the physical properties of all types of unknown chemicals to measure the effectiveness of standard mixtures in NTA performance evaluation, but it is still very useful.

The currently available data acquisition methods are not suitable for automatically recording MS/MS spectral data of unknown components in complex food and environmental samples. Improving analytical throughput and productivity in biotransformation study of food and environmental contaminants is another area that requires continuous efforts. MS/MS spectrum interpretation is still one of bottlenecks in metabolite identification and metabolomics profiling in true unknown analysis, although there are some software packages available on the market. Therefore, it is necessary to develop more software to assist data acquisition, processing, and interpretation to improve the data analysis efficiency and the identification confidence. Identification of isomers is still a challenge. The resolution power of HRMS alone cannot distinguish isomers. There are many isomeric compounds in food and environmental samples, especially in metabolomics study. Therefore, more efforts are needed to improve chromatographic separation methods prior to HRMS analysis to separate and identify isomers in food and environmental samples. The molecular weighs, mass defects, isotope patterns, and fragmentations of truly unknown xenobiotic metabolites in food and environmental samples are not predictable, which is different from drug metabolites. Currently, there is no method that can universally screen all xenobiotics and metabolites in a single injection. Therefore, more NTA methods based on HRMS are needed to identify unknown xenobiotics and metabolites in food and environmental samples.

More reliable data processing tools are needed to enable to automatically assign molecular ions, elemental compositions and formulas, and even structures. The use of commercial or open-source software tools also needs a harmonized data analysis protocol. More tools are needed to automatically interpret complex data and assign molecule formulas when molecular masses exceeds 300 Da and the molecular formula contains more than elements of C, H, N, and O. In addition, more advances are needed in HRMS instruments to provide the isotopic abundance errors (isotopic pattern accuracy) to that data processing tools can quickly and accurately assign

formulas. Compared to higher mass accuracy without isotopic pattern accuracy, mass accuracy with high isotopic pattern accuracy (for example, 2%) can eliminate most false candidates. The declining trend of ion intensity in MS^E spectrum with collision energy can be used as a new additional filter. However, more software tools are needed to be able to automatically record, recognize the decrease of ion intensity in the MS^E spectrum with collision energy, and deconvolution the spectrum mixture to individual chemicals. Furthermore, definition of detectable and identifiable chemical space (chemicals covered in the method) based on an NTA workflow (a sequence of tasks from sample collection and preparation to data analysis) remains challenging and non-standardized. Each step of the NTA workflow, including sample collection, preparation, and NTA data acquisition, processing, and filtering steps will all influence the types of chemicals which are detected and identified. Accordingly, it is challenging to assess whether the non-detection of an analyte in an NTA study indicates true absence in a sample (i.e., blow a detection limit) or is a false negative result caused by limitations of the workflow in which chemicals are missing during the entire analysis procedure from sample preparation to data analysis. Therefore, the definition of criteria for false-positive and false-negative assignments as well as detection limit of NTA method should be standardized in data analysis.

In NTA, sample preparation is usually based on simple extractions, with limited clean-up to minimize the loss of chemicals/metabolites of possible interest from the sample. The sample preparation determines the extractable and detectable chemical space and thereby the identifiable chemical space in NTA results because the preparation step can introduce a bias towards the types of chemicals extracted and detected. Similar to other steps within the NTA workflow, there is currently no consistent approach on how to optimize the sample preparation step, which is critical to obtain reproducible and comparable results.

The current databases, whether they are online databases, commercial databases, or in-house databases, are poorly transferable for their reference spectra. Different libraries define different criteria for determining match scores in data analysis, resulting in that mass annotations based on library matching often inconclusive. This heterogeneity of reference spectra between different platforms hinder the effectiveness of spectral library search in NTA. Therefore, a large platform and server space will be needed in the future to share data by disseminating chromatograms, spectra or suspect lists. In the future, a universal or standardized LC-MS spectral library will be needed for use of HRMS based NTA method in food and environmental analysis.

Considering that there are no standards for unknown compounds in HRMS-based NTA food and environmental analysis, more harmonized prediction tools need to be developed, including retention time (RT) prediction tools and mass fragmentation prediction tools. The predicted RT can help narrow the range of candidate structures by comparing with experimental RTs. The RT prediction models explored so far are limited to current chromatographic methods and cannot be used under all conditions. Further efforts are needed to develop a universal and accurate RT prediction models to increase the confidence of compound identification. Depending on needs,

qualitative information for unknown compounds identified is not enough in exposure assessment and food and environmental safety assessment. To support regulatory activities, there is also a need to develop quantitation prediction tools to provide semi-quantitative level information. So far, there is still a lack of correlation models between the compound concentration and its corresponding mass spectral characteristics, such as ion intensity, peak area, and peak height. The lack of concentration information also hinders the values of NTA data in food quality and safety. Therefore, it is necessary to develop more NTA approaches that can provide qualitative information and quantitative or semi-quantitative information for food quality and safety and environmental safety. Due to the lack of MS/MS spectra for identifying unknown compounds, more in-silico fragmentation prediction tools need to be developed to predict fragmentation patterns and MS/MS spectra for compound identification. The current commercial prediction tools cannot accurately predict fragmentation patterns, nor can they provide suitable predicted MS/MS spectra that match experimental data. There is also a lack of such prediction tools to harmonize the predicted fragments into instrument conditions for the unknown compound.

8 Conclusion

Modern HRMS instruments such as TOF and Orbitrap have been dramatically improved to provide higher resolution, an accurate mass, and higher full scan sensitivity and selectivity than their older counterparts. In addition, the improvement of quantification ability in linear ranges and reproducibility makes HRMS very attractive and effective for both target and non-targeted screening of different groups of food and environmental contaminants. Combination with gas and liquid chromatography and coupled with novel software developments have increased the scope of HRMS in food and environmental analysis, which converts HRMS as a true alternative to conventional triple quadrupole MS instruments in food and environmental analysis to ensure food quality and safety, and environmental safety. Considering that HRMS-based NTA approaches can be used to analyze a large number of compounds simultaneously, additional work is needed to optimize and harmonize its terminology together with NTA workflows, validation, and QA/QC procedures from sample collection and preparation to data analysis. Compared with the workflow and sample preparation, data analysis and data mining are more complicated and challenging due to a large amount of MS data and variable MS spectral quality. Therefore, advance software tools and more efforts are required to minimize the data treatment and increase the data processing speed in NTA processes. Sample preparation needs to reduce matrix effects and retain all unknowns in the final sample. In the next decades, NTA's challenges in the extension and automation of NTA workflows for pattern analysis, compound identification, and accurate quantification are expected to be solved by machine learning from computer capabilities and tools. Regarding food quality and safety and environmental safety and monitoring programs, the possibility of retrospective analysis of full scan data will be the

key benefit of HRMS-based NTA data. This retrospective NTA data allows laboratories to evaluate almost all compounds in a sample, so there is no need to rerun samples to search for "new" or "unexpected" contaminants and metabolites. In the future, improvements and advances in hardware UHPLC and HRMS technologies, software tools and approaches, as well as a larger database combining chemical structures, physicochemical properties, standard LC-HRMS MS/MS spectrum libraries, production tonnages, toxic potential combined with RT prediction models will help to significantly accelerate and automate NTA workflows, thereby increasing the confidence in identifying unknowns. The development of high-throughput procedures that simultaneously screen, identify, and quantify residues, contaminants, and other important chemicals in food and environmental samples will be the next ideal accomplishment. Finally, research institutes, regulatory agencies, and policymakers also need to work together to use NTA data to pave the way for the implementation of NTA techniques in the chemical monitoring and management of food and environmental chemistry.

References

1. IUPAC, Compendium of Chemical Terminology, 2nd edn. (the "Gold Book") (1997) Online corrected version: (2006) resolution in mass spectroscopy. https://doi.org/10.1351/goldbook.R05318
2. IUPAC, Compendium of Chemical Terminology, 2nd edn. (the "Gold Book") (1997) Online corrected version: (2006) resolving power in mass spectrometry. https://doi.org/10.1351/goldbook.R05321
3. Xian F, Hendrickson CL, Marshall AG (2012) High resolution mass spectrometry. Anal Chem 84:708–719
4. Guo Z, Huang S, Wang J, Feng Y-L (2020) Recent advances in non-targeted screening analysis using liquid chromatography – high resolution mass spectrometry to explore new biomarkers for human exposure. Talanta 219:121339. https://doi.org/10.1016/j.talanta.2020.121339
5. Brenton AG, Godfrey AR (2010) Accurate mass measurement: terminology and treatment of data. J Am Soc Mass Spectrom 21:1821–1835
6. Kellmann M, Muenster H, Zomer P, Mol H (2009) Full scan MS in comprehensive qualitative and quantitative residue analysis in food and feed matrices: how much resolving power is required? J Am Soc Mass Spectrom 20:1464–1476
7. Stephens WE (1946) A pulsed mass spectrometer with time dispersion. Phys Rev 69(11–12):691. https://doi.org/10.1103/PhysRev.69.674.2
8. Mamyrin BA (2000) Time-of-flight mass spectrometry (concepts, achievements, and prospects). Int J Mass Spectrom 206:251–266
9. Makarov A (2000) Electrostatic axially harmonic orbital trapping: a high-performance technique of mass analysis. Anal Chem 72(6):1156–1162. https://doi.org/10.1021/ac991131p
10. Hu Q, Noll RJ, Li H, Makarov A, Hardman M, Cooks GR (2005) The orbitrap: a new mass spectrometer. J Mass Spectrom 40(4):430–443. https://doi.org/10.1002/jms.856
11. Hecht ES, Eliuk S, Scigelova M, Makarov A (2019) Fundamentals and advances of orbitrap mass spectrometry. Encyclo Anal Chem. https://doi.org/10.1002/9780470027318.a9309.pub2
12. Getzinger GJ, Ferguson PL (2020) Illuminating the exposome with high-resolution accurate-mass mass spectrometry and nontargeted analysis. Curr Opin Environ Sci Health 15:49–56. https://doi.org/10.1016/j.coesh.2020.05.005

13. Hollender J, van Bavel B, Dulio V, Farmen E, Furtmann K, Koschorreck J, Kunkel U, Krauss M, Munthe J, Schlabach M, Slobodnik J, Stroomberg G, Ternes T, Thomaidis NS, Togola A, Tornero V (2019) High resolution mass spectrometry-based non-target screening can support regulatory environmental monitoring and chemicals management. Environ Sci Eur 31:42. https://doi.org/10.1186/s12302-019-0225-x
14. Yao C, Wang J, Chan P, Feng Y-L (2018) A novel non-targeted screening method for urinary exposure biomarker discovery of phthalates using liquid chromatography-mass spectrometry. Anal Methods 10:959–967
15. Gillet LC, Navarro P, Tate S, Rost H, Selevsek N, Reiter L, Bonner R, Aebersold R (2012) Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. Mol Cell Proteomics 11. https://doi.org/10.1074/mcp.O111.016717
16. Bruderer T, Varesio E, Hopfgartner G (2017) The use of LC predicted retention times to extend metabolites identification with SWATH data acquisition. J Chromatogr A 1071:3–10
17. Zhu X, Chen Y, Subramanian R (2014) Comparison of information-dependent acquisition, SWATH, and MS^{All} techniques in metabolite identification study employing ultrahigh-performance liquid chromatography – quadrupole time of-flight mass spectrometry. Anal Chem 86:1202–1209
18. Roemmelt AT, Steuer AE, Poetzsch M, Kraemer T (2014) Liquid chromatography, in combination with a quadrupole time-of-flight instrument (LC QTOF), with sequential window acquisition of all theoretical fragment-ion spectra (SWATH) acquisition: systematic studies on its use for screenings in clinical and forensic toxicology and comparison with information-dependent acquisition (IDA). Anal Chem 86:11742–11749
19. Bonner R, Hopfgartner G (2016) SWATH acquisition mode for drug metabolism and metabolomics investigations. Bioanalysis 8:1735–1750
20. Arnhard K, Gottschall A, Pitterl F, Oberacher H (2015) Applying 'Sequential windowed acquisition of all theoretical fragment ion mass spectra' (SWATH) for systematic toxicological analysis with liquid chromatography – high-resolution tandem mass spectrometry. Anal Bioanal Chem 407:405–414
21. Bruderer T, Varesio E, Hidasi AO, Duchoslav E, Burton L, Bonner R, Hopfgartner G (2018) Metabolomic spectral libraries for data-independent SWATH liquid chromatography mass spectrometry acquisition. Anal Bioanal Chem 410:1873–1884
22. Hopfgartner G, Tonoli D, Varesio E (2012) High-resolution mass spectrometry for integrated qualitative and quantitative analysis of pharmaceuticals in biological matrices. Anal Bioanal Chem 402:2587–2596
23. Jaeger C, Meret M, Schmitt CA, Lisec J (2017) Compound annotation in liquid chromatography/high-resolution mass spectrometry based metabolomics: robust adduct ion determination as a prerequisite to structure prediction in electrospray ionization mass spectra. Rapid Commun Mass Spectrom 31:1261–1266
24. Crimmins BS, Xia X, Hopke PK, Holsen TM (2014) A targeted/non-targeted screening method for perfluoroalkyl carboxylic acids and sulfonates in whole fish using quadrupole time-of-flight mass spectrometry and MSe. Anal Bioanal Chem 406:1471–1480
25. Cariou R, Omer E, Léon A, Dervilly-Pinel G, Le Bizec B (2016) Screening halogenated environmental contaminants in biota based on isotopic pattern and mass defect provided by high resolution mass spectrometry profiling. Anal Chim Acta 936:130–138
26. Lohne JJ, Turnipseed SB, Andersen WC, Storey J, Madson MR (2015) Application of single-stage orbitrap mass spectrometry and differential analysis software to nontargeted analysis of contaminants in dog food: detection, identification, and quantification of glycoalkaloids. J Agric Food Chem 63:4790–4798
27. Schymanski EL, Jeon J, Gulde R, Fenner K, Ruff M, Singer HP, Hollender J (2014) Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ Sci Technol 48:2097–2098

28. Kind T, Fiehn O (2006) Metabolomic database annotations via query of elemental compositions: mass accuracy is insufficient even at less than 1 ppm. BMC Bioinform 7:234. https://doi.org/10.1186/1471-2105-7-234
29. Kind T, Fiehn O (2010) Advances in structure elucidation of small molecules using mass spectrometry. Bioanal Rev 2:23–60
30. Pluskal T, Uehara T, Yanagida M (2012) Highly accurate chemical formula prediction tool utilizing high-resolution mass spectra, MS/MS fragmentation, heuristic rules, and isotope pattern matching. Anal Chem 84:4396–4403
31. Ibáñez C, Simó C, García-Cañas V, Acunha T, Cifuentes A (2015) The role of direct high-resolution mass spectrometry in foodomics. Anal Bioanal Chem 407:6275–6287. https://doi.org/10.1007/s00216-015-8812-1
32. Lloyd AJ, Beckmann M, Wilson T, Tailliart K, Allaway D, Draper J (2017) Ultra high performance liquid chromatography–high resolution mass spectrometry plasma lipidomics can distinguish between canine breeds despite uncontrolled environmental variability and non-standardized diets. Metabolomics 13:15. https://doi.org/10.1007/s11306-016-1152-0
33. Matsuzawa Y, Higashi Y, Takano K, Takahashi M, Yamada Y, Okazaki Y, Nakabayashi R, Saito K, Tsugawa H (2021) Food lipidomics for 155 agricultural plant products. J Agric Food Chem 69:8981–8990
34. Li Q, Zhao Y, Zhu D, Pang X, Liu Y, Frew R, Chen G (2017) Lipidomics profiling of goat milk, soymilk and bovine milk by UPLC-Q-Exactive orbitrap mass spectrometry. Food Chem 224:302–309
35. Lu H, Chen H, Tang X, Yang Q, Zhang H, Chen YQ, Chen W (2019) Ultra performance liquid chromatography−Q exactive orbitrap/mass spectrometry-based lipidomics reveals the influence of nitrogen sources on lipid biosynthesis of mortierella *alpina*. J Agric Food Chem 67:10984–10993
36. Sun T, Wang X, Cong P, Xu J, Xue C (2020) Mass spectrometry-based lipidomics in food science and nutritional health: a comprehensive review. Compr Rev Food Sci Food Saf 19:2530–2558
37. Wu B, Wei F, Xu S, Xie Y, Lv X, Chen H, Huang F (2021) Mass spectrometry-based lipidomics as a powerful platform in foodomics research. Trends Food Sci Technol 107:358–376
38. Bianco M, Calvano CD, Ventura G, Losito I, Cataldi TRI (2022) Determination of hidden milk allergens in meat-based foodstuffs by liquid chromatography coupled to electrospray ionization and high-resolution tandem mass spectrometry. Food Control 131:108443
39. Chopra A, Ali SA, Bathla S, Rawat P, Vohra V, Kumar S, Mohanty AK (2020) High-resolution mass spectrometer–based ultra-deep profile of milk whey proteome in Indian Zebu (*Sahiwal*) cattle. Front Nutr 7:150. https://doi.org/10.3389/fnut.2020.00150
40. Gavagea M, Van Vlierberghe K, Van Poucke C, De Loose M, Gevaert K, Dieub M, Renard P, Arnould T, Gillard N (2019) Selection of egg peptide biomarkers in processed food products by high resolution mass spectrometry. J Chromatogr A 1584:115–125
41. Bracker J, Brockmeyer J (2018) Characterization and detection of food allergens using high-resolution mass spectrometry: current status and future perspective. J Agric Food Chem 66:8935–8940
42. Marzano V, Tilocca B, Fiocchi AG, Vernocchi P, Mortera SL, Urbani A, Roncada P, Putignani L (2020) Perusal of food allergens analysis by mass spectrometry-based proteomics. J Proteomics 215:103636
43. Yang CT, Ghosh D, Beaudry F (2018) Detection of gelatin adulteration using bio-informatics, proteomics and high-resolution mass spectrometry. Food Addit Contam A 35(4):599–608. https://doi.org/10.1080/19440049.2017.1416680
44. Orduna AR, Husby E, Yang CT, Ghosh D, Beaudry F (2017) Detection of meat species adulteration using high-resolution mass spectrometry and a proteogenomics strategy. Food Addit Contam A 34(7):1110–1120. https://doi.org/10.1080/19440049.2017.1329951

45. Cunsolo V, Muccilli V, Saletti R, Foti S (2014) Mass spectrometry in food proteomics: a tutorial. J Mass Spectrom 49:768–784
46. Korte R, Brockmeyer J (2017) Novel mass spectrometry approaches in food proteomics. Trends Anal Chem 96:99–106
47. Andjelković U, Josić D (2018) Mass spectrometry based proteomics as foodomics tool in research and assurance of food quality and safety. Trends Food Sci Technol 77:100–119
48. Bahmani M, O'Lone CE, Juhász A, Nye-Wood M, Dunn H, Edwards IB, Colgrave ML (2021) Application of mass spectrometry-based proteomics to Barley research. J Agric Food Chem 69:8591–8609
49. Chang WC-W, Wu H-Y, Kan H-L, Lin Y-C, Tsai P-J, Chen Y-C, Pan Y-Y, Liao P-C (2021) Discovery of spoilage markers for chicken eggs using liquid chromatography-high resolution mass spectrometry-based untargeted and targeted foodomics. J Agric Food Chem 69:4331–4341
50. Jia W, Shi Q, Shi L, Qin J, Chang J, Chu X (2020) A strategy of untargeted foodomics profiling for dynamic changes during Fu brick tea fermentation using ultrahigh-performance liquid chromatography-high resolution mass spectrometry. J Chromatogr A 1618:460900
51. Jia W, Dong X, Shi L, Chu X (2020) Discrimination of milk from different animal species by a foodomics approach based on high-resolution mass spectrometry. J Agric Food Chem 68: 6638–6645
52. Jia W, Shi L, Zhang F, Chang J, Chu X (2019) High-throughput mass spectrometry scheme for screening and quantification of flavonoids in antioxidant nutraceuticals. J Chromatogr A 1608: 460408
53. Chang WC-W, Wu H-Y, Yeh Y, Liao P-C (2020) Untargeted foodomics strategy using high-resolution mass spectrometry reveals potential indicators for fish freshness. Anal Chim Acta 1127:98–105
54. Jia W, Wang H, Shi L, Zhang F, Fan C, Chen X, Chang J, Chu X (2019) High-throughput foodomics strategy for screening flavor components in dairy products using multiple mass spectrometry. Food Chem 279:1–11
55. Senyuva HZ, Gökmen V, Sarikaya EA (2015) Future perspectives in Orbitrap™-high-resolution mass spectrometry in food analysis: a review. Food Addit Contam A 32(10):1568–1606. https://doi.org/10.1080/19440049.2015.1057240
56. Rubert J, Zachariasova M, Hajslova J (2015) Advances in high resolution mass spectrometry based on metabolomics studies for food – a review. Food Addit Contam A 32(10):1685–1708. https://doi.org/10.1080/19440049.2015.1084539
57. Herrero M, Simo C, Garcia-Canas V, Ibanez E, Cifuentes A (2012) Foodomics: MS-based strategies in modern food science and nutrition. Mass Spectrom Rev 31:49–69
58. Valdés A, Simó C, Ibáñez C, García-Cañas V (2013) Foodomics strategies for the analysis of transgenic foods. Trends Anal Chem 52:2–15
59. Rychlik M, Kanawati B, Schmitt-Kopplin P (2017) Foodomics as a promising tool to investigate the mycobolome. Trends Anal Chem 96:22–30
60. Hu C, Xu G (2013) Mass-spectrometry-based metabolomics analysis for foodomics. Trends Anal Chem 52:36–46
61. Fu Y, Zhao C, Lu X, Xu G (2017) Nontargeted screening of chemical contaminants and illegal additives in food based on liquid chromatography–high resolution mass spectrometry. TrAC Trends Anal Chem 96:89–98
62. Kaufmann A, Walker S (2013) Post-run target screening strategy for ultra high performance liquid chromatography coupled to orbitrap based veterinary drug residue analysis in animal urine. J Chromatogr A 1292:104–110
63. Quifer-Rada P, Vallverdú-Queralt A, Martínez-Huélamo M, Chiva-Blanch G, Jáuregui O, Estruch R, Lamuela-Raventós R (2015) A comprehensive characterisation of beer polyphenols by high resolution mass spectrometry (LC–ESI-LTQ-orbitrap-MS). Food Chem 169:336–343
64. Schusterova D, Suchanova M, Pulkrabova J, Kocourek V, Urban J, Hajslova J (2019) Can occurrence of pesticide metabolites detected in crops provide the evidence on illegal practices

in organic farming? J Agric Food Chem 67(22):6102–6115. https://doi.org/10.1021/acs.jafc.8b06999
65. Lopez-Ruiz R, Belmonte-Sanchez E, Romero-Gonzalez R, Vidal JLM, Ramírez-Cassinello JJ, Frenich AG (2021) Laboratory study on dissipation and risk assessment of the proinsecticide thiocyclam and its metabolite nereistoxin in tomato using liquid chromatography high resolution mass spectrometry. Food Chem 344:128729. https://doi.org/10.1016/j.foodchem.2020.128729. Epub 2020 Nov 28
66. Rivera-Pérez A, López-Ruiz R, Romero-González R, Frenich AG (2020) A new strategy based on gas chromatography–high resolution mass spectrometry (GC–HRMS-Q-orbitrap) for the determination of alkenylbenzenes in pepper and its varieties. Food Chem 321:126727
67. Müller A, Schulz W, Ruck WKL, Weber WH (2011) A new approach to data evaluation in the non-target screening of organic trace substances in water analysis. Chemosphere 85:1211–1219
68. Wang X, Wang S, Cai Z (2013) The latest developments and applications of mass spectrometry in food-safety and quality analysis. TrAC Trends Anal Chem 52:170–185
69. Galani JHY, Houbraken M, Wumbei A, Djeugap JF, Fotio D, Spanoghe P (2018) Evaluation of 99 pesticide residues in major agricultural products from the western highlands zone of cameroon using QuEChERS method extraction and LC-MS/MS and GC-ECD analyses. Foods 7:184
70. Moreno-González D, Pérez-Ortega P, Gilbert-López B, Molina-Díaz A, García-Reyes JF, Fernández-Alba AR (2017) Evaluation of nanoflow liquid chromatography high resolution massspectrometry for pesticide residue analysis in food. J Chromatogr A 1512:78–87
71. Ishibashi M, Izumi Y, Sakai M, Ando T, Fukusaki E, Bamba T (2015) High-throughput simultaneous analysis of pesticides by supercritical fluid chromatography coupled with high-resolution mass spectrometry. J Agric Food Chem 63:4457–4463. https://doi.org/10.1021/jf5056248
72. Wang J, Chow W, Leung D, Chang J (2012) Application of ultrahigh-performance liquid chromatography and electrospray ionization quadrupole orbitrap high-resolution mass spectrometry for determination of 166 pesticides in fruits and vegetables. J Agric Food Chem 60:12088–12104., dx.doi.org. https://doi.org/10.1021/jf303939s
73. Besil N, Cesio V, Heinzen H, Fernandez-Alba AR (2017) Matrix effects and interferences of different citrus fruit coextractives in pesticide residue analysis using ultrahigh-performance liquid chromatography-high-resolution mass spectrometry. J Agric Food Chem 65:4819–4829. https://doi.org/10.1021/acs.jafc.7b00243
74. Yang P, Chang JS, Wong JW, Zhang K, Krynitsky AJ, Bromirski M, Wang J (2015) Effect of sample dilution on matrix effects in pesticide analysis of several matrices by liquid chromatography-high-resolution mass spectrometry. J Agric Food Chem 63:5169–5177. https://doi.org/10.1021/jf505168v
75. Hu S, Zhao M, Mao Q, Fang C, Chen D, Yan P (2019) Rapid one-step cleanup method to minimize matrix effects for residue analysis of alkaline pesticides in tea using liquid chromatography–high resolution mass spectrometry. Food Chem 299:125146
76. Saito-Shida S, Hamasaka T, Nemoto S, Akiyama H (2018) Multiresidue determination of pesticides in tea by liquid chromatography high-resolution mass spectrometry: comparison between orbitrap and time of-flight mass analyzers. Food Chem 256:140–148
77. Wang F, Li S, Feng H, Yang Y, Xiao B, Chen D (2019) An enhanced sensitivity and cleanup strategy for the nontargeted screening and targeted determination of pesticides in tea using modified dispersive solid-phase extraction and cold-induced acetonitrile aqueous two-phase systems coupled with liquid chromatography-high resolution mass spectrometry. Food Chem 275:530–538
78. Bauer A, Jesús F, Ramos MJG, Lozano A, Fernández-Alba AR (2019) Identification of unexpected chemical contaminants in baby food coming from plastic packaging migration by high resolution accurate mass spectrometry. Food Chem 295:274–288

79. Wong JW, Wang J, Chow W, Carlson R, Jia Z, Zhang K, Hayward DG, Chang JS (2018) Perspectives on liquid chromatography−high-resolution mass spectrometry for pesticide screening in foods. J Agric Food Chem 66:9573–9581
80. Martínez-Domínguez G, Romero-González R, Frenich AG (2016) Multi-class methodology to determine pesticides and mycotoxins in green tea and royal jelly supplements by liquid chromatography coupled to orbitrap high resolution mass spectrometry. Food Chem 197: 907–915
81. Alcántara-Durán J, Moreno-González D, García-Reyes JF, Molina-Díaz A (2019) Use of a modified QuEChERS method for the determination of mycotoxin residues in edible nuts by nano flow liquid chromatography high resolution mass spectrometry. Food Chem 279:144–149
82. Romera D, Mateo EM, Mateo-Castro R, Gómez JV, Gimeno-Adelantado JV, Jiménez M (2018) Determination of multiple mycotoxins in feedstuffs by combined use of UPLC–MS/MS and UPLC–QTOF–MS. Food Chem 267:140–148
83. Zachariasova M, Cajka T, Godula M, Malachova A, Veprikova Z, Hajslova J (2010) Analysis of multiple mycotoxins in beer employing (ultra)-high-resolution mass spectrometry. Rapid Commun Mass Spectrom 24:3357–3367
84. von Bargen KW, Lohrey L, Cramer B, Humpf H-U (2012) Analysis of the fusarium mycotoxin moniliformin in cereal samples using $^{13}C_2$-moniliformin and high-resolution mass spectrometry. J Agric Food Chem 60:3586–3591., dx.doi.org. https://doi.org/10.1021/jf300323d
85. De Paepe E, Wauters J, Van Der Borght M, Claes J, Huysman S, Croubels S, Vanhaecke L (2019) Ultra-high-performance liquid chromatography coupled to quadrupole orbitrap high-resolution mass spectrometry for multi-residue screening of pesticides, (veterinary) drugs and mycotoxins in edible insects. Food Chem 293:187–196
86. Rubert J, León N, Sáez C, Martins CPB, Godula M, Yusà V, Manes J, Soriano JM, Soler C (2014) Evaluation of mycotoxins and their metabolites in human breast milk using liquid chromatography coupled to high resolution mass spectrometry. Anal Chim Acta 820:39–46
87. Beccari G, Caproni L, Tini F, Uhlig S, Covarelli L (2016) Presence of fusarium species and other toxigenic fungi in malting barley and multi-mycotoxin analysis by liquid chromatography-high-resolution mass spectrometry. J Agric Food Chem 64:4390–4399. https://doi.org/10.1021/acs.jafc.6b00702
88. Ates E, Godula M, Stroka J, Senyuva H (2014) Screening of plant and fungal metabolites in wheat, maize and animal feed using automated on-line clean-up coupled to high resolution mass spectrometry. Food Chem 142:276–284
89. Chiaia-Hernandez AC, Krauss M, Hollender J (2013) Screening of lake sediments for emerging contaminants by liquid chromatography atmospheric pressure photoionization and electrospray ionization coupled to high resolution mass spectrometry. Environ Sci Technol 47:976–986., dx.doi.org. https://doi.org/10.1021/es303888v
90. Bade R, Bijlsma L, Miller TH, Barron LP, Sancho JV, Hernández F (2015) Suspect screening of large numbers of emerging contaminants in environmental waters using artificial neural networks for chromatographic retention time prediction and high resolution mass spectrometry data analysis. Sci Total Environ 538:934–941
91. Brack W, Hollender J, de Alda ML, Müller C, Schulze T, Schymanski E, Slobodnik J, Krauss M (2019) High-resolution mass spectrometry to complement monitoring and track emerging chemicals and pollution trends in European water resources. Environ Sci Eur 31:62
92. Hollender J, Bourgin M, Fenner KB, Longrée P, McArdell CS, Moschet C, Ruff M, Schymanski EL, Singer HP (2014) Exploring the behaviour of emerging contaminants in the water cycle using the capabilities of high resolution mass spectrometry. Chimia 68:793–798. https://doi.org/10.2533/chimia.2014.793
93. Alygizakis NA, Samanipour S, Hollender J, Ibanez M, Kaserzon S, Kokkali V, van Leerdam JA, Mueller JF, Pijnappels M, Reid MJ, Schymanski EL, Slobodnik J, Thomaidis NS, Thomas KV (2018) Exploring the potential of a global emerging contaminant early warning network

through the use of retrospective suspect screening with high-resolution mass spectrometry. Environ Sci Technol 52:5135–5144
94. Picó Y, Barceló D (2015) Transformation products of emerging contaminants in the environment and high-resolution mass spectrometry: a new horizon. Anal Bioanal Chem 407:6257–6273. https://doi.org/10.1007/s00216-015-8739-6
95. Richardson SD (2010) Environmental mass spectrometry: emerging contaminants and current issues. Anal Chem 82:4742–4774
96. Cariou R, Omer E, Leon A, Dervilly-Pinel G, Le Bizec B (2016) Screening halogenated environmental contaminants in biota based on isotopic pattern and mass defect provided by high resolution mass spectrometry profiling. Anal Chim Acta 936:130–138
97. Tang C, Tan J (2018) Quasi-targeted analysis of halogenated organic pollutants in fly ash, soil, ambient air and flue gas using gas chromatography-high resolution mass spectrometry with isotopologue distribution comparison and predicted retention time alignment. J Chromatogr A 1555:74–88
98. Bogdal C, Alsberg T, Diefenbacher PS, MacLeod M, Berger U (2015) Fast quantification of chlorinated paraffins in environmental samples by direct injection high-resolution mass spectrometry with pattern deconvolution. Anal Chem 87:2852–2860. https://doi.org/10.1021/ac504444d
99. Liu Y, D'Agostino LA, Qu G, Jiang G, Martin JW (2019) High-resolution mass spectrometry (HRMS) methods for nontarget discovery and characterization of poly- and per-fluoroalkyl substances (PFASs) in environmental and human samples. Trends Anal Chem 121:115420
100. Du B, Tian Z, Peter KT, Kolodziej EP, Wong CS (2020) Developing unique nontarget high-resolution mass spectrometry signatures to track contaminant sources in urban waters. Environ Sci Technol Lett 7:923–930. https://doi.org/10.1021/acs.estlett.0c00749
101. Alvim CB, Mendoza-Roca JA, Bes-Pia A (2020) Wastewater treatment plant as microplastics release source – quantification and identification techniques. J Environ Manage 255:109739
102. Fries E, Dekiff JH, Willmeyer J, Nuelle M-T, Ebert M, Remy D (2013) Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy. Environ Sci: Processes Impacts 15:1949–1956
103. Dümichen E, Eisentraut P, Bannick CG, Barthel A-K, Senz R, Braun U (2017) Fast identification of microplastics in complex environmental samples by a thermal degradation method. Chemosphere 174:572–584
104. Fischer M, Scholz-Bottcher BM (2017) Simultaneous trace identification and quantification of common types of microplastics in environmental samples by pyrolysis-gas chromatography-mass spectrometry. Environ Sci Technol 51:5052–5060
105. Zhang X, Mell A, Li F, Thaysen C, Musselman B, Tice J, Vukovic D, Rochman C, Helm PA, Jobst KJ (2020) Rapid fingerprinting of source and environmental microplastics using direct analysis in real time-high resolution mass spectrometry. Anal Chim Acta 1100:107–117. https://doi.org/10.1016/j.aca.2019.12.005
106. Kern S, Fenner K, Singer HP, Schwarzenbach RP, Hollender J (2009) Identification of transformation products of organic contaminants in natural waters by computer-aided prediction and high-resolution mass spectrometry. Environ Sci Technol 43(18):7039–7046. https://doi.org/10.1021/es901979h
107. Li Z, Maier MP, Radke M (2014) Screening for pharmaceutical transformation products formed in river sediment by combining ultrahigh performance liquid chromatography high resolution mass spectrometry with a rapid data-processing method. Anal Chim Acta 810:61–70
108. Calza P, Medana C, Sarro M, Rosato V, Aigotti R, Baiocchi C, Minero C (2014) Photocatalytic degradation of selected anticancer drugs andidentification of their transformation products in water by liquidchromatography–high resolution mass spectrometry. J Chromatogr A 1362:135–144

Omics Approaches in Food and Environmental Analysis

Marinella Farré

Contents

Abstract Environmental-OMICS includes the applications of genomics, transcriptomics, proteomics, and metabolomics to understand better genetics, toxicity mechanisms, and modes of action in response to acute and chronic exposure to chemical pollution on aquatic and terrestrial organisms, and to understand which molecular events initiate these effects. These are essential goals in toxicology to predict adverse responses better or look for more efficient remediation approaches. In parallel and closely related, foodomics is a new discipline (Cifuentes, J Chromatogr A 1216:7109, 2009) applying the same omics technologies to study food and nutrition. It is a global discipline that integrates compound profiling assessment in food, food authenticity, and biomarker-detection related to food quality or safety, including contaminants in food, the development of transgenic foods, investigations on food bioactivity, and food effects on human health.

In both cases, the state-of-the-art technologies to assess effects and new mass spectrometry (MS) approaches combined with bioinformatics are crucial to answer

M. Farré (✉)
Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain
e-mail: mfuqam@cid.csic.es

Yolanda Picó and Julian Campo (eds.), *Mass Spectrometry in Food and Environmental Chemistry*, Hdb Env Chem (2023) 119: 187–224, DOI 10.1007/698_2022_893,

Published online: 18 October 2022

the main questions driving environmental-omics and foodomics, which are included in the One-Health concept. Human health is cross related to our environment, the organisms in this environment, and the food chain.

The primary aim of the present chapter is to provide an overview of the different strategies that have been used in recent years in the environmental-omics and foodomics with the common driver of the One-Health concept. The advantages and limitations will be discussed as well, and finally, new trends presented.

Keywords Foodomics, Genomics, Lipidomics, Metabolomics, Nutrigenomics, Proteomics, Toxicogenomics, Transcriptomics

1 Genomics, Toxicogenomics, Transcriptomics, and Nutrigenomics

Microarrays are high-throughput analytic devices for simultaneously monitoring quantitative transcription of thousands of genes in parallel, providing comprehensive genome-scale expression. This technology uses synthesized oligonucleotide targets on a solid platform that acts to hybridize with complementary probes generated with fluorescent tags from a biological sample (Fig. 1).

In the last 20 years, impressive advances have been produced in *environmental toxicogenomics* thanks to microarrays and RNA-seq for gene expression analysis. In most cases, model species are used, and then the represented genes are targeted, but for non-model species, uncharacterized targets continue being a challenge, even though the improvement in annotation of microarrays. Gene expression profiling has been used in ecotoxicology to study the effects of different contaminants both organic (pesticides, pharmaceuticals, industrial origin compounds, endocrine disruptors, and nanomaterials, among others) and inorganic such as metals. The platforms using complementary DNA (cDNA) strands were used to study chemicals during the last decade. On the other hand, RNA technologies, such as RNA-seq, entail massive parallel sequencing of transcripts using methods developed by Illumina (NextSeq, TruSeq); and Thermo Fisher Scientific (Ion Torrent); and Pacific Biosystems, among many others. Longer reads or whole genome and targeted sequencing, RNA sequencing, and epigenetics are now possible. In Table 1, there is a summary of the most used Whole Genome Sequencing platforms.

For example, Gómez-Sagasti et al. [1] studied the early gene expression profiles in *Escherichia coli* cells exposed to three concentrations of a mixture of heavy metals by cDNA microarrays. At the lowest metal dose, the effects were an activation of metal-specific transporters and a mobilization of glutathione transporters involved in metal sequestration. At the intermediate dose, an overexpression of ROS scavengers and heat shock proteins were observed. Finally, at the highest dose, *E. coli* cells showed repression of genes related to DNA mutation correctors such as MutY glycopeptidases. In another example, the changes in the genomic expression

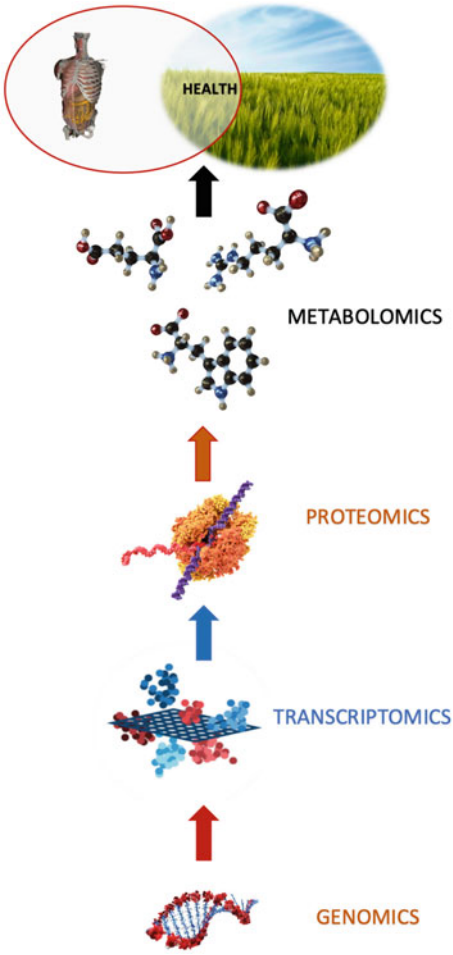


Fig. 1 Summary of main omic fields applied to environmental-omics and foodomics

Table 1 Summary of commonly used Whole Genome platforms

Platform	Sequencing technology	Read length	Output	Error rate	Type of instrument
Illumina	Sequencing by synthesis	Short reads 1 × 36 bp–2 × 300 bp	0.3–1000 Gb	Low	Benchtop 2–29 h
Ion torrent	Sequencing by synthesis	Short reads 200–400 bp	0.6–15 Gb	Low	Benchtop 2–4 h
PacBio	Single molecule sequencing by synthesis	Long reads Up to 60 kb	0.5–10 Gb	High	Large scale 0.5–4 h
Oxford nanopore	Single molecule	Long reads Up to 100 kb	0.1–20 Gb	High	Portable 1 min–48 h

profile of marine medaka fish, *Oryzias javanicus* exposed to 4-nonylphenol were studied [2]. The genes differentially expressed were associated with oestrogenic signalling, immune responses, nerve cell maturation, cellular stress and detoxification, tumour transformation, glucose metabolism, RNA processing, lipid biosynthesis, and iron metabolism. These genes could be used as molecular biomarkers of endocrine-disrupting chemicals' biological responses. Sanchez et al. used transcriptomics in male zebrafish after 96-h exposition to study the mode of action of the fungicide chlorothalonil [3]. Gene expression analysis revealed changes in transcriptional sub-networks related to cell division, reproduction, immunity, DNA damage, and xenobiotic clearance.

In addition to laboratory exposures, transcriptomics is becoming more widely applied in field-based experiments of pollutants in the aquatic media. Collí-Dulá et al. used an oligonucleotide microarray to study perfluoroalkyl substances (PFASs) effects on wild largemouth bass [4]. In this study, transcriptomic profiling was conducted in the liver and testis of fish collected from five different lakes to determine molecular signatures. PFASs in tissues were associated with the expression of genes related to lipid metabolism, energy production, RNA processing, protein production/degradation, and contaminant detoxification, all of which were consistent with biomarker responses observed in other studies with PFASs. One of the primary outcomes of this study was how to distinguish the responses due to PFASs compared to other types of pollutants. It should be pointed out that for field studies is necessary the site characterization, with water quality characteristics such as temperature, dissolved oxygen, pH, humic substances, among other variables, to determine better the relative proportion of these factors to the transcriptional responses observed [5].

Transcriptomics can be employed for monitoring pollution over time and to assess biological responses before and after site remediation. Martyniuk et al. [6] used largemouth bass placed into ponds located in muck farms in the Lake Apopka, Florida region [6]. These ponds had a high contaminant load, primarily organochlorine pesticides (OCPs) that were used about 50 years ago on crops. The results revealed that largemouth bass inhabiting the ponds for 4 months showed significantly higher levels of organochlorine pesticides in their muscle, such as DDE, dieldrin, and methoxychlor compared to background levels. Moreover, these high contaminant loads in the muscle corresponded to lower plasma 17β-estradiol but there were no significant changes in gonadosomatic index or plasma vitellogenin levels. On the other hand, transcriptomics profiling suggested that genes involved in reproduction and immune function were significantly changed in fish placed in the mesocosm compared to largemouth bass collected from different reference sites. This study provided new information about the gene regulatory networks affected by OCPs being the most outstanding observation that these contaminants affect the immune system. Recently, Mehinto et al. [7] characterized the toxicological responses induced by persistent organic pollutants in wild-caught horny head turbot (*P. verticalis*). To do so, horny head turbot was intraperitoneally injected with a single dose of polychlorinated biphenyl (PCB) or polybrominated diphenyl ethers (PBDEs) congeners prepared using environmentally realistic mixture proportions.

After 96-h exposure, the livers were excised and analysed using transcriptomic approaches and analytical chemistry. Concentrations of PCBs and PBDEs measured in the livers indicated clear differences across treatments. Distinct gene profiles were characterized for PCBs and PBDEs exposed fish, with significant differences observed in the expression of genes associated with immune responses, endocrine-related functions, and lipid metabolism. This study provided further evidence of the potential of transcriptomics to improve aquatic health assessment and identify causative agents.

During recent years, toxicogenomics has been as well applied to assess plants responses to different exposures. Heavy metal contamination has become one of the important determinants of crop productivity recently. Heavy metal triggers various stimuli in plants, leading to complex mechanisms at the genetic, molecular, biochemical, and physiological levels. Phytoremediation based approaches stand second in bioremediation technologies meant for clean-up of soils/sites contaminated with heavy metals. Recent development in plant genomics, transcriptomics, proteomics, and metabolomics aided in understanding the role of several genes, transcription factors, and metabolites involved in conferring metal tolerance [8]. This branch bridges food safety and environmental analysis.

Genomics is a powerful tool for food safety analysis especially in foodborne bacteria outbreaks. In particular, the Next Generation Sequencing (NGS) combined with bioinformatic approaches is a step forward in food microbiology. The whole genome sequencing (WGS) of single isolates is a new approach that allows detailed comparison of individual strains. There are two main approaches:

- single nucleotide polymorphism (SNP) analysis and
- genomic multi-locus sequence typing (MLST)

Metabarcoding and metagenomics, applied to total DNA isolated from either foods or from the production environment, allow the identification of complete microbial populations. Metagenomics identifies the gene content and when these approaches are coupled to transcriptomics or proteomics, allows the identification of the biochemical activity of microbial populations. The technology has shown a rapid development, with continuous improvement in quality and the reduction of costs and nowadays is having a major influence on food microbiology. Therefore, WGS of food pathogens has been introduced into public health surveillance rapidly compared with other methodological advancements [9]. Initially NGS was employed for the retrospective analyses of outbreaks of foodborne illnesses, whilst now has been introduced for prospective surveillance of bacterial foodborne pathogens in Denmark, The United Kingdom, France, and the USA [10–12]. It should be pointed out that after 1 year of implementation in the USA successful results have been shown. The outbreaks were detected earlier and the source more often, and the total number of outbreaks increased [13].

In a recent study, Mahoney et al [14]. evaluated the association between *Listeria monocytogenes* genomic characteristics and adhesion to polystyrene at 8°C. *L. monocytogenes* can be adhered to surfaces establishing itself in food production facilities. This study evaluated the adhesion ability of *L. monocytogenes* and

associations between the genetic factors such as internalin A (inlA) genotypes, stress survival islet 1 (SSI-1) genotype, and clonal complex (CC) and the observed phenotypes. The results indicated that premature stop codons in the virulence gene inlA were associated with increased cold adhesion, while invasion enhancing deletion in inlA was associated with the decrease. The results suggested that there is an evolutionary trade-off between virulence and adhesion in *L. monocytogenes*. These results provided understanding of *L. monocytogenes* adhesion in food production surfaces enabling to develop strategies to reduce their occurrence in the food system.

Genomics can be used to assess the origin of some bacterial foodborne transmission. For example, Group B Streptococcus (GBS) outbreak, associated with freshwater fish consumption, occurred in Singapore in 2015. GBS has not been generally recognized as a foodborne pathogen. Therefore, its occurrence in food is not usually tested. A study by Chau et al. [15] was conducted to determine the frequency and distribution of GBS sequence types in local retail food in Singapore. The results in food from retailed stores were compared with those from regional fish isolate collection and published sequence types of human isolates. GBS was detected in 3.8% of the prepared seafood samples and 18% of the unprepared food samples. GBS was most frequently detected in fish among the unprepared food sampled, followed by meat, non-fish seafood, and vegetables. GBS sequence type 283 was identified in a sample of uncooked oyster. Seven other known sequence types (1, 3, 7, 10, 23, 24, and 103) and three newly assigned sequence types (1,647, 1,648 and 1,649) were isolated from the studied foods.

Food pathogen genomics is a rapidly developing field and comprises specialities, such as bioinformatics, which have not been subjected to standardization previously. For this reason, there is a need for standardization to assure that data generated is accurate, reliable, and globally comparable regardless of the sequencing platform, the bioinformatic approach, and software used. In that sense, different quality assurance instruments are developing including proficiency tests for microbial WGS analysis. Other initiatives include characterized sets of strains available for evaluating the performance of bioinformatic pipelines. For example, outbreak benchmark dataset including sequence data, metadata, and corresponding known phylogenetic trees for some bacterial species such as *L. monocytogenes*, *Escherichia coli*, and *Campylobacter jejuni* have been launched (https://github.com/WGS-standards-and-analysis/Datasets) [16].

Although WGS has revolutionized the molecular typing of pathogens, the main challenges to be addressed are:

- Further work on standardization is requested.
- Investigation into the role of environmental niches on the mutation rates of pathogens should be performed.
- Exploration of the value of mobile genetic element WGS analysis should also be more used.
- Industrial implementation should increase: WGS of bacterial isolates completely changes the way that subtyping has traditionally been performed. This, together

with the significant analytical costs along with the knowledge and competency requirements are currently barriers for its wider use by industry.

Two approaches with NGS technologies are used to study the microbial communities without bacterial culture:

- Amplicon sequencing or *Metabarcoding*, which is based on the amplification and sequencing of specific marker gene families [17]; and
- *Metagenomics*: the random shotgun sequencing of the whole genomic content of communities.

Metabarcoding requires the isolation of DNA directly from samples. Extracted DNA undergoes targeted PCR amplification of phylogenetic marker genes. Massive parallel sequencing of amplicons generates an array of profiling information about the microbiota associated with food products. Finally, the sequencing data is processed with bioinformatic pipelines to structure and annotate the raw information into knowledge.

The potency to follow the succession of microbial populations over time at various taxonomic levels is one of the central benefits of the metabarcoding approach. Thanks to that metabarcoding provides a cost-effective overview of the taxonomic composition of a sample and has already been applied to a variety of food products, e.g., to study the microbiology of fermented food [18, 19], and has also been used for characterizing the microbiota of food spoilage (e.g. the proliferation of lactic acid bacteria in fresh cut lettuce, leading to acidification and loss of structure [20]).

Metagenomics generates sequencing information from the genetic material in a sample, permits identification of individual strains, and can allow the prediction of functions encoded by microbial communities. The use of metagenomics is being explored for the detection, identification, and characterization of pathogens in food [21].

In addition to pathogens detection, metagenomics and metabarcoding hold great potential for the survey of food production, assessing food safety, authenticity [22, 23], and quality. Relevant applications have been published during the last years in food authenticity. Liu et al. [24] presented High-Throughput Sequencing (HTS)-based metabarcoding method successfully applied to pollen/honey identification to determine floral composition that can be used to identify the geographical origins of honeys. In another work, a DNA-barcode assay with fatty acid profile analysis was performed to authenticate the botanical origin of olive oil [25]. Real-time PCR (qPCR) is a central tool in food genomics which can be used to determine product authenticity. In Agrimonti et al. [26], a qPCR for characterization of microbial populations and type of milk in dairy food products was presented. Another important field of application is the improvement of certain food industry processes, for example, improvement in fermentations. Pereira et al. [27] carried out the selection of autochthonous lab strains of unripe green tomato aiming at the production of highly nutritious lacto-fermented ingredients. Genomics has been also applied to data-driven decentralized breeding because increases the accuracy in the prediction of crop productions in difficult environments. Data-driven decentralized

breeding could identify genotypes with enhanced local adaptation providing superior productive performance across seasons.

A branch of foodomics is nutrigenomics that focuses on the study of the effects of foods and their constituents on gene expression. Nutrigenomics assesses the impact of specific nutrients on health through the expression of genetic information [28–33].

2 Proteomics

Proteomics is the large-scale study of proteins, the entire proteome, of a cell, tissue, or organism under a specific, defined set of conditions. Proteomics is based on three basic pillars: (1) a method to fractionate complex protein or peptide mixtures, (2) MS to acquire the data necessary to identify individual proteins, and (3) bioinformatics to analyse and assemble the MS data. Thanks to instrumental and technological developments proteomics experienced impressive progress during the last decade.

In recent years, there are three main proteomic approaches established:

- Protein separation in gel (bottom-up approach)
- Shotgun (bottom-up approach)
- Mass spectrometry analysis (top-down approach)

Chromatographic and electrophoretic separations prior protein digestion are called bottom-up approaches, whereas the analysis of intact protein by means of separation with chromatography coupled to mass spectrometry is the top-down approach. The top-down approach in combination with small protein biomarkers (below 30 kDa) can be implemented to identify and quantify protein biomarkers in routine analysis [34].

One of the primary challenges in proteomics is the wide difference of proteins in cell types and individuals and the difference between most and less abundant proteins. Nowadays, different approaches are available to reduce proteome complexity including fractionation, depletion, as well as enrichment of low-abundant proteins. Following the separation and enrichment, MS represents the most-powerful tool in proteomics because it can be used under non-targeted schemes [35]. Current MS techniques allow the analysis of large-scale proteins and peptides. Improved MS with better sensitivity and superior mass accuracy and resolution aims to identify and quantify complex protein (peptides) mixtures in a single experiment. Main mass analysers used in proteomics are time-of-flight (TOF), orbitrap, Fourier transform ion cyclotron resonance (FT-ICR), and ion trap (IT), which are usually combined in one mass spectrometer (triple quadrupole (QqQ), Q-IT, Q-TOF, TOF-TOF, IT-FTMS, etc.).

2.1 Environmental Proteomics

It opens a new window for environmental sciences. Currently, environmental proteomics is being developed to provide information about molecular responses to contaminants in living organisms [36], and for the identification of more specific and sensitive markers of pollution for routine monitoring.

In general, for environmental studies proteomics consists in the selection of sentinel organisms on the basis of their susceptibility and extent to exposure, then the proteome study comparing exposed and non-exposed populations to assess cellular and molecular alterations. For example, for the biomonitoring of marine pollution the most abundant candidate species are invertebrates, highlighting among them the bivalve molluscs for their key role in the ecosystems and for their great value to aquaculture [37]. Moreover, bivalves are used as test species in biomonitoring due to ubiquitous distribution, easy accessibility, high filtering capacity as well as increased resistance to a wide range of pollutants, including emerging contaminants such as microplastics [38], nanoparticles [39], and other organic pollutants [40]. For the freshwater environments, the most common organisms studied are fish, crustaceans, and molluscs but there are mainly two organisms that stand out: the bryophyte *Fontinalis antypiretica* [41] and the zebra mussel *Dreissena polymorpha* [42]. It should be mentioned that there are far fewer biomonitoring studies of freshwater pollution than of marine pollution. More recently, native crustacean species have been used as bioindicators of freshwater ecosystems with good results [43].

In general, few studies are based on plants as bioindicator organisms due to technical reasons related to the extraction of complex proteins and to the fact that vegetal tissues have low concentrations of proteins and, in general, a high number of interfering substances are present. Moreover, another limitation is the lack of model plant species, which is limited to maize, rice, and wheat, and the lack of curated protein databases for non-model species.

Environmental proteomics has been specially used to assess contamination in marine environments. The traditional biochemical methods and gel-based proteomics have been particularly used to search protein biomarkers. Among them the two-dimensional gel electrophoresis (2-DE) followed by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) are the more employed techniques. The limitation, however, of these approaches is that only a small subset of proteins is analysed resulting in highly biased results.

In Table 2, a summary of proteomic studies is presented. The proteomic studies carried out in marine environments were mainly based on the use of bivalves. Protein expression was strongly affected by pollutants such as metals, affecting structural proteins such as actin and tubulin. For example, Cu, Zn, Cr, and Ni have been related to impairments in the structural components in oysters [56, 57]. Other organic contaminants such as pharmaceuticals have been shown to cause changes in the same structural proteins in freshwater clam *Corbicula fluminea* after 1 day exposure to wastewater from a psychiatric hospital [58]. In another work, Navon et al. [59]

Table 2 Summary of recent proteomic studies to assess the impact of contaminants in biota

Pollutants	Organism	Technology	Alterations identified	Reference
As and Cd (1213.9 and 26.9 mg/L, respectively, for 96 h)	Amphipod (*Gammarus sp.*)	2D-DiGE	Energetic metabolism	[44]
Au nanoparticles (80 mg/L for 96 h)	Gilt-head bream (*Sparus aurata*)	2-DE and MALDI-TOF-TOF MS	Energy metabolism, stress response, cytoskeleton structure	[45]
Bisphenol A	Zebrafish (*Danio rerio*)	Shotgun proteomics	Oxidative stress response, metabolic shifts, and degradome perturbations	[46]
Cd (0.1, 0.3, and 1 mg/L for 15 days)	Farrer's scallop (*Chlamys farreri*)	iTRAQ	Carbohydrate and lipid metabolism	[47]
Cd (5 and 50 mg/L for 14 days)	Bastard halibut (*Paralichthys olivaceus*)	iTRAQ	Oxidative phosphorylation, bile acid biosynthesis Stress resistance Apoptosis	[48]
Co-exposure Cu (II)/Cd (II) and the antibiotics chlortetracycline and florfenicol	Synechocystis sp. PCC 6803, a cyanobacterium	Label-free quantitative (LFQ) proteomics assays UPLC-MS/MS, PRM	Photosynthetic proteins were down-regulated, and ribosome was the primary target	[49]
DDT and benzo (a) pyrene (10 mg/L and 10 mg/L, respectively, for 7 days)	Asian green mussel (*Perna viridis*)	iTRAQ	Cytoskeleton structure, gene expression, material and energy metabolism, reproduction and development, stress response, signal transduction, apoptosis	[50]
Microplastics (800 mg/L for 52 days)	Blue mussel (*Mytilus edulis*)	Label-free quantification (LFQ)	Immunity and detoxification response	[51]
Microplastics (MPs) and antibiotic resistance genes (ARGs) in contaminated soils	Earthworms	SDS-PAGE electrophoresis and HPLC-HRMS	100 mg/kg of 10 μm MPs changed the microenvironment of earthworm gut, built a new homeostatic process, and thus increased the abundance of key bacterial that carried a variety of ARG	[52]

(continued)

Table 2 (continued)

Pollutants	Organism	Technology	Alterations identified	Reference
Norfloxacin antibiotic in soil	Rhodococcus biphenylivorans TG9T	TMT kit and LC-MS/MS analysis	Inhibited DNA replication	[53]
Polychlorinated biphenyls (PCBs) (30 mg/L for 3 weeks)	Mediterranean mussel (*Mytilus galloprovincialis*)	2-DE and LC-MS analyser	Protein polymerization actin filament severing Myosin II binding protein-containing complex binding	[54]
Zn (50 mg/L and 500 mg/L Zn for 30 days)	Portuguese oyster (*Crassostrea angulata*)	Nano-UPLC-MS/MS	Zn and Ca transport, phosphate and energy metabolism, immune regulation, oxidative stress responses, gene expression regulation, fat metabolism	[55]

used 'shotgun' proteomics to identify differentially expressed proteins in *Herdmania momus*, after 14-day exposure to the antidepressant carbamazepine in laboratory conditions. Individuals were exposed to 5 or 10 μg/L in addition to a control treatment. Out of 199 identified proteins, 24 were differentially expressed (12%) between the treatment groups, and thus can potentially be developed as biomarkers for carbamazepine contamination. Again, one of the most affected proteins was tubulin followed by myosin [59].

Another significant group of proteins altered by pollution are the proteins involved in the metabolism. These proteins are valuable biomarkers [60]. For example, the proteomic analysis and biochemical alterations in marine mussel gills after exposure to the organophosphate flame retardant, tris(1,3-dichloro-2-propyl) phosphate (TDCPP), showed the inhibition of the acetylcholinesterase (AChE) activity, at both, after 7 and 28 days of exposure. Proteomic analysis revealed subtle effects of TDCPP in mussel gills, since few proteins (<2% of the analysed proteome) were significantly affected. But the epimerase family protein SDR39U1, an enzyme that could be involved in detoxification processes, was up-regulated at both exposure times, and the down-regulation of receptor-type tyrosine-protein phosphatase N2-like (PTPRN2) after 7 days of exposure, which is involved in neurotransmitter secretion and might be related to the neurotoxicity described for this compound [61]. Zhang et al. [62] studied the effect of two concentrations of 6-μm polystyrene beads (0.023 and 0.23 mg/L in seawater) to establish the multigenerational effect of microplastics on the marine copepod *Tigriopus japonicus*. The effects were followed in two-generation exposure (F0-F1) followed by one-generation recovery (F2) in clean seawater. The proteome profiling was conducted for the F1 and F2 copepods under control and 0.23 mg/L microplastics treatment to investigate within-generation proteomic response and transgenerational proteome plasticity. The proteomic analysis demonstrated that microplastics exposure increased several cellular biosynthesis processes and, in turn, reduced energy

storage due to the trade-off, hence compromising survival and reproduction of the treated copepods in F1. Furthermore, the two-generational effect of microplastics in copepods had significant transgenerational proteome plasticity, as shown by the increased energy metabolism and stress-related defence pathway, which accounts for regaining the compromised phenotypic traits during recovery (i.e. F2). In another proteomic study to assess the impact of microplastic pollution in marine organisms, blue mussels (*Mytilus edulis*) [51] were used. The repeated exposure, over a period of 52 days in an outdoor mesocosm setting, showed that microplastics exposure altered the haemolymph proteome and, polyethylene resulted in more changes to protein abundances than the bioplastic based on polylactic acid. Many of the proteins affected are involved in vital biological processes, such as immune regulation, detoxification, metabolism, and structural development [51].

2.2 Proteomics in Food Analysis

Nowadays, main applications of proteomics in food analysis under the concept of foodomics are:

- The determination of food allergens
- To trace food quality, composition, and origin
- Nutriproteomics

Different proteins have been identified as food allergens in dairy products, peanuts, soybeans, eggs, cereals, or fruits, for instance. Thus, *proteomics is a useful tool for the identification of allergens in food products* [35]. The bottom-up proteomics strategy has been widely used. Under this approach, the first step consists in the protein digestion in order to produce a set of derived peptides from the proteins in the sample and their identification as a part of a particular protein. MALDI-MS have been very much employed because it is well suited for peptide mass fingerprinting (PMF), as well as selected fragmentation of various precursors using collisional-induced dissociation (CID) or post-source decay (PSD).

Celiac disease, gluten hypersensitivity, wheat-dependent exercise-induced anaphylaxis (WDEIA), and wheat allergy are consequence of wheat, barley, and rye proteins. The most abundant gluten proteins are glutenins and gliadins. These proteins (prolamins), with high contents of proline and glutamine, are responsible for celiac disease. A variety of proteomic studies have been carried out to identify and quantify the gluten proteins from processing products ensuring gluten-free foods. For that reason, special efforts have been paid to elucidate a curated gluten protein sequence database to support proteomic technologies [63]. Wheat quality has been improved due to genetic improvement programmes but by modifications at the genotype level.

Other important groups of food allergens are proteins contained in legumes, nuts, and seeds that can cause anaphylactic reactions. The allergens are mainly proteins of storage, such as 2S albumins, 7S globulins, and 11S globulins. MRM was used to

characterize the allergens present in lentil cultivars determining trace amounts of certain peptides such as vincilin, legumin, lectin and lipid transfer proteins [64]. In a similar manner the contents in allergens, basically storage proteins, from different cultivars of soy were studied by SRM [65]. Shotgun proteomics could be a suitable technique of choice to select the cultivar with lesser allergen proteins in lentil seed. Moreover, the allergenicity of soy protein could be reduced by high hydrostatic pressure. Indeed, significant changes were produced in 7S and 11S globulin, hence reducing the allergenicity in children [66]. However, Ribeiro et al. [67] found minor differences of allergenic potential of 13 hazelnut (*Corylus avellana L.*) varieties.

Other allergens that have been widely studied are those contained in fruits and vegetables. Most adverse reactions are associated with pollen because of 'cross-allergenicity' [68, 69].

Another group of food allergens are present in animal-based foods in general coming from milk, eggs, and fish. Allergies caused by meat are scarce and they have been only described in atopic children. Most cases are related to specific IgE to galactose-α-1,3-galactose, an oligosaccharide of non-primate mammals [70]. Proteomic studies have been carried out to assess red meat allergy against the carbohydrate α-Gal epitope [71]. On the other hand, most common proteins related to egg allergenicity are ovomucoid (Gal d1), ovalbumin (Gal d2), ovotransferrin (Gal d3), and lysozyme (Gal d4), and in the yolk, α-livetin (Gal d5) and YGP42 (Gal d6) [72].

Also, seafood includes many allergenic proteins being the more important ones parvalbumin, tropomyosin, and arginine kinase, inducing immunological and clinical cross-reactivity [73]. Most of the allergic reactions are produced by parvalbumins.

Among animal-based allergens also milk allergy is a common abnormal response of the immune system to proteins in milk such as α-lactalbumin (also called Bos d4), β-lactoglobulin (Bos d5), and casein (Bos d8) based on IgE measurements. However, other minor proteins, such as the bovine serum albumin (BSA), lactoferrin, and immunoglobulins could also produce allergic reactions [74, 75]. Different proteomic studies have been carried out to assess the different allergenicity after milk processed foods. For example, it has been demonstrated that heating treatment together with other food components could reduce the allergenicity of cow milk [76]. In the case of whey proteins, the loss of the allergenic effect is produced after 30 min of heating at 65°C [77]. Additional studies were focused on the search of peptides for the detection of casein and whey milk allergens from baked cookies. Nineteen peptides of four casein proteins and two whey proteins were identified using PRM [78].

In proteomic studies, one important step to assess food allergens is the extraction step. In this sense important amount of research has been carried out during the last years. A simple extraction protocol of allergenic proteins in food commodities such as milk, egg, hazelnut, and lupine seeds was recently presented by Calvano et al. [79]. Bottom-up approaches based on sodium dodecyl sulphate (SDS) gel electrophoresis separation followed by in-gel digestion or direct in-solution digestion of whole samples are described. Then, the analysis was performed by MALDI-MS and MS/MS along with a comparison of data obtained by using the most widespread matrices for proteomic studies, namely, α-cyano-4-hydroxy-cinnamic acid (CHCA)

and α-cyano-4-chloro-cinnamic acid (CClCA). Some approaches persecute to improve the extraction without the use of denaturing agents or detergents and optimized protein/enzyme ratio to reduce matrix interference and increase detection capability of target peptides. For example, Stella et al. [80] validated a new approach to assess four allergens on fish or swine processed food combining: (1) an extraction buffer not containing denaturing agents or detergents, (2) a defatting step with n-hexane, (3) an optimized protein/enzyme ratio to reduce matrix interference, and (4) the addition of heavy labelled internal standard peptide to improve method repeatability. The method was proved to be robust enough to detect and quantify proteins from milk, egg, soy, and crustaceans simultaneously with LOQ values below 5 μg/g in complex matrices. The method validation was according to the requirements of Regulation (EC) 333/2007. All information collected for each allergen under consideration was included in a targeted parallel reaction monitoring (PRM) method to analyse four allergens simultaneously [80].

Another relevant application of proteomics in food is related to its quality, traceability, and authenticity [22, 81]. Adulteration of cheese using less expensive milk is one of the authenticity problems in the dairy industry. The method of reference to assess the presence of cow milk in ewe or goats cheese is based on the detection of the caseins bands for cow γ2- and γ3 on an IEF gel [22]. However, this method presents a series of limitations. In the case of percentages lower than 1%, the marker bands for bovine milk are not visible when staining the gel with Coomassie blue [22]. Another limitation has been reported for cheese with concentrations below 5% cow's milk; the marker bands could be not detected using the most common staining methods. Moreover, interfering bands can cause false-positive responses and the need for an excellent electrophoretic separation of γ-caseins [22]. Proteomic approaches based on MS-based protein profiling, using either MALDI-TOF or electrospray mass spectrometry (ESI-MS), have been extensively investigated recently. Protein profiling obtained by MALDI-TOF was used for the investigation of the adulteration of ewe and buffalo milk with bovine milk [82], and in the adulteration of mozzarella cheese. The approach has been used to assess the adulteration of donkey and goat milk with cow, ewe, and buffalo milk at levels down to 0.5% [83].

The addition of UHT milk to raw or pasteurized milk has also been detected by MALDI-TOF MS with LOD of 10% [84]. ESI-MS has been employed to assess β-lactoglobulin to detect bovine milk in ovine milk [85] by means of high performance liquid chromatography, electrospray ionization and mass spectrometry (HPLC-ESI-MS) or capillary electrophoresis-MS. The presence of cow milk was detected at levels not lower than 5%.

Seasonality of buffalo milk production, rapid cheese decay and transport of its preserving liquid have relevant practical/economic consequences for mozzarella production; consequently, a progressive diffusion of cheese products realized with frozen curd or frozen milk. Arena et al. developed a MALDI-TOF-MS polypeptide profiling to assess specific markers of its freshness [86].

Proteomics have been applied to authenticate processed meat foods. For example, a method based on nano-LC-QTOF-MS/MS was applied to quantify highly

processed meat proteins to authenticate processed meat products [87]. Twenty new, heat-stable peptides unique to chicken, duck, and goose were identified as markers, with significant scores. This method permitted the detection of 1% (w/w) of chicken and 1% (w/w) pork in a mixture of the meat of three species. The abundance of proteins was computed by absolute protein expression (APEX) proteomics tool, which is a spectral matching technique [87]. In another work [88], two-dimensional O'Farrell electrophoresis with isoelectrofocusing in ampholine and immobiline pH gradients, the detection of proteins on two-dimensional electropherograms by staining with Coomassie R-250 and silver nitrate, and mass spectrometric identification of proteins by means of MALDI-TOF and MS/MS methods were used to confirm protein and peptide authenticity, and also to detect biomarkers of proteolytic changes in meat after slaughter. A quantitative proteomic distances study between three Spanish bovine breeds (Asturiana de los Valles, AV; Retinta, RE; and Rubia Gallega, RG) was performed from two-dimensional electrophoresis profiles of meat samples of longissimus thoracic muscle at 2-h post-mortem [89]. In total, 18 differentially abundant myofibrillar and sarcoplasmic proteins/isoforms contributing to proteomic distances between breeds were identified by MS/MS [89]. Other applications of proteomics for food authentication have been applied to oilseed [90, 91], and in cereal and vegetable crops [92, 93].

Nutriproteomics is a promising tool bridging diet and diseases in nutritional research. Comparative proteomics strategies have been applied to glean insight on the effect of specific natural compounds, nutrients, or diet. Most of these applications are based on bottom-up proteomic approaches; in particular, classical 2-DE separation of proteins and MS detection of the in-gel digested proteins. However, this field is still rather limited. Dietary antioxidants have been studied as chemical compounds candidates against cancer and inhibition of tumour progression. Three combined micronutrients, vitamin E, selenium, and lycopene were studied by [94], of which vitamin E and lycopene have recognized antioxidant activity in mice [94]. In the same field, the antiangiogenic effects of *Lindera obtusiloba Blume* (Hwangmaemok, HMM), which is a plant in the *Lauraceae* family have been studied. The effects of HMM extract on endothelial cell proliferation, migration, and neovascularization in chick chorioallantoic membrane (CAM) were investigated together with the molecular mechanism of antiangiogenesis using a ProteoChip-based proteomics technology [95]. In a more recent study, new strategies for breast cancer prevention are discussed [96]. Nutriproteomics is a promising tool to characterize the nutritional content of food and characterize and search functional ingredients. For example, the sweet chestnut (*Castanea sativa Miller*) is a tree species whose edible fruit was a staple food for many centuries until their consumption was displaced by the introduction of potato and cereal crops. Nowadays, there is a renewed interest in this fruit, partly due to being gluten-free. To describe and evaluate the sweet chestnut germplasm in Portugal a nutriproteomics study was carried out. Two-dimensional electrophoresis and MS were used to further investigate the chestnut proteome. Most of the proteins were assigned as having functions in nutritional storage activity [97]. During the last decade, the interest by isoflavones increased as functional ingredients. For the first time Rowell, Carpenter, & Lamartiniere demonstrated the

usefulness of proteomics for the discovery of novel pathways that might be involved in cancer prevention by isoflavones [98]. Currently a high number of studies have been carried out to assess the proteome and health characteristics of isoflavones. Epidemiological data indicates that regular intake of isoflavones from soy reduces the incidence of oestrogen-dependent and ageing-associated disorders, such as menopause disorders, osteoporosis, cardiovascular diseases, and cancer. Equol, produced from daidzein, is the isoflavone-derived metabolite with the greatest oestrogenic and antioxidant activity [99]. Other studies were more focused on the study of which isoflavones can have higher effects in cancer treatments. For example, the effects of isoflavone ME-344 on the disruption of redox homeostasis and mitochondrial function were characterized [100]. Proteomic studies can help to personalize certain treatments such as for cancer. In this sense, the importance of isoflavone dose and oestrogen receptor status in breast cancer cells were studied by Ilies et al. [101].

3 Lipidomics

Lipidomics is defined as the large-scale characterization of pathways and networks of lipids in biological systems. The word lipidome is used to describe the complete lipids profile in an organism, tissue, or cell.

As happens with proteomics, the advances in MS during the last decades increased the resolution and accuracy in untargeted lipidomic analysis. Lipidomics represents a powerful tool for the identification of all detectable lipids and their metabolites, including unknown chemicals [102].

The optimal workflow to understand the lipid profile and their functions by lipidomics is summarized as: the first step is sample collection and preparation, derivatization, LC separation, MS analysis, quality control, data processing, and data interpretation [103].

It is necessary to provide appropriate storage conditions based on the physical and chemical states of the sample to minimize degradation of lipids during sampling and sample preparation, such as storing at low temperatures (−20°C, −80°C, or liquid nitrogen) and avoiding exposure to O_2 during storage. In addition, to minimize the matrix effect and interference substances, extraction methods are employed before LC/MS approaches such as liquid–liquid extraction (LLE) or solid-phase extraction (SPE). Moreover, contamination problems should be avoided.

Specialized derivatization strategies have been used to enhance the selectivity and sensitivity of MS-based analysis [104]. The combination of derivatization chemistry with MS/MS scan modes (e.g. neutral loss, precursor-ion, or product-ion) and ionization efficiency has greatly improved structural characterization of isobaric lipid molecules and multiplexed absolute/relative quantification in MS/MS-based lipid profiling.

3.1 Environmental Lipidomics

Lipidomics has been used to assess changes produced by environmental stressors and contaminants in environmental biota [105]. During the recent years, lipidomics has been applied in environmental toxicology. It has been applied to assess oxidative stress produced by xenobiotics and to assess the biota responses to environmental factors, such as ocean acidification [106], climate change [107], and contaminants [108]. For example, the changes in the profile of lipidomics produced in the oysters (*Crassostrea hongkongensis*) exposed to copper (Cu) were studied by Chang et al. [109]. The changes in the lipidome profile of the digestive gland of exposed oysters showed induced phospholipid changes and an increase in polyunsaturated fatty acids that represent precursors of inflammatory mediators [109].

Lipidomics studies provide indication of the perturbation of oxidation pathways, and they have a great promise for a cumulative risk assessment. However, lipidomics is in early steps of developments and only a few untargeted data processing software programs can comprehensively cover the scope of oxidized lipid species for annotation and quantitation [110].

Environmental lipidomics has been applied in wildlife studies to identify lipid biomarkers indicative of environmental stress, and pollution. In aquatic organisms, fatty acid profiling has been used. For example, sewage release has been linked with shifts in the fatty acid profiles of freshwater clams [111]. In another study, the changes in fatty acids profile of caged mussels from a polluted in comparison with those from a clean reference site were used to identify lipid biomarkers of pollution [112].

On the other hand, during the last century, a reduction in communicable diseases has increased the life expectancy, while the mortality due to immune system diseases, Alzheimer's and multiple sclerosis, or inflammatory diseases from environmental exposures has increased, with the 70% of these diseases that can be linked to xenobiotic exposures and the diet [113]. In a similar manner, xenobiotic exposures, infections, and other environmental factors are the major cause of 80–95% of cancers. Lipidomics can play a central role in improving the understanding of the mechanisms ruling these immune disorders. It is important to assess the environmentally induced oxidation of lipids because of their cumulative risk and toxicity. Moreover, a class of biologically induced oxidized lipids, oxylipins, can indicate common responses to environmental xenobiotics that involve immune system dysregulation and inflammation [114]. Oxylipins may therefore serve as general markers of inflammatory-related environmental injuries. Nevertheless, the correlation between an imbalance in lipid homeostasis and the abundance of different molecular species is a poorly explored area of research nowadays [115]. In addition, pollutant effects on immunomodulatory lipids are an intriguing field of investigation. And the effects of contaminant exposure on haemocyte lipidomics related to immune functions remain unexplored to date.

Another application of lipidomics is bioremediation. Lipidomics, in combination with other omic techniques, can be used to characterize both the mechanisms of

toxicity to an organism and the mechanism of tolerance and bioremediation. This new level of understanding may lead to achieve the development of hybrid plants with tolerance to xenobiotics, and favourable characteristics for bioremediation applications. For example, the expression of the mammalian Cytochrome P450 2E1, a gene involved in the metabolism of xenobiotics in the liver, has been expressed in numerous genetically engineered plants, drastically increasing their plants for contaminants degradation [116].

Moreover, lipidomics has been used to characterize biota and their metabolic activity over extended time scales, providing information about the global climate change, and nutrient cycles. Lipids can be preserved in fossilized substrates, sediments, and glaciers, thus providing valuable information on ecosystem processes and their influence at geological timescales not obtainable via visual examination of fossil records [117].

Fatty acids are stable over geological timescales, and they can be used to characterize microbial communities in distinct environments over time and across multiple geographical locations. While most lipidomics-based research studies analyse fatty acid fragments to characterize microbial communities, the literature is currently shifting to the analysis of lipids [118].

Most environmental lipidomics studies have been based on the characterization of fatty acid profiles by GC-MS, but there are many advantages to the study of intact lipids, which possess specific membrane properties and functions, serve as signalling molecules, and can be more representative of certain organisms. Moreover, the expansion to intact lipids studies can be a new route for biomarker discovery. It should be mentioned that the limited number of environmental lipidomics studies is not concordant with their potential, but nowadays, there are a series of bottlenecks to overcome, such as high-level expertise needed for data processing and interpretation, the cost of the analysis using high-resolution mass spectrometers, and the need for characterizing the natural shifts in the baseline lipidome across various species and genotypes over time, and identifying lipid structures unique to specific organisms.

3.2 Lipidomics in Food

Lipidomics approaches enable large-scale and comprehensive studies of lipids. Therefore, lipidomics has been employed to optimize reaction conditions for the proper modification of structured lipids in the food industry, the characterization of lipids in food safety, and evaluation of biofunctional and nutrition levels of lipids in living organisms. For example, there are great differences in physicochemical, biochemical, and nutritional properties among different edible oils from diverse sources, such as plants, animals, and microorganisms or their blending and modification [84]. The nutritional value and the properties of edible oils are affected not only by the fatty acid composition, but also by the positional distribution of acyl groups bonded to glycerol. A shotgun lipidomic application was used to determine triacylglycerols in soybean seeds. This approach was based on the use of

electrospray ionization (ESI)-MS/MS with ten neutral loss scans performed to quantify 93 major triacylglycerols, but also new species were detected [119]. In another example, a chiral HPLC-atmospheric pressure chemical ionization (APCI)-MS method was employed to determine the composition of triacylglycerols enantiomers and regioisomers in hazelnut oil. In this case two cellulose-tris-(3,5-dimethylphenylcarbamate) columns connected in a series with a gradient of hexane-2-propanol mobile phase were used. Unsaturated fatty acids were preferentially esterified in sn-2 position in hazelnut oil [120]. Using 2D-LC coupled with atmospheric pressure chemical ionization MS (APCI-MS), triacylglycerols of high-oleic and normal peanut oils were profiled and statistically evaluated. The main differences in the content of triacylglycerols between high-oleic and normal peanut oils were found in 18:1–18:1–18:1 (OOO), 18:1–16:0–18:1 (OPO), and 16:0–18:1–18:2 (POL) species [121]. In another example, a comparative analysis of the lipids profiles in three edible bivalve species (clams, oysters, and mussels) permitted the identification of more than 600 different lipids belonging to 14 classes [122]. Despite this study was essentially carried out to establish a relationship between the nutritional value of each species, the application of lipidomics provides the baseline data for further studies on the effects of pollutants on the lipidome. Moreover, lipidomics has been successfully applied in authentication studies. For instance, the authentication of different types of milk [123].

The continued improvement of high-resolution mass spectrometry (HRMS) approaches has boosted the use of lipidomics in different fields of the food industry. Nevertheless, lipidomics is far to be well implemented in production environments. The main needs are more available and easy use of bioinformatic applications and computational methods to characterize the structure and abundance of complex lipids, and to help to interpret bio-function and metabolic pathways. Finally, improvements in the spatial resolution and scan speed of MS imaging equipment will greatly enhance the ability to measure and assess the spatial distribution of lipids in tissues and single cells.

4 Metabolomics

Metabolomics is the study of the low molecular weight metabolites that are generally $<$1,000 Da in cells, tissues, biofluids, or whole organisms by the application of advanced analytical techniques [124]. This includes primary metabolites which are involved in the development and reproduction of an organism, and secondary metabolites which are produced by bacteria, fungi, and plants and have various ecological functions [125].

Metabolomics is considered as the downstream process of genomics, transcriptomics, and proteomics, and the changes of metabolite levels directly relate to biochemical activity and the phenotype [126].

The fundamental metabolic pathways such as those involved in energy cycle, proteins, and lipids are conserved from bacteria to eukaryotes [127].

The primary analytical techniques applied to assess the metabolomic datasets are nuclear magnetic resonance (NMR) spectroscopy and MS because of their ability to detect small molecules. There are two main workflows in metabolomics:

- Target metabolomics which studies a predetermined set of metabolites [128].
- Non-targeted metabolomic analysis is the non-biased study of as many metabolites that can be identified by the analytical approach and databases [128].

Non-targeted metabolomic analysis frequently uses high-resolution MS or NMR spectroscopy, while targeted metabolomics often uses MS as the analytical method of choice [129].

4.1 Environmental Metabolomics

Environmental metabolomics is the study of metabolic response of organisms as a result of their interactions with the environment [130]. Metabolomics studies have been applied to determine the response in front of various environmental stressors such as UV light [131], atmospheric carbon dioxide concentrations [132], drought [133, 134] and contaminants [135–140] among others.

These experiments can be carried out under controlled laboratory experiments with targeted species or in-field studies to evaluate how an organism is impacted when exposed to an ecosystem under environmental stress.

Environmental metabolomics provides for a more accurate biomonitoring and risk assessment. The general workflow to achieve these objectives is:

- study design,
- exposure,
- sample preparation and metabolite extraction,
- data collection,
- data analysis, and finally a biological interpretation of the analysed data.

4.1.1 Study Design

This step involves the selection of the type of experiment to be carried out, at laboratory level or in-field study, and the selection of exposed organisms. Environmental metabolomics studies are done using a variety of organisms that include from microorganisms to plants and animals.

Microorganisms have been the organisms of choice in many metabolomic studies because of their easy manipulation under controlled conditions. The response of microorganisms to contaminants such as polycyclic aromatic hydrocarbons (PAHs) [141] and tetrachloro bisphenol A [142] or also to environmental stressors, for example, acidophilic metal-rich environments [143] have been determined. Earthworms have been studied to assess the metabolomic impact of certain contaminants

in soil. For example, in soils contaminated with arsenic [144], nanomaterials [145, 146], and pesticides [147, 148]. Plants are as well used to investigate the metabolic impact of contaminants in soils. For example, how plants respond to PAHs or metals from remediation efforts [149], the impact of mineral deficiency [150], of UV-B radiation [131], and drought [133]. Crustaceans, bivalves, and fish such as medaka, rainbow trout, salmon, and fathead minnow are the most common used aquatic organisms. Among them, it should be highlighted the use of bivalves because they have a sessile lifestyle and can accumulate contaminants, and therefore an analysis of the metabolic profile of bivalves may reflect the contamination at the site of collection. There are a series of considered model organisms, for example, rat and mouse for mammals, the zebrafish (*Danio rerio*) for aquatic vertebrates, the water flea (*Daphnia magna*) for aquatic invertebrates, the *Arabidopsis thaliana* for plants, the yeast *Saccharomyces cerevisiae* for eukaryotes, and *Escherichia coli* for prokaryotes.

4.1.2 Exposure

In both cases, laboratory and field studies, the number of individuals, type of control, type of exposure, and the reference sites should have careful planning. In addition, since there is natural variation in biological samples an adequate replication is needed for proper statistical analysis [151].

In field studies, the organisms that represent the exposed groups may be collected at contaminated or stressed locations and may be compared to organisms collected at more pristine or less stressed locations, which serve as the group from a reference site. In addition, field studies can be carried out using two different schemes, one involves the sampling of free-living organisms in the environment and the second one involves deploying organisms into environments that are under stress.

Environmental metabolomics studies that sample free-living organisms at a field site are a step forward in environmental monitoring because they better represent the individual variability, which may stem from genetic differences. Moreover, field populations may be exposed to multiple stressors. However, this is also a challenge because it is difficult to distinguish cause and effect, and it may be difficult to distinguish between gender and different life stages. Field-based studies also face the difficulty of locating an ideal reference site that can act as control with minimal stressors but where the natural settings are analogous to the impacted site.

On the other hand, field-deployed organisms have an excellent value for environmental risk assessment. Laboratory populations and the experimental conditions (temperature, light, and diet) are strictly controlled, and they are maintained constant to limit any perturbations to the metabolome. Laboratory studies have the benefit of standard protocols that are available as guidance documents for aquatic toxicity testing and soil toxicity testing that should be followed for laboratory work (OECD, 2002, 2004). For these reasons, controlled conditions are optimal for determining the mode of action of contaminants as the metabolic perturbations can be directly accredited to the stressor [152]. There are standard reporting requirements for

metabolomics experiments of mammalian/in vivo work [153], and for metabolomics experiments of microbial and in vitro work [154]. Although the metabolic disturbances from exposure to stressors in laboratory conditions can be extrapolated to predict the hazard in environmental settings, care must be taken to avoid overestimating or underestimating risk [155].

4.1.3 Sample Collection, Sample Preparation, and Metabolite Extraction

Since the metabolic responses across cells, tissues, organs, and the whole organism may be different, the decision of which biological sample to analyse should be made according to the research carried out. The metabolomics analysis of a certain tissue can give information about the state of an organ and different tissues of an organism containing different baseline metabolic profiles. Sometimes, the collection of a biofluid such as blood or urine is minimally invasive and can provide information about the overall biological state of an organism.

As proper selection of biological samples the proper sample preparation is important to avoid disturbances in the metabolome that may occur during the sample preparation. Sample preparation procedures may need to quickly halt enzymatic activity with freezing in liquid nitrogen and samples must be kept cold throughout any storage. In some cases, water should be removed (e.g. by lyophilization) for analysis by NMR spectroscopy and the solvent extracts of cell cultures need to be dried and reconstituted in an NMR buffer. NMR-based metabolomics most frequently uses a deuterium oxide phosphate-based buffer which extracts polar metabolites. The phosphate buffer maintains a constant pH to minimize variations in chemical shift and a deuterated solvent allows for a lock signal in NMR spectroscopy.

The metabolite extraction procedure for MS analysis typically uses solvents of varying polarity to extract polar and nonpolar metabolites. Also, the metabolite extraction method depends on the biological matrix chosen to be analysed, and there are well-developed protocols for metabolite extractions, for example, from mammalian and bacterial cell cultures [156], and biological fluids such as blood and urine [157].

4.1.4 Data Collection and Analytical Techniques

As mentioned before, MS and NMR spectroscopy are the techniques of choice. However, both NMR spectroscopy and MS analytical techniques encounter some challenges, and there is no single analytical technique that can identify and quantify the whole metabolome. Several studies propose direct infusion mass spectrometry (DIMS) coupled to high-resolution mass analysers such as Orbitrap mass analysers because of the rapid and non-targeted analysis [158–160]. However, ion suppression and the complexity of the mass spectra without sample separation are disadvantages

of DIMS [158]. Therefore, MS is usually coupled to LC, GC, or capillary electrophoresis (CE) for analyte separation prior to mass detection, which can aid in metabolite identification using the retention time. GC-MS of polar metabolites typically requires chemical derivatization. However, the quantitation of derivatized metabolites may be difficult as the derivatized metabolites may not be constant. CE-MS involves the separation of highly polar and ionic metabolites due to different migration speeds of the metabolites [161]. CE-MS based metabolomics studies often couple capillary electrophoresis to a TOF mass analyser [161]. LC-MS analysis includes the selection of the chromatographic column, solvents for the mobile phases, potential additives to the mobile phase, and mobile-phase gradients for separation by LC. Most MS-based metabolomics studies use LC with electrospray ionization (ESI) that is suitable for the ionization of a range of metabolites. Non-targeted metabolomics studies for global profiling frequently use high-resolution and high accuracy mass analysers such as Orbitrap analysers. The main advantage of using MS for environmental metabolomics is that MS can detect and quantify metabolites at very low concentrations in the femtomolar to atto-molar range, and has a high dynamic range and resolution. The limitation is that MS only detects metabolites that can promptly ionize.

NMR spectroscopy can identify unknown metabolites unambiguously, can distinguish isomers, and can be used for structure elucidation of unknown compounds [162]. The most used NMR method in environmental metabolomics is one-dimensional (1D) proton (^{1}H) NMR spectroscopy. Another relevant advantage of NMR spectroscopy is that it has non-destructive data acquisition, for instance, NMR methods such as ^{1}H high-resolution magic-angle spinning NMR (^{1}H HR-MAS NMR) can be performed on intact tissues, although this technique has been mainly used in clinical studies [163]. In vivo NMR spectroscopy using flow-based systems with solution-state NMR [164] and comprehensive multiphase NMR [165] is also emerging for environmental metabolomics applications of living organisms. The main limitation of NMR spectroscopy is the sensitivity, only the most abundant metabolites at concentrations >1 mM can be detected [166]. Also, the resolution in 1D ^{1}H NMR spectroscopy is low and although two-dimensional (2D) NMR methods increase resolution, the acquisition time is much. However, nowadays there are technical developments that can increase the sensitivity and resolution of NMR spectroscopy for metabolomics analysis, such as para-H_2-induced hyperpolarization, cryoprobe technology, and microprobe technology.

4.1.5 Data Analysis, and Finally a Biological Interpretation of the Analysed Data

Before the statistical analysis and the pattern detection with chemometrics models, and the previous step of pre-processing the raw spectra collected from NMR spectroscopy or MS is required.

After this previous step, the multivariate statistical analysis is performed to simplify and facilitate the interpretation of the large data sets obtained. The most

commonly used method is an unsupervised statistical approach, the principal component analysis (PCA), that has no bias or previous knowledge about the treatment group. PCA is used to determine general patterns in the metabolic profiles of the different treatment groups. The outcome is a scores plot where each point in a two-dimensional or three-dimensional space is the spectrum of a single sample. The samples with similar features in their metabolome are grouped in the same cluster to identify group membership and visualize the data quickly. Finally, the averaged PCA scores plot is made where the scores of all the spectra of one treatment group are averaged and plotted as average values with standard error. PCA loadings plots indicate the metabolites that contribute to the metabolic variation between treatment groups.

On the other hand, to generate predictions about the data supervised statistical methods such as partial least squares (PLS), PLS regression (PLS-r), PLS discriminant analysis (PLS-DA), and orthogonal (O)-PLS-DA models are used. The data from classified samples are considered the training set, which are used to predict the data from unclassified samples, the test set. PLS models require proper validation to avoid overfit of the data and result in the false appearance of group separation. Validation includes leave-one-out cross validation, K-fold cross validation, Monte Carlo cross validation, and double cross validation. In order to test the predictive ability of the PLS model, an independent validation can be carried out with a different data set. To determine the statistical significance of the model, frequently a permutation test is used. This test gives random group assignments to evaluate the prediction accuracy.

Metabolite data can be presented by reporting the absolute concentrations of metabolites across all treatment groups or by reporting the percentage of change of each metabolite in the exposed groups relative to a control. To determine whether the difference of a metabolite concentration between two independent treatment groups is statistically significant, the main univariate statistical tests are the Student's t-test for data with a normal distribution and the Wilcoxon Mann–Whitney test for data that are not normally distributed. When more than two groups with normal distribution are compared, to determine the significance the analysis of variance (ANOVA) test with post-hoc analysis can be used. The result of all these statistical tests is a P-value. If the P-value is below a significance level that is generally 0.05, then the difference between two or more group means is accepted. Also, if the P-value is below the selected significance level, then no significant difference between the two groups is considered.

4.1.6 Data Analysis, and Finally a Biological Interpretation

The biological interpretation of the metabolic changes is challenging because metabolites can be part of several metabolic pathways. The Online databases of metabolic data include the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Database that shows many metabolic pathways and the individual metabolites in each step of a pathway [167]. The MetaboAnalyst 4.0 has several data

interpretation tools for both MS and NMR spectroscopy data, such as the metabolite pathway analysis (MetPA) where the changes of the individual metabolites are correlated to metabolic pathway information. In this case, the users can enter the list of metabolites with their abundances from two treatment groups, select the model organism among 21 models which MetaboAnalyst 4.0 has the metabolic pathway library from databases as the KEGG. Then, it is necessary to specify the parameters for pathway analysis, including the algorithm for pathway enrichment analysis and the algorithm for topological analysis. The output is presented as a map-style network visualization system and a list of metabolic pathways in order of their statistical significance.

Metabolomics offers a picture of the biochemistry of the organism at any one time. Environmental metabolomics allows characterizing the interaction that occurs between organisms and the surrounding environment. In particular, it has been used to study the interaction between organisms and contaminants that are present in the environment and may have toxic effects. Environmental metabolomics has been as well applied for biomarkers discovery, but this is a relatively new field. It has been in the last 5–10 years when the application of metabolomics for analysing biological samples in environmental monitoring has strongly attracted the attention of research. Consequently, an increasing number of papers are currently being published generating a high amount of data that need to be compiled and harmonized to get relevant information. In Table 3, a summary of recent works in this field is summarized. As can be seen the previous workflows are used, but it should be pointed out that MS analysis together with bioinformatic tools is recently the combination of choice. The main advantage of environmental metabolomics is assess the subacute impact of contamination at realistic concentrations of exposure and the ability to assess complex mixtures and provide integrated responses of these complex mixtures and other environmental stressors.

4.2 Metabolomics in Food Safety and Quality Control

Metabolomics has already been successfully applied in various fields of food science, showing promise in maintaining food safety and food quality including authenticity and food traceability.

Several food safety issues including the emergence of novel pathogens in food, adulteration, risk assessment of genetically modified foods, and the detection of chemical contaminants have been gaining increased attention. For these reasons, food metabolomics has growing as a powerful tool to comprehensively assess quality and safety aspects of food, providing valuable information on the authenticity of food ingredients [181–183].

For example, a quality classification of Spanish olive oils was proposed by Sales et al. [184], using untargeted GC-QTOF-MS with atmospheric pressure chemical ionization and metabolomics-based statistical approach. A novel integrated non-targeted metabolomic analysis was used to establish significant metabolite

Table 3 Examples of recent metabolomic studies in the environment

Organism	Contaminant	Exposure route	Timing of exposure	Sample types	Metabolic biomarkers	Reference
Adult zebrafish (*Danio rerio*)	Tributyltin	Living in tributyltin solution	Adult	Zebrafish embryos extract	↓ Alanine, 4-Aminobutanoate, L-Valine, Taurine, Creatine, L-Methionine, L-Carnitine, 6-Succinoa-minopurine,6-Pyruvoyltetrahydrop-terin, Hypoxanthine, Adenosine monophosphate, Guanosine monophosphate, 5β-Cyprinolsulfate, Oxidized glutathione, PS (44:12) ↑ L-Glutamine, 5-Oxo-D-proline, L-Tyrosine, L-Acetylcarnitine, 4-Aminohippuric acid, Neopterin, Inosine, Methylhexadecanoic acid, dehydrodiconiferyl alcohol, LysoPC (18:1), LysoPC (18:0), PC (32:1), PC (36:5)	[168]
C57BL/6 J mice	DEHP	Gavage	Postnatal day 56–84	Blood	↑ LTB4 8S-HETE Arachidonic acid, Bishomo-γ-linolenic acid, γ-Linolenic acid, Linolenic acid	[169]
Caenorhabditis elegans	Atrazine	Living in solution	Larvae	Nematodes extract solution	↓ Phosphatidylcholine, CDP-choline, GMP, 7,8-Dihydroneopterin 2′,3′- cyclic-p, 2-Oxobutanoate, Neopterin, 7,8-Dihydroneopterin ↑ Salicin 2-Hydroxyethyl-ThPP	[170]
Daphnia magna	Bisphenol A (BPA) Bisphenol F (BPF) and bisphenol S (BPS)	Living in solution	Adult	Organism	↓ Choline, cysteine, glutamic acid, histamine, histidine, isoleucine, leucine, malic acid, and phenylalanine	[171]
Female Wistar rats	di-(2-ethylhexyl) phthalate (DEHP)	Gavage	Postnatal day 28–58	Urine	↓ Kynurenic acid, N6-methyladenosine, L-isoleucyl-L-proline, xanthurenic acid, hippuric acid, tetrahydrocortisol, citric acid, phenylpropionylglycine, cPA(18:2/ 0:0), LysoPC (14:1)	[172]

Kunming mice	(DEHP)	Injection	Postnatal day 0–21	Plasma	↓ PC, PC-(O), PC-(P), LPC18:0, and PIs ↑ PE, PI, PS, and CE	[173]
Lemna minor L.	Metribuzin, glyphosate	Solution		Leaf	↑ γ-aminobutyric acid (GABA), salicylate, caffeate, α,α-trehalose, and squalene	[174]
Lettuce (*Lactuca sativa*)	Imidacloprid and fenvalerate	Water solution		Roots	↑ Arginine, cysteine, homoserine, and 4-hydroxyisoleucine ↓ Glucose, raffinose, maltotetraose, maltopentaose, and stachyose ↓ Organic acids and polyphenolic compounds	[175]
Mytilus edulis	PFOA	Living in PFOA seawater		Gland	↓ LysoPC, taurine, Glutamine, oleic acid ↑ chenodeoxycholate, MDA, pyruvate, serine, glutamate, cysteine, glycine	[176]
Specific pathogen free grade male Sprague Dawley rats	Cd	Gavage		Blood/ kidney	↓ UA, Hypoxanthine (Hyp), Xanthosine, Deoxyinosine, 12-HETE, Guanidinosuccinic acid, Taurocholic acid, Hydroxyphenylacetylglycine, ATP ↑ Formiminoglutamic acid, arachidonic acid	[177]
Sprague Dawley rats	PFOS	Gavage	Postnatal day 0–21	Brain section	↓ Phosphatidylcholines, ↑ GABA, taurine, Gly, Met, Pro, Ser, and T4-Hydroxyproline, N-methyl-D-aspartate (NMDA)	[178]
Sprague Dawley rats	Bisphenol A	Gavage	Postnatal day 42–56	Urine	↓ Riboflavin, Biotin ↑ Methylated amino acids	[179]
Triticum aestivum L	Oxytetracycline and microplastic-polyethylene	Water solution	Seedling growth stage	Leaf samples	↑ Carboxylic acids Glyoxylate and dicarboxylate metabolism, amino acid metabolism and isoquinoline alkaloid biosynthesis	[180]

variations between different lettuce varieties [182]. Another metabolomic approach was proposed by Jandrić et al. [181], for the authentication of Indian citrus fruit. The method permits to distinguish adulterated foods.

Metabolomics has been used to authenticate organic foods. For example, the effects of organic food intervention on biomarkers of exposure to pesticides [185–187]. Metabolomics can as well be used as an analytical technique for food processing evaluation [188] such as in fermented food [189–192].

Thanks to the fast development of analysis techniques, metabolomics has achieved significant progress. Promising findings bridging the biological systems information and food analysis have been achieved. Nowadays, it is possible to analyse more than 1,000 metabolites in a single run and integrate various analytical methods. Like any other omics field, the application of metabolomics approaches in food security, quality, and traceability remains far from achieving its maximal potential and suffers from some bottlenecks, including sensitivity and compound identification limitations. Appropriate statistical approaches for the vast amount of data and metabolite coverage and function continue being requested. Moreover, the practical application and their integration on productive schemes are still nowadays low. Therefore, it is essential to enhance the sensitivity and precision of instruments to enlarge the coverage of metabolites and increase data quality with the combination of different analytical methods and platforms.

5 Conclusions

Omic technologies provide a holistic view of the molecules that make up a cell, tissue, or organism. Omic technologies aimed primarily at the universal detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics), lipids (lipidomics), and metabolites (metabolomics) in a specific biological sample in a non-targeted and non-biased manner. These systems, primarily when integrated, can provide information on the subacute responses to stressors or offer complete information about processes. Omics can also be referred to as high-dimensional biology; the integration of these techniques is called systems biology. Therefore, when these techniques are integrated into food and nutrition, foodomics effectively provides analytic information about the effects of food on human health, it can help to improve food processing, quality control, traceability, and authentication. In Fig. 2, a summary of main analytical techniques in foodomics is presented. In the field of environmental sciences, omic technologies will provide a better understanding of the impact of complex mixtures of contaminants integrated with other environmental stressors. Moreover, the combination of these disciplines in food and environmental sciences brings together the necessary tools for a more confident risk assessment under the one health initiative. Nevertheless, omic strategies still have many challenges, such as the bioinformatic tools that are still evolving. There is a lack of standardized methodologies. Thus, it is necessary to establish carefully designed experiments, accompanied by appropriate analytical techniques and

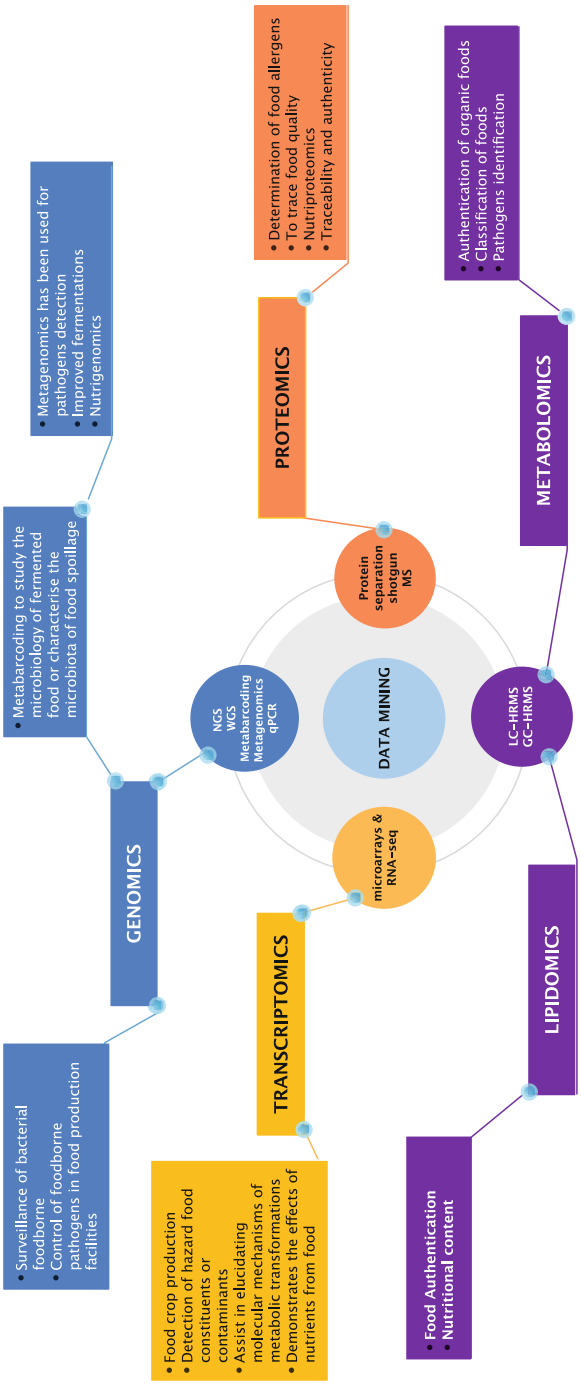


Fig. 2 Summary of main analytical techniques used in food metabolomics. *NGS* next generation sequencing, *WGS* whole genome sequencing, *LC-HRMS* liquid chromatography coupled to high-resolution mass spectrometry, *GC-HRMS* gas chromatography coupled to high-resolution mass spectrometry

statistical analyses, which will assist to compare results tackling many of these challenges, with the potential to generate reliable validated data to answer the biological questions.

Acknowledgements This work was supported by IMAGE project PID2020-116789RB-C41from the Spanish Ministry of Science and Innovation. IDAEA-CSIC is a Severo Ochoa Centre of Research Excellence (Spanish Ministry of Science and Innovation; Project CEX2018-000794-S).

References

1. Gómez-Sagasti MT et al (2014) cDNA microarray assessment of early gene expression profiles in Escherichia coli cells exposed to a mixture of heavy metals. Cell Biol Toxicol 30(4):207–232
2. Won H, Woo S, Yum S (2014) Acute 4-nonylphenol toxicity changes the genomic expression profile of marine medaka fish, Oryzias javanicus. Mol Cell Toxicol 10(2):181–195
3. Sánchez Garayzar AB et al (2016) Hepatic gene expression profiling in zebrafish (Danio rerio) exposed to the fungicide chlorothalonil. Comp Biochem Physiol Part D Genom Proteom 19: 102–111
4. Collí-Dulá RC et al (2016) Molecular impacts of perfluorinated chemicals (PFASs) in the liver and testis of male largemouth bass (Micropterus salmoides) in Minnesota Lakes. Comp Biochem Physiol Part D Genom Proteom 19:129–139
5. Bahamonde PA et al (2016) Defining the role of omics in assessing ecosystem health: perspectives from the Canadian environmental monitoring program. Environ Toxicol Chem 35(1):20–35
6. Martyniuk CJ et al (2016) High contaminant loads in Lake Apopka's riparian wetland disrupt gene networks involved in reproduction and immune function in largemouth bass. Comp Biochem Physiol Part D Genom Proteom 19:140–150
7. Mehinto AC et al (2021) Transcriptomic response patterns of hornyhead turbot (Pleuronichthys verticalis) dosed with polychlorinated biphenyls and polybrominated diphenyl ethers. Comp Biochem Physiol Part D: Genom Proteom 38:100822
8. Jamla M et al (2021) Omics approaches for understanding heavy metal responses and tolerance in plants. Curr Plant Biol 27:100213
9. Koutsoumanis K et al (2019) Whole genome sequencing and metagenomics for outbreak investigation, source attribution and risk assessment of food-borne microorganisms. EFSA J 17(12):e05898
10. Allard MW et al (2016) Practical value of food pathogen traceability through building a whole-genome sequencing network and database. J Clin Microbiol 54(8):1975–1983
11. Moura A et al (2016) Whole genome-based population biology and epidemiological surveillance of Listeria monocytogenes. Nat Microbiol 2(2):16185
12. Jagadeesan B et al (2019) The use of next generation sequencing for improving food safety: translation into practice. Food Microbiol 79:96–115
13. Jackson BR et al (2016) Implementation of nationwide real-time whole-genome sequencing to enhance listeriosis outbreak detection and investigation. Clin Infect Dis 63(3):380–386
14. Mahoney DBJ et al (2022) Associations between Listeria monocytogenes genomic characteristics and adhesion to polystyrene at 8°C. Food Microbiol 102:103915
15. Chau ML et al (2022) Group B streptococcus in retail food – beyond ST283 and raw fish. Food Control 133:108625
16. Timme RE et al (2017) Benchmark datasets for phylogenomic pipeline validation, applications for foodborne pathogen surveillance. PeerJ 5:e3893

17. Forbes JD et al (2017) Metagenomics: the next culture-independent game changer. Front Microbiol 8(JUL):1069
18. Bokulich NA, Mills DA (2012) Next-generation approaches to the microbial ecology of food fermentations. BMB Rep 45(7):377–389
19. Parente E et al (2016) FoodMicrobionet: a database for the visualisation and exploration of food bacterial communities based on network analysis. Int J Food Microbiol 219:28–37
20. Paillart MJM et al (2017) Bacterial population dynamics and sensorial quality loss in modified atmosphere packed fresh-cut iceberg lettuce. Postharvest Biol Technol 124:91–99
21. Leonard SR et al (2016) Strain-level discrimination of Shiga toxin-producing Escherichia coli in spinach using metagenomic sequencing. PLoS One 11(12):e0167870
22. Ortea I, O'Connor G, Maquet A (2016) Review on proteomics for food authentication. J Proteome 147:212–225
23. Creydt M, Fischer M (2018) Omics approaches for food authentication. Electrophoresis 39(13):1569–1581
24. Liu S et al (2022) Tracing the origin of honey products based on metagenomics and machine learning. Food Chem 371:131066
25. Uncu AT et al (2017) Barcode DNA length polymorphisms vs fatty acid profiling for adulteration detection in olive oil. Food Chem 221:1026–1033
26. Agrimonti C et al (2019) Application of real-time PCR (qPCR) for characterization of microbial populations and type of milk in dairy food products. Crit Rev Food Sci Nutr 59(3):423–442
27. Pereira N et al (2021) Selection of autochthonous lab strains of unripe green tomato towards the production of highly nutritious lacto-fermented ingredients. Foods 10(12):2916
28. Zhu Z et al (2021) Nutrigenomics reveals potential genetic underpinning of diverse taste preference of Chinese men. Genes Genom 43(6):689–699
29. Utkina AS, Karagodin VP (2021) Nutrigenomics as a tool for optimizing the composition of specialized food products by the efficiency criterion. IOP Conf Ser Earth Environ Sci 677(4): 042050
30. Brennan L, De Roos B (2021) Nutrigenomics: lessons learned and future perspectives. Am J Clin Nutr 113(3):503–516
31. Bordoni L et al (2021) Nutrigenomics of dietary lipids. Antioxidants 10(7):994
32. Anitha A et al (2021) Vitamins and cognition: a nutrigenomics perspective. Curr Nutr Food Sci 17(4):348–362
33. Ahluwalia MK (2021) Nutrigenetics and nutrigenomics—a personalized approach to nutrition. Adv Genet 108:277–340
34. Tholey A, Becker A (2017) Top-down proteomics for the analysis of proteolytic events - methods, applications and perspectives. Biochim Biophys Acta, Mol Cell Res 1864(11): 2191–2199
35. Cifuentes A (2009) Food analysis and foodomics. J Chromatogr A 1216(43):7109
36. Li C et al (2018) Molecular biological methods in environmental engineering. Water Environ Res 90(10):1371–1391
37. Campos A et al (2012) Proteomic research in bivalves: towards the identification of molecular markers of aquatic pollution. J Proteome 75(14):4346–4359
38. Ward JE et al (2019) Selective ingestion and egestion of plastic particles by the blue mussel (Mytilus edulis) and eastern oyster (Crassostrea virginica): implications for using bivalves as bioindicators of microplastic pollution. Environ Sci Technol 53(15):8776–8784
39. Zha S et al (2019) Immunotoxicity of four nanoparticles to a marine bivalve species, Tegillarca granosa. J Hazard Mater 377:237–248
40. Rodil R et al (2019) Legacy and emerging pollutants in marine bivalves from the Galician coast (NW Spain). Environ Int 129:364–375
41. Debén S et al (2015) Inland water quality monitoring with native bryophytes: a methodological review. Ecol Indic 53:115–124

42. Magni S et al (2018) Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater zebra mussel Dreissena polymorpha (Mollusca: Bivalvia). Sci Total Environ 631–632:778–788
43. Bertrand L et al (2018) Native crustacean species as a bioindicator of freshwater ecosystem pollution: a multivariate and integrative study of multi-biomarker response in active river monitoring. Chemosphere 206:265–277
44. Vellinger C et al (2016) Investigating the emerging role of comparative proteomics in the search for new biomarkers of metal contamination under varying abiotic conditions. Sci Total Environ 562:974–986
45. Barreto A et al (2020) Effects of gold nanoparticles in gilthead seabream—a proteomic approach. Aquat Toxicol 221:105445
46. Molina AM et al (2021) Proteomic profile of the effects of low-dose bisphenol A on zebrafish ovaries. Food Chem Toxicol 156:112435
47. Zhang H, Zhai Y (2020) Integrated transcriptomic and proteomic analyses of the tissues from the digestive gland of Chlamys farreri following cadmium exposure. J Cell Biochem 121(2): 974–983
48. Lu Z et al (2020) iTRAQ-based proteomic analysis on the mitochondrial responses in gill tissues of juvenile olive flounder Paralichthys olivaceus exposed to cadmium. Environ Pollut 257:113591
49. You X et al (2022) Interactions between antibiotics and heavy metals determine their combined toxicity to Synechocystis sp. J Hazard Mater 424:127707
50. Song Q et al (2017) Toxic responses of Perna viridis hepatopancreas exposed to DDT, benzo (a)pyrene and their mixture uncovered by iTRAQ-based proteomics and NMR-based metabolomics. Aquat Toxicol 192:48–57
51. Green DS et al (2019) Exposure to microplastics reduces attachment strength and alters the haemolymph proteome of blue mussels (Mytilus edulis). Environ Pollut 246:423–434
52. Xu G, Yu Y (2021) Polystyrene microplastics impact the occurrence of antibiotic resistance genes in earthworms by size-dependent toxic effects. J Hazard Mater 416:125847
53. Yu C et al (2022) Antibiotic tolerance and degradation capacity of the organic pollutant-degrading bacterium Rhodococcus biphenylivorans TG9T. J Hazard Mater 424:127712
54. Ambrosio L et al (2018) Accumulation of polychlorinated biphenyls in mussels: a proteomic study. J Food Prot 81(2):316–324
55. Luo L et al (2017) Differential effects of zinc exposure on male and female oysters (Crassostrea angulata) as revealed by label-free quantitative proteomics. Environ Toxicol Chem 36(10):2602–2613
56. Li Y, Wang WX (2021) Integrated transcriptomics and proteomics revealed the distinct toxicological effects of multi-metal contamination on oysters. Environ Pollut 284:117533
57. Flores-Nunes F et al (2015) Changes in protein expression of pacific oyster Crassostrea gigas exposed in situ to urban sewage. Environ Sci Pollut Res 22(22):17267–17279
58. Bebianno MJ et al (2016) Proteomic changes in Corbicula fluminea exposed to wastewater from a psychiatric hospital. Environ Sci Pollut Res 23(6):5046–5055
59. Navon G, Novak L, Shenkar N (2021) Proteomic changes in the solitary ascidian Herdmania momus following exposure to the anticonvulsant medication carbamazepine. Aquat Toxicol 237:105886
60. Gomes T et al (2014) Proteomic response of mussels Mytilus galloprovincialis exposed to CuO NPs and Cu2+: an exploratory biomarker discovery. Aquat Toxicol 155:327–336
61. Sánchez-Marín P et al (2021) Proteomic analysis and biochemical alterations in marine mussel gills after exposure to the organophosphate flame retardant TDCPP. Aquat Toxicol 230: 105688
62. Zhang C et al (2019) Transgenerational proteome plasticity in resilience of a marine copepod in response to environmentally relevant concentrations of microplastics. Environ Sci Technol 53(14):8426–8436

63. Bromilow S et al (2017) A curated gluten protein sequence database to support development of proteomics methods for determination of gluten in gluten-free foods. J Proteome 163:67–75
64. Shaheen N et al (2019) Proteomic characterization of low molecular weight allergens and putative allergen proteins in lentil (Lens culinaris) cultivars of Bangladesh. Food Chem 297: 124936
65. McClain S et al (2018) Variation in seed allergen content from three varieties of soybean cultivated in nine different locations in Iowa, Illinois, and Indiana. Front Plant Sci 9:1025
66. Li H et al (2018) High hydrostatic pressure reducing allergenicity of soy protein isolate for infant formula evaluated by ELISA and proteomics via Chinese soy-allergic children's sera. Food Chem 269:311–317
67. Ribeiro M et al (2020) Natural variation of hazelnut allergenicity: is there any potential for selecting hypoallergenic varieties? Nutrients 12(7):2100
68. Cardona EEG et al (2018) Novel low-abundance allergens from mango via combinatorial peptide libraries treatment: a proteomics study. Food Chem 269:652–660
69. Nikolić J et al (2018) Employment of proteomic and immunological based methods for the identification of catalase as novel allergen from banana. J Proteome 175:87–94
70. Wilson JM, Platts-Mills TAE (2019) Red meat allergy in children and adults. Curr Opin Allergy Clin Immunol 19(3):229–235
71. Apostolovic D et al (2014) Immunoproteomics of processed beef proteins reveal novel galactose-α-1,3-galactose-containing allergens. Allergy 69(10):1308–1315
72. Sogawa K et al (2018) Search for a novel allergen in hen's egg allergy using an IgE immunoblotting assay. Int Arch Allergy Immunol 176(3–4):189–197
73. Ruethers T et al (2018) Seafood allergy: a comprehensive review of fish and shellfish allergens. Mol Immunol 100:28–57
74. D'Auria E et al (2018) Precision medicine in cow's milk allergy: proteomics perspectives from allergens to patients. J Proteome 188:173–180
75. Qi K et al (2019) A rapid immobilized trypsin digestion combined with liquid chromatography – tandem mass spectrometry for the detection of milk allergens in baked food. Food Control 102:179–187
76. Bavaro SL et al (2019) Modulation of milk allergenicity by baking Milk in foods: a proteomic investigation. Nutrients 11(7):1536
77. Abbring S et al (2020) Loss of allergy-protective capacity of raw cow's milk after heat treatment coincides with loss of immunologically active whey proteins. Food Funct 11(6): 4982–4993
78. Ramachandran B, Yang CT, Downs ML (2020) Parallel reaction monitoring mass spectrometry method for detection of both casein and whey Milk allergens from a baked food matrix. J Proteome Res 19(8):2964–2976
79. Calvano CD et al (2021) Proteomic analysis of food allergens by MALDI TOF/TOF mass spectrometry. Methods Mol Biol 2178:357–376
80. Stella R et al (2020) LC-HRMS/MS for the simultaneous determination of four allergens in fish and swine food products. Food Chem 331:127276
81. Cajka T et al (2016) Advances in mass spectrometry for food authenticity testing: an omics perspective. In: Advances in food authenticity testing. Woodhead Publishing, pp 171–200
82. Cozzolino R et al (2001) Identification of adulteration in milk by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Mass Spectrom 36(9):1031–1037
83. Di Girolamo F et al (2014) A sensitive and effective proteomic approach to identify she-donkey's and goat's milk adulterations by MALDI-TOF MS fingerprinting. Int J Mol Sci 15(8):13697–13719
84. Pinto G et al (2012) Lactosylated casein phosphopeptides as specific indicators of heated milks. Anal Bioanal Chem 402(5):1961–1972
85. Muller L et al (2008) Capillary electrophoresis-mass spectrometry - a fast and reliable tool for the monitoring of milk adulteration. Electrophoresis 29(10):2088–2093

86. Arena S, Salzano AM, Scaloni A (2016) Identification of protein markers for the occurrence of defrosted material in milk through a MALDI-TOF-MS profiling approach. J Proteome 147: 56–65
87. Montowska M, Fornal E (2017) Label-free quantification of meat proteins for evaluation of species composition of processed meat products. Food Chem 237:1092–1100
88. Vostrikov NL, Chernukh IM (2018) Identification of tissue-specific proteins and peptides forming innovative meat products corrective properties to confirm authenticity of meat raw materials. Foods Raw Mater 6(1):201–209
89. Rodríguez-Vázquez R et al (2020) Measuring quantitative proteomic distance between Spanish beef breeds. Food Chem 315:126293
90. Kotecka-Majchrzak K et al (2021) Proteomic analysis of oilseed cake: a comparative study of species-specific proteins and peptides extracted from ten seed species. J Sci Food Agric 101(1):297–306
91. Kotecka-Majchrzak K et al (2021) Multispecies identification of oilseed-and meat-specific proteins and heat-stable peptide markers in food products. Molecules 26(6):1577
92. Lerma-García MJ et al (2019) Proteomic fingerprinting of apple fruit, juice, and cider via combinatorial peptide ligand libraries and MS analysis. Electrophoresis 40(2):266–271
93. Agregán R et al (2021) Proteomic advances in cereal and vegetable crops. Molecules 26(16): 4924
94. Cervi D et al (2010) Micronutrients attenuate progression of prostate cancer by elevating the endogenous inhibitor of angiogenesis, platelet Factor-4. BMC Cancer 10:258
95. Park YM et al (2017) Nutriproteomic analysis of Hwangmaemok-induced antiangiogenic effect using antibody-arrayed protein chip assay. J Med Food 20(6):586–591
96. Sellami M, Bragazzi NL (2020) Nutrigenomics and breast cancer: state-of-art, future perspectives and insights for prevention. Nutrients 12(2):512
97. Ribeiro M et al (2020) Nutriproteomics survey of sweet chestnut (Castanea sativa miller) genetic resources in Portugal. Food Biosci 36:100622
98. Rowell C, Carpenter DM, Lamartiniere CA (2005) Chemoprevention of breast cancer, proteomic discovery of genistein action in the rat mammary gland. J Nutr 135(12 Suppl):2953s–2959s
99. Mayo B, Vázquez L, Flórez AB (2019) Equol: a bacterial metabolite from the daidzein isoflavone and its presumed beneficial health effects. Nutrients 11(9):2231
100. Zhang L et al (2019) Isoflavone ME-344 disrupts redox homeostasis and mitochondrial function by targeting heme oxygenase 1. Cancer Res 79(16):4072–4085
101. Ilieş M et al (2020) From proteomics to personalized medicine: the importance of isoflavone dose and estrogen receptor status in breast cancer cells. J Pers Med 10(4):1–15
102. Cajka T, Fiehn O (2016) Toward merging untargeted and targeted methods in mass spectrometry-based metabolomics and lipidomics. Anal Chem 88(1):524–545
103. Chen H et al (2017) Lipidomics in food science. Curr Opin Food Sci 16:80–87
104. Ryan E, Reid GE (2016) Chemical derivatization and ultrahigh resolution and accurate mass spectrometry strategies for "shotgun" lipidome analysis. Acc Chem Res 49(9):1596–1604
105. Koelmel JP et al (2020) Environmental lipidomics: understanding the response of organisms and ecosystems to a changing world. Metabolomics 16(5):56
106. Sogin EM et al (2016) Metabolomic signatures of increases in temperature and ocean acidification from the reef-building coral, Pocillopora damicornis. Metabolomics 12(4):71
107. Ahuja I et al (2010) Plant molecular stress responses face climate change. Trends Plant Sci 15(12):664–674
108. Zhang J et al (2016) Low-level environmental phthalate exposure associates with urine metabolome alteration in a Chinese male cohort. Environ Sci Technol 50(11):5953–5960
109. Chan CY, Wang W-X (2018) A lipidomic approach to understand copper resilience in oyster Crassostrea hongkongensis. Aquat Toxicol 204:160–170

110. Koelmel JP et al (2017) LipidMatch: an automated workflow for rule-based lipid identification using untargeted high-resolution tandem mass spectrometry data. BMC Bioinformatics 18(1): 331
111. Rocchetta I et al (2014) Effects of sewage discharges on lipid and fatty acid composition of the Patagonian bivalve Diplodon chilensis. Mar Pollut Bull 79(1):211–219
112. Albergamo A et al (2016) Free fatty acid profiling of marine sentinels by nanoLC-EI-MS for the assessment of environmental pollution effects. Sci Total Environ 571:955–962
113. Jörg S et al (2016) Environmental factors in autoimmune diseases and their role in multiple sclerosis. Cell Mol Life Sci 73(24):4611–4622
114. Pakiet A et al (2019) Changes in lipids composition and metabolism in colorectal cancer: a review. Lipids Health Dis 18(1):29
115. Abhyankar V et al (2018) Drosophila DNA/RNA methyltransferase contributes to robust host defense in aging animals by regulating sphingolipid metabolism. J Exp Biol 221(22): jeb187989
116. Zhang L, Routsong R, Strand SE (2019) Greatly enhanced removal of volatile organic carcinogens by a genetically modified houseplant, pothos ivy (Epipremnum aureum) expressing the mammalian cytochrome P450 2e1 gene. Environ Sci Technol 53(1):325–331
117. El Ouahabi A, Grimalt JO (2017) Marine sedimentary lipidomics of the glacial-interglacial changes during the lower Pleistocene (SW Iberian Margin). Environ Sci Pollut Res 24(34): 26605–26614
118. Sollai M et al (2019) A combined lipidomic and 16S rRNA gene amplicon sequencing approach reveals archaeal sources of intact polar lipids in the stratified Black Sea water column. Geobiology 17(1):91–109
119. Li M, Butka E, Wang X (2014) Comprehensive quantification of triacylglycerols in soybean seeds by electrospray ionization mass spectrometry with multiple neutral loss scans. Sci Rep 4(1):6581
120. Lísa M, Holčapek M (2013) Characterization of triacylglycerol enantiomers using chiral HPLC/APCI-MS and synthesis of enantiomeric triacylglycerols. Anal Chem 85(3):1852–1859
121. Dong XY et al (2015) Triacylglycerol composition profiling and comparison of high-oleic and normal peanut oils. J Am Oil Chem Soc 92(2):233–242
122. Li H et al (2020) Comparative lipid profile of four edible shellfishes by UPLC-triple TOF-MS/MS. Food Chem 310:125947
123. Li Q et al (2017) Lipidomics profiling of goat milk, soymilk and bovine milk by UPLC-Q-exactive orbitrap mass spectrometry. Food Chem 224:302–309
124. Gao K et al (2019) Direct determination of free state low molecular weight compounds in serum by online TurboFlow SPE HPLC-MS/MS and its application. Talanta 194:960–968
125. Palazzotto E, Weber T (2018) Omics and multi-omics approaches to study the biosynthesis of secondary metabolites in microorganisms. Curr Opin Microbiol 45:109–116
126. Johnson CH, Ivanisevic J, Siuzdak G (2016) Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol 17(7):451–459
127. Peregrín-Alvarez JM, Sanford C, Parkinson J (2009) The conservation and evolutionary modularity of metabolism. Genome Biol 10(6):R63
128. Bingol K (2018) Recent advances in targeted and untargeted metabolomics by NMR and MS/NMR methods. High Throughput 7(2):9
129. Emwas AH (2015) The strengths and weaknesses of NMR spectroscopy and mass spectrometry with particular focus on metabolomics research. Methods Mol Biol 1277:161–193
130. Bundy JG, Davey MP, Viant MR (2008) Environmental metabolomics: a critical review and future perspectives. Metabolomics 5(1):3
131. Zhang X et al (2018) Measurement of metabolite variations and analysis of related gene expression in Chinese liquorice (Glycyrrhiza uralensis) plants under UV-B irradiation. Sci Rep 8(1):6144

132. Creydt M et al (2019) Effects of elevated CO2 concentration on leaves and berries of black elder (Sambucus nigra) using UHPLC-ESI-QTOF-MS/MS and gas exchange measurements. J Plant Physiol 234–235:71–79
133. Liang SM et al (2021) Metabolomics analysis reveals drought responses of trifoliate Orange by arbuscular mycorrhizal fungi with a focus on terpenoid profile. Front Plant Sci 12:740524
134. Zhang X et al (2021) Widely targeted metabolomics reveals the different metabolic changes in leaves and roots of two cotton varieties under drought stress. J Agron Crop Sci 207(6): 1041–1049
135. Wu C et al (2022) Integrated microbiology and metabolomics analysis reveal plastic mulch film residue affects soil microorganisms and their metabolic functions. J Hazard Mater 423: 127258
136. Gu J et al (2019) 1 H NMR-based metabolomic analysis of nine organophosphate flame retardants metabolic disturbance in hep G2 cell line. Sci Total Environ 665:162–170
137. Jin R et al (2020) Perfluoroalkyl substances and severity of nonalcoholic fatty liver in children: an untargeted metabolomics approach. Environ Int 134:105220
138. Brew DW et al (2020) Metabolomic investigations of the temporal effects of exposure to pharmaceuticals and personal care products and their mixture in the eastern oyster (Crassostrea virginica). Environ Toxicol Chem 39(2):419–436
139. Fu Q et al (2021) Metabolomic profiling and toxicokinetics modeling to assess the effects of the pharmaceutical diclofenac in the aquatic invertebrate Hyalella azteca. Environ Sci Technol 55(12):7920–7929
140. Sheikholeslami MN et al (2020) Untargeted metabolomics changes on Gammarus pulex induced by propranolol, triclosan, and nimesulide pharmaceutical drugs. Chemosphere 260: 127479
141. Li X et al (2019) New insights into the responses of soil microorganisms to polycyclic aromatic hydrocarbon stress by combining enzyme activity and sequencing analysis with metabolomics. Environ Pollut 255:113312
142. Tian J et al (2017) The toxic effects of Tetrachlorobisphenol A in Saccharomyces cerevisiae cells via metabolic interference. Sci Rep 7(1):2655
143. Mosier AC et al (2013) Metabolites associated with adaptation of microorganisms to an acidophilic, metal-rich environment identified by stable-isotope-enabled metabolomics. MBio 4(2):e00484-12
144. Tang R et al (2020) A new perspective on the toxicity of arsenic-contaminated soil: tandem mass tag proteomics and metabolomics in earthworms. J Hazard Mater 398:122825
145. Zhu Y et al (2020) Integration of transcriptomics and metabolomics reveals the responses of earthworms to the long-term exposure of TiO2 nanoparticles in soil. Sci Total Environ 719: 137492
146. Xu K et al (2021) Toxicity of three carbon-based nanomaterials to earthworms: effect of morphology on biomarkers, cytotoxicity, and metabolomics. Sci Total Environ 777:146224
147. Zhang R, Zhou Z (2019) Effects of the chiral fungicides metalaxyl and metalaxyl-M on the earthworm Eisenia fetida as determined by1H-NMR-based untargeted metabolomics. Molecules 24(7):1293
148. Yang X et al (2021) The responses of the growth, cytochrome P450 isoenzymes activities and the metabolomics in earthworms to sublethal doses of dichlorvos in soil. Ecotoxicol Environ Saf 207:111547
149. Sivaram AK et al (2019) Metabolomics reveals defensive mechanisms adapted by maize on exposure to high molecular weight polycyclic aromatic hydrocarbons. Chemosphere 214:771–780
150. Sung J et al (2015) Metabolomic profiling from leaves and roots of tomato (Solanum lycopersicum L.) plants grown under nitrogen, phosphorus or potassium-deficient condition. Plant Sci 241:55–64
151. Simmons DBD et al (2015) Omics for aquatic ecotoxicology: control of extraneous variability to enhance the analysis of environmental effects. Environ Toxicol Chem 34(8):1693–1704

152. Gómez-Canela C et al (2016) Targeted metabolomics of Gammarus pulex following controlled exposures to selected pharmaceuticals in water. Sci Total Environ 562:777–788
153. Griffin JL et al (2007) Standard reporting requirements for biological samples in metabolomics experiments: mammalian/in vivo experiments. Metabolomics 3(3):179–188
154. van der Werf MJ et al (2007) Standard reporting requirements for biological samples in metabolomics experiments: microbial and in vitro biology experiments. Metabolomics 3: 189–194
155. Murphy CA et al (2018) Incorporating suborganismal processes into dynamic energy budget models for ecological risk assessment. Integr Environ Assess Manag 14(5):615–624
156. Dietmair S et al (2010) Towards quantitative metabolomics of mammalian cells: development of a metabolite extraction protocol. Anal Biochem 404(2):155–164
157. Bruce SJ et al (2009) Investigation of human blood plasma sample preparation for performing metabolomics using ultrahigh performance liquid chromatography/mass spectrometry. Anal Chem 81(9):3285–3296
158. Ghaste M, Mistrik R, Shulaev V (2016) Applications of Fourier transform ion cyclotron resonance (FT-ICR) and orbitrap based high resolution mass spectrometry in metabolomics and lipidomics. Int J Mol Sci 17(6):816
159. Southam AD et al (2017) A complete workflow for high-resolution spectral-stitching nanoelectrospray direct-infusion mass-spectrometry-based metabolomics and lipidomics. Nat Protoc 12(2):255–273
160. Taylor NS, White TA, Viant MR (2017) Defining the baseline and oxidant perturbed lipidomic profiles of Daphnia magna. Metabolites 7(1):11
161. Ramautar R, Somsen GW, de Jong GJ (2019) CE-MS for metabolomics: developments and applications in the period 2016–2018. Electrophoresis 40(1):165–179
162. Bingol K, Brüschweiler R (2015) Two elephants in the room: new hybrid nuclear magnetic resonance and mass spectrometry approaches for metabolomics. Curr Opin Clin Nutr Metab Care 18(5):471–477
163. Battini S et al (2017) Metabolomics approaches in pancreatic adenocarcinoma: tumor metabolism profiling predicts clinical outcome of patients. BMC Med 15(1):56
164. Tabatabaei Anaraki M et al (2018) Development and application of a low-volume flow system for solution-state in vivo NMR. Anal Chem 90(13):7912–7921
165. Mobarhan YL et al (2016) Comprehensive multiphase NMR applied to a living organism. Chem Sci 7(8):4856–4866
166. Nagana Gowda GA et al (2018) NMR-guided mass spectrometry for absolute quantitation of human blood metabolites. Anal Chem 90(3):2001–2009
167. Kanehisa M et al (2016) KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 44(D1):D457–D462
168. Ortiz-Villanueva E et al (2018) Assessment of endocrine disruptors effects on zebrafish (Danio rerio) embryos by untargeted LC-HRMS metabolomic analysis. Sci Total Environ 635:156–166
169. Li Y et al (2020) Hepatotoxicity study of combined exposure of DEHP and ethanol: a comprehensive analysis of transcriptomics and metabolomics. Food Chem Toxicol 141: 111370
170. Yin J et al (2020) Non-targeted metabolomic profiling of atrazine in Caenorhabditis elegans using UHPLC-QE orbitrap/MS. Ecotoxicol Environ Saf 206:111170
171. Oliveira Pereira EA et al (2021) Metabolomics reveals that bisphenol pollutants impair protein synthesis-related pathways in Daphnia magna. Meta 11(10):666
172. Dong X et al (2017) Urinary metabolomic profiling in rats exposed to dietary di(2-ethylhexyl) phthalate (DEHP) using ultra-performance liquid chromatography quadrupole time-of-flight tandem mass spectrometry (UPLC/Q-TOF-MS). Environ Sci Pollut Res Int 24(20): 16659–16672

173. Huang Y et al (2019) DEHP and DINP induce tissue- and gender-specific disturbances in fatty acid and lipidomic profiles in neonatal mice: a comparative study. Environ Sci Technol 53(21): 12812–12822
174. Kostopoulou S et al (2020) Assessment of the effects of metribuzin, glyphosate, and their mixtures on the metabolism of the model plant Lemna minor L. applying metabolomics. Chemosphere 239:124582
175. Zhang Y et al (2022) Metabolic disturbance in lettuce (Lactuca sativa) plants triggered by imidacloprid and fenvalerate. Sci Total Environ 802:149764
176. Li F et al (2021) Integrated analysis of physiological, transcriptomics and metabolomics provides insights into detoxication disruption of PFOA exposure in Mytilus edulis. Ecotoxicol Environ Saf 214:112081
177. Guan T et al (2021) Metabolomics analysis of the effects of quercetin on renal toxicity induced by cadmium exposure in rats. Biometals 34(1):33–48
178. Reardon AJF et al (2019) Neurodevelopmental and metabolomic responses from prenatal coexposure to perfluorooctanesulfonate (PFOS) and methylmercury (MeHg) in Sprague-Dawley rats. Chem Res Toxicol 32(8):1656–1669
179. Wang X et al (2021) Hepatoprotective effects of oridonin against bisphenol A induced liver injury in rats via inhibiting the activity of xanthione oxidase. Sci Total Environ 770:145301
180. Bao Y et al (2022) Stress response to oxytetracycline and microplastic-polyethylene in wheat (Triticum aestivum L.) during seed germination and seedling growth stages. Sci Total Environ 806:150553
181. Jandrić Z et al (2017) Authentication of Indian citrus fruit/fruit juices by untargeted and targeted metabolomics. Food Control 72:181–188
182. Yang X et al (2018) A novel integrated non-targeted metabolomic analysis reveals significant metabolite variations between different lettuce (Lactuca sativa. L) varieties. Hortic Res 5:33
183. Yao R et al (2018) Quality variation of goji (fruits of Lycium spp.) in China: a comparative morphological and metabolomic analysis. Front Pharmacol 9:151
184. Sales C et al (2017) Quality classification of Spanish olive oils by untargeted gas chromatography coupled to hybrid quadrupole-time of flight mass spectrometry with atmospheric pressure chemical ionization and metabolomics-based statistical approach. Food Chem 216: 365–373
185. Cubero-Leon E, De Rudder O, Maquet A (2018) Metabolomics for organic food authentication: results from a long-term field study in carrots. Food Chem 239:760–770
186. Konstantinou C et al (2022) Use of metabolomics in refining the effect of an organic food intervention on biomarkers of exposure to pesticides and biomarkers of oxidative damage in primary school children in Cyprus: a cluster-randomized cross-over trial. Environ Int 158: 107008
187. Martínez Bueno MJ et al (2018) A non-targeted metabolomic approach to identify food markers to support discrimination between organic and conventional tomato crops. J Chromatogr A 1546:66–76
188. Utpott M et al (2022) Metabolomics: an analytical technique for food processing evaluation. Food Chem 366:130685
189. Park MK, Kim YS (2021) Mass spectrometry based metabolomics approach on the elucidation of volatile metabolites formation in fermented foods: a mini review. Food Sci Biotechnol 30(7):881–890
190. Adebo OA et al (2021) Application of gas chromatography–mass spectrometry (GC-MS)-based metabolomics for the study of fermented cereal and legume foods: a review. Int J Food Sci Technol 56(4):1514–1534
191. Gao Y et al (2021) Metabolomics approaches for the comprehensive evaluation of fermented foods: a review. Foods 10(10):2294
192. Singh D, Lee S, Lee CH (2017) Metabolomics for empirical delineation of the traditional Korean fermented foods and beverages. Trends Food Sci Technol 61:103–115

Thermal Desorption and Pyrolysis Combined with Gas Chromatography–Mass Spectrometry in Food and Environmental Chemistry

Julian Campo and Yolanda Picó

Contents

Abstract Pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) has confirmed to be a versatile technique that benefits food and environmental analyses. It has been used for the chemical characterization of materials and compounds that are not suitable for traditional gas chromatography (GC) because of their large size. The controlled thermal degradation carried out during pyrolysis is able to break down macromolecules into volatile fragments easier to identify because they become separable by GC and detectable by mass spectrometry (MS). A wide array of applications has been reported using Py–GC–MS, from characterization of macromolecules (polymers, paints, lacquers, adhesives, plastic, synthetic fibers, organic matter, etc.) in a variety of disciplines including forensics, history, engineering, and, of course, food and environmental sciences. In recent years, this technique has

J. Campo and Y. Picó (✉)
Environmental and Food Safety Research Group-University of Valencia (SAMA-UV), Desertification Research Centre – CIDE (Spanish National Research Council, University of Valencia, Generalitat Valenciana), Valencia, Spain
e-mail: Julian.campo@uv.es; Yolanda.pico@uv.es

Yolanda Picó and Julian Campo (eds.), *Mass Spectrometry in Food and Environmental Chemistry*, Hdb Env Chem (2023) 119: 225–248, DOI 10.1007/698_2022_887,

Published online: 18 October 2022

experienced an important increase due to its capability for the chemical fingerprinting of organic matter, and the identification and characterization of nano-, microplastics used for food package and present in environmental and food samples. In this chapter, we describe current Py–GC–MS instrumentation and working modes and summarize recent applications in food and environmental analysis with special emphasis on its strengths and limitations.

Keywords Curie-point, Evolved gas analysis, Microfurnace, Reactive pyrolysis, Thermal degradation

1 Introduction

Pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) can characterize macromolecules [1] because pyrolysis transforms non-volatile macromolecules into their structural units, which are normally small and volatile, while GC provides resolving power and MS identification capability, broadening the range of applications [2]. The International Union of Pure and Applied Chemistry (IUPAC) defines Py–GC as "a version of reaction chromatography in which a sample is thermally decomposed to simpler fragments before entering the column," and Py–GC–MS as "the characterization in an inert atmosphere of a material or a chemical process in which chemical degradation reaction(s) is/are induced by thermal energy" [3].

Pyrolysis provides the thermal decomposition of the sample at relatively low pressure and at temperatures between 500 and 1,400°C originating characteristics volatile units in the presence of an inert gas (the most common is helium). Chemical changes in the macromolecules are induced by high temperatures with progressive bond breakages going from the weakest to the strongest. Among reactions implicated are depolymerization (resulting in basic units and/or oligomers), random excision (resulting in random small fragments as all the C-C bonds have the same energy), and the elimination of side groups (cleaved of the polymer bond). Other possible but minor reactions include chard formation, oxidation cross-linking, cyclization, isomerization, and hydrogenation [2]. The fragmentation of molecules caused by pyrolysis depends on the relative strength of the bonds and the ability of the formed free radicals to provide steady products [4, 5]. For instance, big complex hydrocarbon molecules can be broken down during pyrolysis into simpler and smaller molecules of char, liquid, and gas (Fig. 1) [6].

The units formed in the pyrolyzers are separated by chromatography, using fused silica capillary columns, and identified by MS (using mass spectral libraries or selecting few characteristic ions). Py–GC–MS is well-matched with most of the treatments used with environmental and food samples but can also eliminate these processes as it is capable to analyze directly macromolecules and/or polymers [7–9]. One important advantage of this technique is the small quantity of sample used (5–200 μg) although samples must be dried.

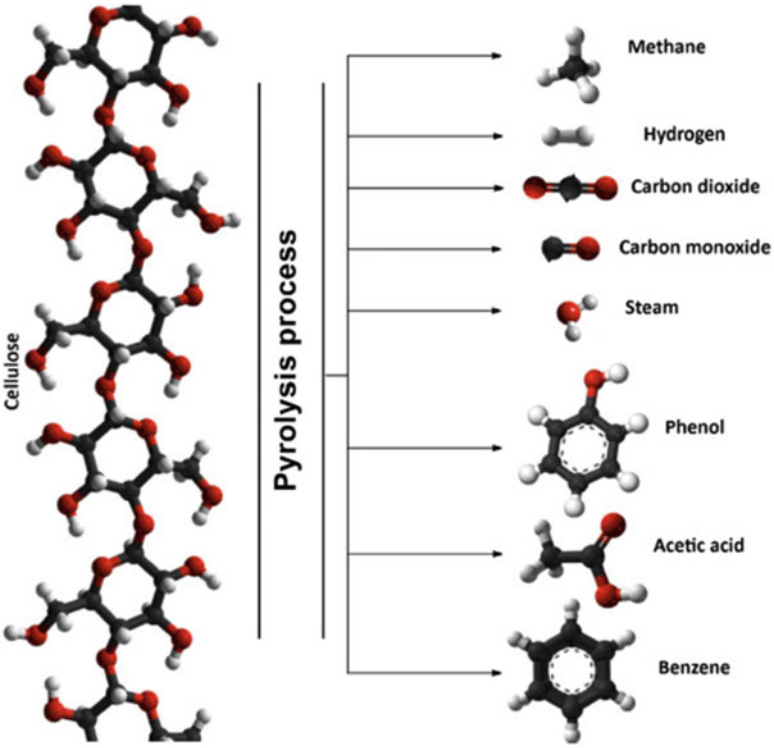


Fig. 1 Decomposition of large hydrocarbon molecules into smaller ones during pyrolysis [6]

Py–GC–MS enables the analysis of macromolecules as polymers, copolymers and additives, packaging materials, fibers, coatings, electronic intermediates, paints, lacquers, paper or wood derivatives, bio-oils and biomass, food, drugs, surfactants, fragrances, etc. [9–15]. The applications of Py–GC–MS include research, quality control, and characterization of materials as well as forensic analyses, conservation and restoration of cultural heritage, biotechnology, geology, and agriculture among others. Publications on Py–GC–MS are mostly focused on its applications such as the authentication of extra virgin olive oils [11] or the characterization, for example, of bio-oils [4, 12, 14, 16], polymers [1, 17, 18], lacquer films [9, 15], or lignite tars [19]. There are also a number of publications, including several reviews, dealing with soil organic matter and its chemical fingerprint [8, 20–26], and specially with the occurrence and fate of micro- and nano-plastics (MPs and NPs, respectively) in the environment [27–35], which has gained great attention recently.

In this chapter, we highlight new instrumental advances that combined with the multiple workflows of Py–GC–MS have broad food and environmental applications, mainly in relation to compositional analysis, quality control of authenticity, adulteration, and contamination detection (especially by plastic polymers), and characterization of organic matter.

2 Py–GC–MS: Instrumentation and Working Modes

2.1 Working Modes in Py–GC–MS

The technological evolution of Py–GC–MS has mostly be focused on devices design, inertness of their components, versatility of the working modes, and reproducibility of the obtained data, and it has already been reported in several review articles [2, 36]. However, there are several important new developments in the pyrolysis devices (microfurnace chambers, quartz liners for the sample, etc.) as well as in the GC–MS process itself (time-of-flight (TOF) MS in the low- or high-resolution mode, comprehensive GCxGC, etc.) that deserve to be highlighted.

Currently, there are different possibilities to work with Py–GC–MS depending on the analyses' objectives summarized as (Fig. 2):

Evolved gas analysis (EGA-MS) (Fig. 2a): the degradation products are separated from macromolecules based on the temperature at which they are formed rather than at their volatilization temperature. This is achieved using a slow temperature ramp in the pyrolyzer and a short and narrow (2.5 m, 0.15 mm i.d.) deactivated capillary tube without a stationary phase, which connects directly the GC injector and the MS-detector. During the GC oven is held constant at 300°C, the pyrolyzer is heated by a temperature program from 40 to 500°C. The resulting chart is called an EGA thermogram. EGA-MS is a previous step in which the number of peaks and the temperature zone for each peak are vital information in determining correct temperatures for the other working modes (i.e., single-shot, double-shot, and heart-cut).

Single-shot analysis (Fig. 2b): the pyrolysis is performed at a single temperature (normally >500°C depending on the sample analyzed), which increases as fast as

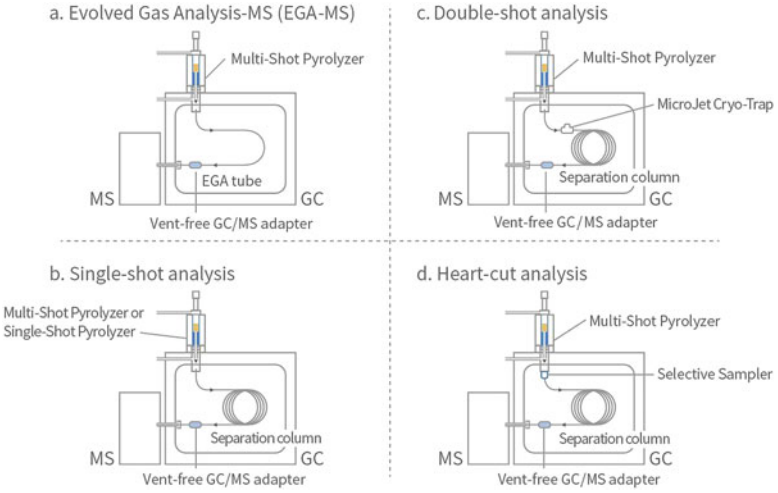


Fig. 2 System configuration for four analytical techniques (courtesy of Frontier Laboratories Japan Ltd.)

possible (in current instruments <20 ms). The macromolecules are fragmented in the pyrolyzer, and the volatile pyrolysis products, known as pyrolyzates, are separated in the chromatographic column.

Double-shot analysis (Fig. 2c): it includes two stages, the first one involves thermal desorption of the volatile compounds (of low molecular weight analyzed at low temperature, ~80–350°C) examined then by GC–MS. In the most recent instruments, these volatile compounds are cryo-trapped temporarily at the head of a separation column by liquid nitrogen. In the second step, the residual sample left after desorption (in which the non-volatile macromolecules remain) is pyrolyzed at high temperatures (~500–800°C), and the pyrolyzate is again analyzed by GC–MS.

Heart-cut analysis (HC/EGA-GC-MS) (Fig. 2d): it is the two-dimensional way of working in Py–GC. The EGA is used to produce a thermogram, and each temperature zone of interest is analyzed independently by heart cutting evaporating components that are selectively introduced to the GC column (by means of a selective sampler), where they are temporarily trapped prior to the analysis by GC–MS (e.g., cryo-trapped as described above). This method allows searching both the specific components in a highly complex matrix and the whole composition of a complex system. While extremely useful, this technique can add complexity and required-time for the analysis.

Reactive Pyrolysis–GC–MS: with this technique the sample undergoes a chemical derivatization reaction in the pyrolysis chamber avoiding extensive sample preparation (the most used derivatizing agent is tetramethylammonium hydroxide). This derivatization may be instead of, or in addition to, heat-induced pyrolysis of the macromolecule into smaller fragments.

In addition to these analytical techniques, modern pyrolyzers can also be used only for thermal desorption of analytes (TD/Py–GC–MS) as in the first step of double-shot analyses. The system configurations for the four analytical techniques can also include a vent-free GC–MS adapter enabling column exchange without venting the MS.

2.2 Recent Advances in Py–GC–MS Instrumentation

Py–GC–MS can deliver quantitative results with high accuracy and precision. As in any other analytical method, quantification of determined compounds requires standards, isotopically labeled internal standards, and the selection of the proper ions [12, 26]. The main disadvantage attributed to Py–GC–MS is its poor reproducibility because of factors such as sample heterogeneity, slow transfer of the pyrolyzate to the chromatographic column, and catalytic events in the pyrolyzer that may alter the chemical nature of the sample. Other drawbacks of the technique are its destructive character, and the difficulty of interpreting the pyrograms obtained due to the large number of reactions that might take place and the density of peaks in the pyrograms [36]. Consequently, recent advances have been focused on developing new approaches to overcome these problems by improving the reproducibility of the

pyrolyzer, enhancing the separation of the compounds, and improving the identification of the reactions products.

2.2.1 Pyrolyzer

Pyrolysis systems have been classified according to their heating mechanisms as: pulse-mode pyrolyzers (filament, Curie-point or induction, and laser) and continuous-mode pyrolyzers (furnace or microfurnace pyrolyzer) (Fig. 3). Current environmental and food applications of Py–GC–MS are mostly focused on microfurnace pyrolyzers because this type of pyrolyzer can work with higher amounts of samples, which is important for the analysis of minority compounds. Improvements in this field have been related to the control of the pyrolysis temperature (rapid rise, reproducibility) and the connection of the pyrolyzer with the GC, which must be as direct as possible (to prevent pyrolyzates back into the injector where they can undergo secondary pyrolysis).

The filament type pyrolyzer heats a sample tube using a filament (Fig. 3a). This technique has some drawbacks in temperature accuracy and reproducibility since some types of samples may be not uniformly heated. Moreover, high boiling compounds may be absorbed in the transfer line carrying the pyrolyzates to GC, which make difficult to introduce them to a GC column. Some progress has been done in developing better and more effective sample holders able to short the analyte diffusion path and to reduce peak broadening. Considerable effort has also been made in the implementation of a technology ensuring accurate monitoring of the filament resistance to guarantee truthful temperature control (heat-transfer variations can affect the temperature of the sample). Modern instruments can perform programmed heating in addition to pulsed pyrolysis, to obtain sequential pyrolysis of a sample combining different temperature ramps, and velocities of heating. Other improvements search to avoid evaporation of the volatile and semi-volatile compounds and denaturation, degradation, or thermofixation of the samples because of sample-preheating (essential in this technique). Consequently, thermal desorption in last instruments is carried out by modular systems or complicated trapping sequences [37, 38].

The Curie-point pyrolyzer (Fig. 3b) is similar to the filament pyrolyzer (preheating of the sample is also necessary) but different temperature steps cannot be programmed as required for EGA-MS analysis, since the temperature ceases to rise when the Curie-point of the metal holding the sample is reached. Then, the EGA analysis is impossible.

The vertical furnace/microfurnace pyrolyzer is a hollow quartz tube covered by a heater device for rapid heating and cooling (Fig. 3c). Modern microfurnaces are equipped with hollow ceramic heaters and powerful cooling fans, able to achieve rapid heating and cooling, together with temperature sensors for the sample. Changes in the sample cup materials for inertness have been made recently such as the use of quartz or chemically bonded quartz thin layers. Microfurnaces avoid the problems caused by the preheating of the filament and Curie-point pyrolyzers by

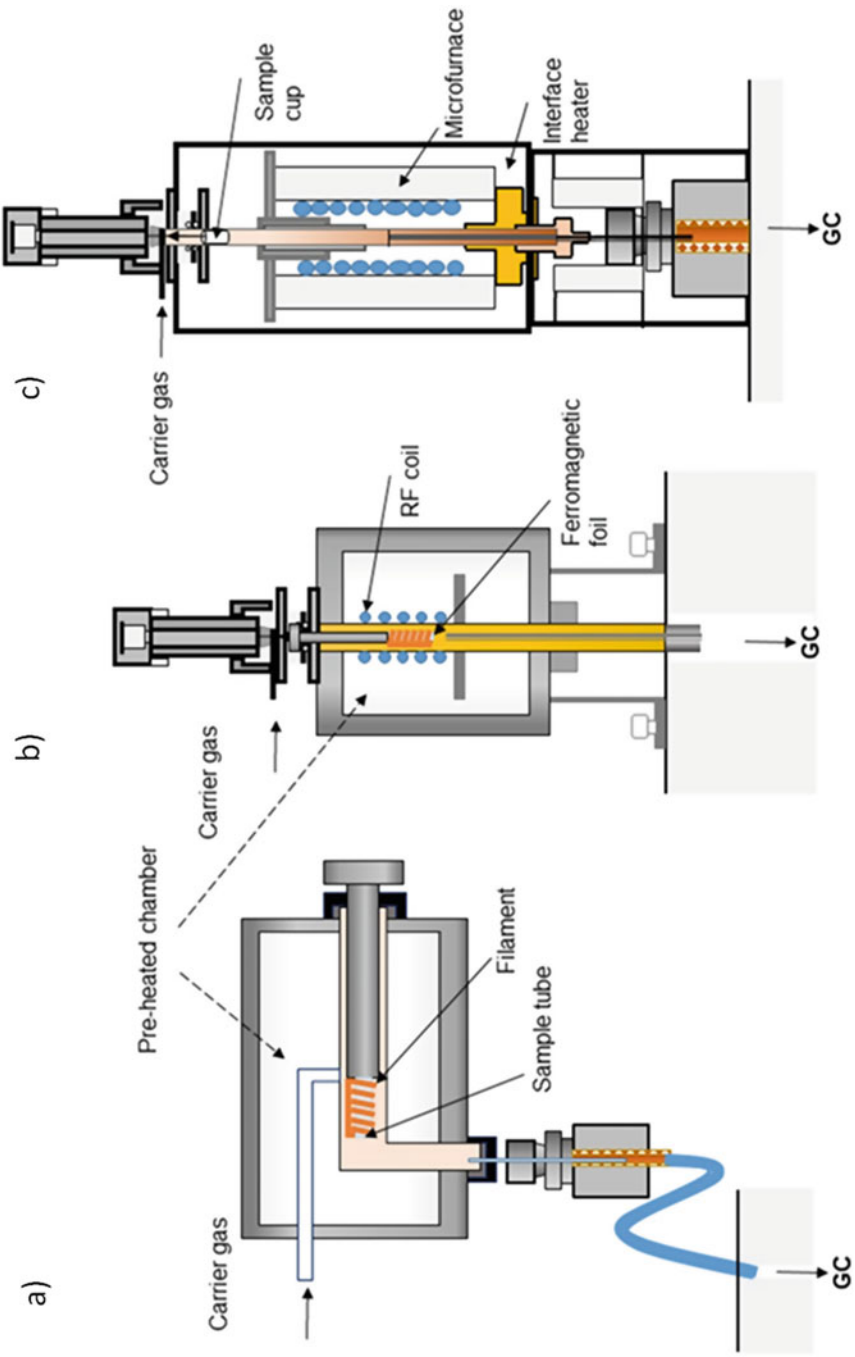


Fig. 3 Pyrolysis systems (pyrolyzers) classified according to their heating mechanisms: (**a**) filament, (**b**) Curie-point, (**c**) microfurnace [2]

placing the sample in an inert deactivated cup, held at ambient temperature (in helium) at the same time that the furnace is preheated to the pyrolysis temperature, and then letting drop the cup into the microfurnace. The sample is purged of oxygen before heated avoiding oxidation reactions [5, 39, 40].

The microfurnace pyrolyzer has several advantages over other types of techniques: a) accurate furnace set temperature in a wide-ranging temperature or continuous ramp-up heating, b) direct interface to the GC injector, which allows the complete introduction of all molecular weight compounds (low to high), and c) the sample just needs to be simply placed in a sample cup and it is ready for analysis. Worth mentioning is the Multi-Shot Pyrolyzer EGA/PY-3030D with a low mass ceramic heater allowing pyrolysis temperatures up to 1,050°C, rapid heating (600°C/min) and cooling (100°C/min), very precise temperature control in the microfurnace (40–1,050°C ± 0.1°C), simple handling of samples through weighing in sample cup (approx. 50–200 μg), and no condensation of gases at cold spots (no dead volumes) during transition to GC due to an interface-heating.

In relation to the connection of the pyrolyzer to the GC, the main concern is to transfer the analytes in a tight "plug." This means heating the sample as fast as possible, in order to have the resulting volatiles in a very short time with narrow and well separated peaks. Cryo-trapping capabilities using liquid nitrogen are available for Py–GC–MS in both pulse- and continuous-mode pyrolyzers [41, 42].

2.2.2 Gas Chromatography–Mass Spectrometry

Although most Py–GC–MS applications have been made with conventional GC separation, there is no limitation in terms of their possible modes. There are two major trends in the use of this technique: (ultra-)fast chromatography and comprehensive two-dimensional GC (GCxGC) [4, 12, 14, 16, 19]. Fast GC seeks to reduce the analysis time by using different combinations of narrower and shorter columns, with lower phase thickness and/or higher carrier gas flow, as well as by high temperature heating ramp rates (up to 250°C/min) and oven cool downs (450 to 50°C in <3 min). However and probably due to the complexity of pyrolyzates, there are few publications reporting this combination [4, 12]. On the other hand, GCxGC provides better resolution power and higher peak capacity by adding a second GC column, with different polarity and connected through a modulator (usually a cryo-modulator), which traps the eluting fractions coming from the first column and injects them into the second one achieving true two-dimensional separations [14, 16, 19]. In the future, this technique could add a third dimension of separation if it is used together with heart-cut EGA [2]. Nevertheless, it is important to highlight that the improvement possibly gained by the combination of these techniques will require enhanced data processing tools due to the great amount of information generated and the time needed to interpret it.

The detection technique of pyrolyzates is typically mass spectrometry (MS) often accomplished by a single quadrupole mass analyzer due to its robustness and capacity to detect the fragmentation products. The molecules detected by MS are

identified through their mass spectra using commercial libraries (e.g., NIST/EPA/NIH, Wiley, MPW, Norman Mass Bank, m/z Cloud) or by means of reference substances [4]. Despite triple quadrupole instruments (QqQ) are nowadays the most recommended systems for the analysis of target compounds (because of their higher sensitivity and specificity), it is not commonly used in combination with Py–GC since the compounds formed in the pyrolysis are mostly unknown and the application of the QqQ is less evident [7]. In contrast, high-resolution mass spectrometry (HRMS) and mainly (quadrupole) time-of-flight (TOF or QqTOF) has become essential in Py–GC–MS analyses since these detectors provide information on the most probable empirical formula of both the analyzed molecule and its characteristic fragments. Although HRMS application focuses on using selected ion monitoring, the analysis of the full spectra in the development of the method is mandatory [12, 26].

3 Applications of Py–GC–MS in Food and Environmental Analysis

Py–GC–MS can be used in a wide array of applications from characterization of materials (polymers, lacquer films, etc.) to quality control (of food and daily commodities) including also forensic analyses in environmental sciences among others. In recent years, the most interesting applications of Py–GC–MS in environmental samples are (1) the chemical fingerprinting of organic matter (OM) [8, 20–26] and (2) the identification, characterization, and fate of MPs and NPs [28–32, 34, 35, 43, 44]. Furthermore, Py–GC–MS main application in food are related to (1) the determination of geographical origin, (2) detection of fraudulently added substances, and (3) quality control.

3.1 Characterization of Food

Because of its versatility, Py–GC–MS offers ample possibilities to be exploited in multiple applications related to food industry, from food authentication to the analysis of composition, quality, and additives, including contaminants as well. One of the first studies using Py–GC–MS in food analysis was published in 1999 [10]. It presented the results obtained by the European Commissions' Joint Research Centre for the detection of added whey proteins in milk, the ripening stages of cheese, the characterization of vinegar and wine, as well as the assessment of the geographical origin of cocoa butters.

Nowadays, the isotope ratio mass spectroscopy (IRMS) for determination of the stable isotope composition of light elements is an interesting approach to discriminate production areas and to validate the origin of vegetables and fruits. The

analysis of $\delta^{13}C$ in discrete oil components using a compound-specific isotope analysis (CSIA) is an accurate method compared to the isotope assessment in bulk samples. Direct analytical Py-CSIA was used for the authentication of extra virgin olive oils avoiding chemical and/or physical pre-treatments [11]. The δ2H value in nine pyrolysis compounds detected in all extra virgin olive oils ranged from −112 to −267 mUr. These compounds were chosen as likely surrogate descriptors of the olive oil geographic origin as they were significantly correlated with longitude and annual temperature by means of Principal Component Analysis and Multiple Linear Regression. Similarly, and because of its capacity to analyze macromolecules, Curie-point-Py–GC–MS has been used to characterize the solvent extracts (ethyl-acetate, n-butanol, raw theabrownin) from a type of Pu-erh tea and Dian Hong black tea. Results showed substantial different chemical compositions among solvent extracts from both teas, and proved the great characterization capacity of the technique [45].

Py–GC–MS has demonstrated to be a useful tool able to provide precise fingerprinting but also detailed information about quality, composition, and additives in active packages of biogenic polymers employed in food industry. One of these materials is the oregano essential oil, which is used due to its bioactive properties. However, because of oregano essential oils high volatility, characteristics like aging and preparation conditions of films can produce losses of active agent. Py–GC–MS was used to characterize a bioactive polylactic acid with polybutylene succinate (95:5) film extruded with variable amounts of oregano essential oil [13]. Bioplastic pyrolysis enables identifying lactide enantiomers and monomer units from the polylactic acid and unambiguous molecular markers from the polybutylene succinate fraction. Oregano essential oil pyrolysis revealed aromatics and terpenes with terpinene, cymene, and thymol/carvacrol peaks that were identified as diagnostic peaks showing significant linear correlation coefficients with the amount of oregano essential oil included in the bioplastic matrix.

Other applications of Py–GC–MS in food industry are focused on the determination of contaminants. Using this technique, the thermal stability of five pharmaceuticals commonly detected in food (chloramphenicol, florfenicol, lincomycin hydrochloride, diphenhydramine hydrochloride, and carbamazepine) was analyzed [46]. Results showed a number of volatile thermal degradation products (some of them previously unidentified) with a technique simpler than others reported earlier. The test was conducted at 250°C (i.e., above actual cooking temperatures of 180–220°C) for 20 s only, a very short period of time (compared to cooking times). However, it seems to reveal information about the degradation products generated at lower temperatures or longer cooling times. Authors concluded that in future studies, lower pyrolysis temperatures should be explored to simulate the general cooking conditions and provide some insight in the fate of drug residues in food during processing. In this regard, the effect of pyrolysis temperature from 150 to 550°C with 100°C-increments was studied in raw or cooked fish muscle samples (*Oreochromis niloticus*) to detect cylindrospermopsin and its degradation products by both (1) applying each temperature to a different sample (single-shot pyrolysis) or (2) sequentially applying each temperature to the same sample (multi-shot pyrolysis) (Fig. 4) [47]. Relative abundance of degradation products was found

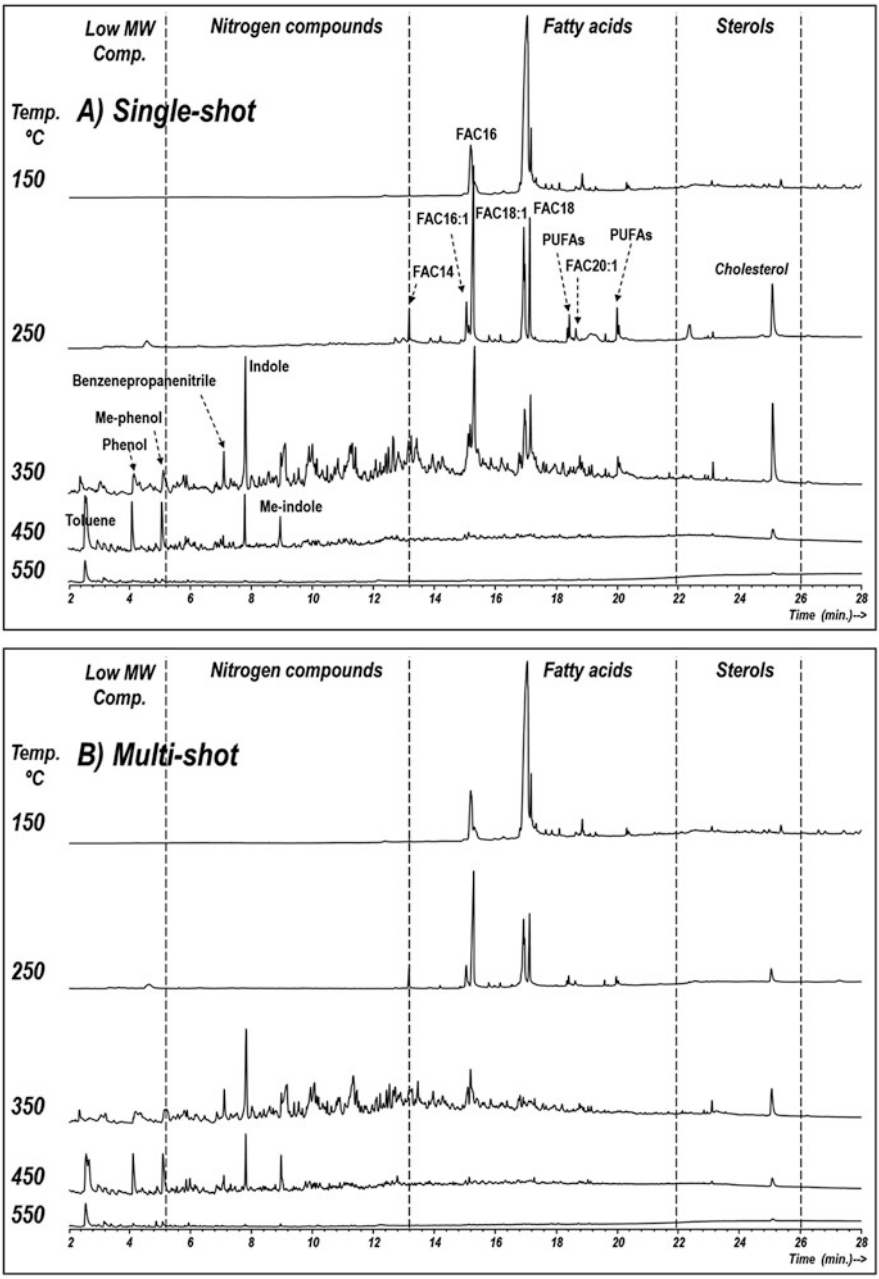


Fig. 4 Analytical pyrolysis of tilapia muscle. Total current ion chromatograms (TIC) performed at (**a**) single-shot mode, i.e. different sample at different temperatures and (**b**) multi-shot mode, i.e. same sample at increasing temperatures. Chromatograms are divided in sections of preferential compound classes and major compounds detected are labeled on the corresponding peaks. *FA:* fatty acid, *PUFA:* polyunsaturated fatty acid, *Me:* methyl [47]

to be different depending on the cooking process (microwaving, broiling, boiling, and steaming) being boiling the only cooking technique that showed to decrease the relative percentage of cylindrospermopsin compared to the control group.

Thermal desorption using Py–GC–MS was used together with a novel sorbent developed for sorptive enrichment of dilute analytes from liquid samples [48]. The extraction element is made of titanium open tubular tube (30 mm × 1.2 mm i.d. × 1.6 mm o.d.) coated with a chemically bonded layer of polydimethylsiloxane (PDMS 500 μm in thickness). This sorbent was used to develop a method to extract and analyze bethrodine (a herbicide) in water samples over a concentration range from 2.5 to 2.5 × 10^4 ng/L. A detection limit of 0.5 ng/L was achieved with fairly good reproducibility of the measurements (relative standard deviation, R.S.D., below 7.5% at 10 ng/L). This procedure was also applied for the simultaneous determination of five preservatives – benzoic acid, sorbic acid, and methyl, ethyl, and propyl esters of *p*-hydroxybenzoic acid – in soft drinks, yogurts, and sauces [49]. The method attained limits of detection between 0.002 and 0.2 mg/L, and it was applied to real samples spiked at levels of between 20 and 100 mg/L showing good recoveries (92–106%) and precisions (R.S.D.s of 0.9–4.6% ($n = 5$)).

3.2 Determination of MPs and NPs

Environmental contamination by MPs and NPs is a global problem with a worldwide-recognized dimension as proven by the large number of studies published in recent years [28–35, 43, 50–55]. Py–GC–MS has become one of the most promising techniques to assess MPs and NPs in environmental samples since it is able to detect low MP dimensions, and even NPs (the most difficult ones because of their size). It is more sensitive and less affected by sample impurities than other methods. Py–GC–MS has been used to identify MPs in soil [56, 57], soil amended with solid waste compost [58], plants [27], bio solids [59], river sediments [40, 60], beach sediments [61], coastal sediments [30, 62], tidal flat sediment [63], suspended particulate matter [40], wastewater [64], sea water [65, 66], surface water [28, 52, 67, 68], salt [63, 69], bivalves [66, 70–72], fish [30, 43, 47, 51, 73], and other types of biota [52, 73]. Py–GC–MS has also been applied to determine NPs in water [74–76].

MPs can be directly assessed from the samples, such as soil [58], but they are usually separated and concentrated from the matrix by wet digestion (using oxidants, acids, or enzymes [62, 77]), or by density differences (with solutions of NaCl [61], NaI, $ZnCl_2$, NaBr [69], or sodium polytungstate [60]), with a final filtration. In samples with high OM content, as wastewater, sequential filtration can help to isolate the MPs without clogging the pore filters [64]. Very recently, pressurized liquid extraction has been applied to solubilize MPs in solvents such as dichloromethane or tetrahydrofuran [44, 59]. NPs can also be extracted from aqueous samples by filtration working in the range of nanoscale (24 nm–52 nm) [75]. Ultrafiltration has also been used to separate MPs and NPs from water attaining lower sizes (5–50 nm) [74] Other techniques as Triton X-45-based cloud-point

extraction has been tested to pre-concentrate NPs providing an enrichment factor of 500 without disturbing their actual morphology and sizes [76].

In the determination of MPs and NPs by Py–GC–MS, the pyrolyzer most frequently used is the microfurnace [59–63, 69, 76], because of its higher sample capacity, though filament [57, 58, 75] and Curie-point [57, 58, 75] pyrolyzers have also been applied. A comparison of micro furnace and Curie-point pyrolyzers to assess MPs in the environment proved that the former can process more sample quantity due to its larger sample cups allowing the transfer of pre-concentrated MPs directly from the glass fiber filters [63]. The double-shot pyrolysis is the preferred mode since the first-shot can act as a clean-up step avoiding interferences as those of OM [40, 57, 59, 62, 76], despite none of the OM compounds can interfere with the indicators selected for polypropylene (PP), polyethylene terephthalate (PET), polycarbonate (PC), poly-(methyl methacrylate) (PMMA), and polystyrene (PS).

The GC–MS is commonly used with a quadrupole mass analyzer although TOF analyzer has also been reported in studies of MPs and NPs in water [75] and wastewater samples [34]. In the former study, TOF was operated in nominal mass units not in accurate mass, similar to a quadrupole but offering an increased sensitivity because all the ions accumulated are transmitted to the TOF detector. In most cases, selected ion monitoring (SIM) mode is the only working mode that attains sufficient sensitivity to detect MPs at low concentrations. It is important to keep in mind that other organic molecules (e.g., organic matter) can be present in the extract/sample leading to a number of non-volatile pyrolysis products, which enhance the risk of interferences with specific indicators of compounds, boost the maintenance frequency, and hamper a reliable quantification. As it has been explained, compounds in the pyrograms can be identified by means of mass spectral databases, custom databases containing pre-acquired pyrograms of plastic references, or by comparison with the published scientific literature [61, 68]. In general, to identify and quantify MPs and NPs, specific indicator compounds are selected for each type of plastic as presented extensively by Picó and Barceló [2]. These indicator compounds were chosen after assessing their specificity against a number of natural materials as chitin, wood, pine needles, cellulose, humic acid, etc. [59]. However, this method of data processing is very time consuming. In this sense, the research moves toward the design of software for automatic qualitative analysis of this information as an automated algorithm developed and implemented in the F-Search software [18]. The process is based on the generation of summated mass spectra (SMS) for each polymer, obtained by extracting specific *m/z* and retention index coordinates of the characteristic pyrolysis products. The identification of a polymer is performed by comparing its SMS with those of a built-in library. After validation, the algorithm was tested on a reference sample containing 11 types of synthetic polymers providing relative standard deviations around 10%. The lowest estimated amount of polymer detectable in a sample, was lower than 1 μg for most polymers.

Reactive pyrolysis after TMAH derivatization has been able to reduce organic interferences and because this technique improves the detection sensitivity for PET and PC [60, 69, 77]. When pyrolysis is used in combination with TMAH, the

pyrolytic behavior of polyethylene (PE), PP, PS, and polyvinylchloride (PVC) remained unaffected while that of PET, PMMA, PC, and polyamide (PA6) changed. As an example of the complexity of the technique and the problems originated by interferences, determination of PS requires the selection of a characteristics PS trimer (*m/z* 312), even after derivatization, because chitin (widely present in the natural environment) releases styrene (*m/z* 104) during pyrolysis (Fig. 5) [76].

The first international attempt (17 laboratories from eight different countries) to compare the suitability of the methods commonly used in MPs analysis showed the performance of Py–GC–MS within the field as well as the main obstacles faced by researchers [78]. Different techniques as microscopy, Fourier-transform infrared microspectroscopy (μ-FTIR), Raman microspectroscopy (μ-Raman), thermal extraction and desorption or Py–GC–MS, scanning electron microscopy, and particle counter were compared in relation to the total particle number, polymer type, number of particles, and/or the particle mass of each polymer type. For the identification of polymer type, μ-Raman and Py–GC–MS performed best. The quantification of polymer mass for identified polymer types was questionable for Py–GC–MS, whereas other methods failed to determine the correct polymer mass. Quantification of particle number per identified polymer type was evaluated successful for μ-FTIR and the quantification of total particle numbers was best for microscopy and to a lesser extent for μ-FTIR [78].

The performance of Py–GC–MS has also been compared to μ-FTIR and μ-Raman, as most successful complementary approaches in the identification of MPs [60]. Py–GC–MS identified copolymers as PE-PP or PE-PP-PA6 that could be difficult to distinguish with μ-Raman without chemometrics approach leading to results with a finer identification [70]. Chemical information provided by Raman and FTIR spectra is limited compared to the one of Py–GC–MS. This is because the selectivity of vibrational spectroscopy is not enough to identify specific monomers and co-monomers, mixtures, additives, and degradation products [79]. Py–GC–MS is able to characterize MPs at a molecular level identifying synthetic polymers as well as the possible presence of additives in contrast to the second common limitation of Raman spectroscopy and ATR-FTIR that provided spectra relative to a surface portion of the MP fragments [60, 70]. The advantage of Py–GC–MS over FTIR spectroscopy is that both polymer types and organic plastic additives can be studied in a single run [80]. Due to Py–GC–MS important advantages, new statistical approaches to tackle polymer variability and matrix interference are being developed [79]. This augurs that pyrolysis will exponentially increase its applicability for analyzing MPs and NPs in the environment.

3.3 Characterization of Organic Matter

Py–GC–MS has also demonstrated to be a powerful tool for analyzing the molecular fingerprint of organic matter (OM) in soils and sediments [5, 23, 24, 39, 40, 81–83], as well as to analyze changes in the dissolved organic matter (DOM) fraction [84–

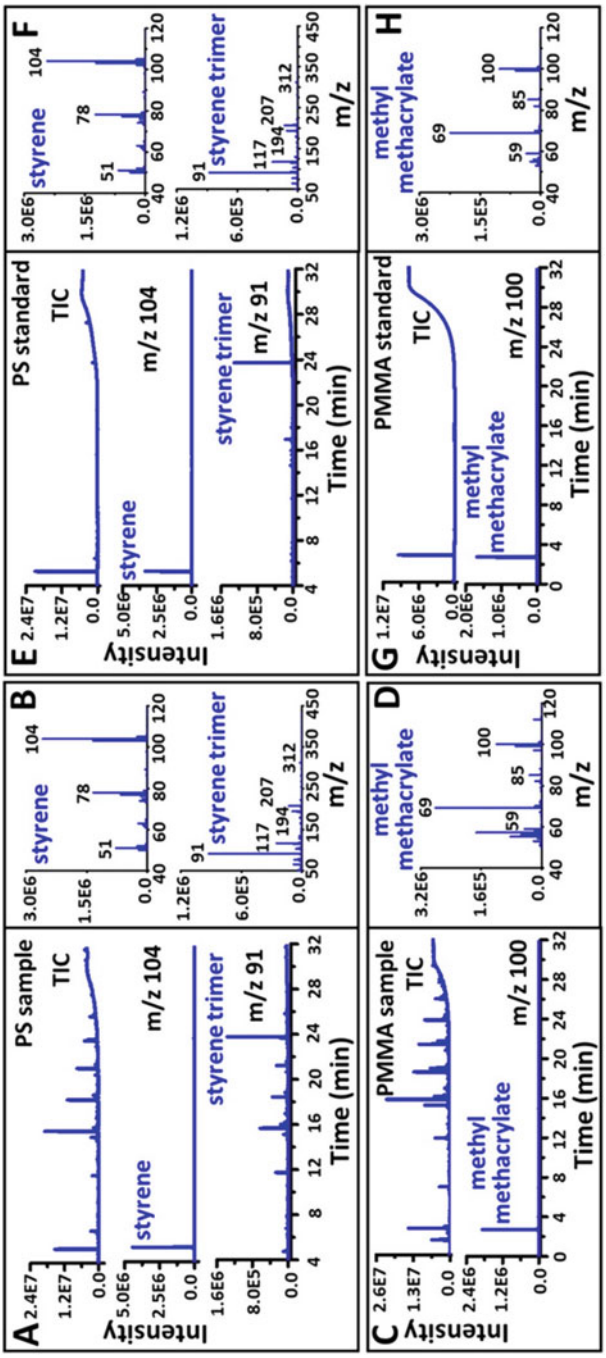


Fig. 5 Py–GC–MS chromatograms of PS- and PMMA-nanoplastic samples and standards: (**a**) Total ion chromatogram (TIC) and selected-indicator-ion chromatograms (SIC) of the PS-nanoplastic sample. (**b**) Mass spectrum of the PS-nanoplastic-sample pyrolytic product. (**c**) TIC and SIC of PMMA-nanoplastic sample. (**d**) Mass spectrum of the PMMA-nanoplastic-sample pyrolytic product. (**e**) TIC and SIC of the PS-nanoplastic standard. (**f**) Mass spectrum of the PS-nanoplastic-standard pyrolytic product. (**g**) TIC and SIC of the PMMA-nanoplastic standard. (**h**) Mass spectrum of the PMMA-standard pyrolytic product [76]

86]. While in water the sample should be filtrated through a glass filter to later isolate and enrich the DOM, by reverse phase mechanisms, as SPE [84] and pre-HPLC [85], in soil and sediment the sample just need to be dried, sieved, and pulverized [5, 39, 40]. DOM can also be extracted from soils using a lysimeter-pump or by shaking soil with water [87].

The study of OM includes the characterization of a diversity of compounds including polysaccharides, amino sugars, proteins, polyhydroxy aromatics, lipids, lignin, etc. Other compounds as humic acids and fulvic acids need prior extraction and clean-up following standard methods (these include elimination of the free OM by flotation with H_3PO_4, demineralization with $Na_4P_2O_7$ to release the humic substances complexed with oxides, then, extraction with basic solutions as NaOH, and separation by precipitation in an acidified solution, pH < 2). Humic acids are later purified by washing with several acidic solutions [83] and/or dialyzed into cellophane bags [20, 88]. The three types of pyrolyzers described previously have been applied to characterize OM: micro furnace [39, 40, 83, 89, 90], platinum heated filament pyrolyzer [5, 20, 86, 87, 91], and Curie-point [85, 88]. Most of these studies have only used the single-shot at temperatures between 500°C and 800°C [5, 20, 39, 40, 83, 85, 87–91]. The double-shot pyrolysis that makes accessible both free volatiles and high molecular weight structures has been hardly used to characterize OM (probably because OM is formed by non-volatile compounds). The reactive pyrolysis has also been tested in few studies [87, 92] since it simplifies the pyrogram providing less fragments. However, a comparison of the ability of both techniques to characterize DOM in soil from the Three Gorges Reservoir area stated that Py–GC–MS fingerprinting is more helpful to quantify microbial DOM than reactive pyrolysis [86], which is useful to assess the molecular features of polyphenolic (cinnamic acids, lignin, and tannin) and aliphatic (cutin and suberin) fractions.

OM characterization by GC–MS is generally performed using a 30 m capillary column and a simple quadrupole in scan mode. Compounds detected in a pyrogram can range between 100 and 400 and are identified by means of a mass spectral database or library as previously described. Their relative abundance can be calculated by normalizing the peak areas of each individual compound to the total area of all the peaks detected. In general, to avoid the high number of pyrolyzates in a sample, the best is to focus the study on a few good biomarkers giving information about the process of interest. For instance, reactive Py–GC–MS has been used to identify branched-chain fatty acids in humic acids to describe microbial activities occurring during composting processes [92]. Methoxyphenols (12 major guaiacyl- and syringyl-type compounds) have also been detected by Py–GC–MS in topsoil samples since they are distinctive molecules advising on the occurrence and degree of alteration of lignin, and have also been proposed as indicators of the soil's capacity to storage C [82, 89].

Studies about changes in OM after different disturbances as forest fires [21–23, 81] or land use changes [5, 82] have also been performed. The thermal transformation of soil OM caused the thermal breakdown and cracking of n-alkane compounds, as was revealed by the increase of the ratio of short-to-long chains and the alteration of the typical odd-over-even carbon predominance indexes [21, 22]. Analyses

carried out by using a vertical microfurnace type double-shot pyrolyzer indicated that the fire resulted in an enrichment of aromatic compounds, nitrogen (N) constituents, lignin-derived compounds, and polysaccharides [23, 81]. The effect of land use changes in OM compounds has been studied using different approaches including Py–GC–MS. Zhang et al. [5] used a heated platinum filament pyrolyzer directly connected to a GC–ion trap MS to analyze the effects of freshwater wetlands conversion to sugarcane (*Saccharum officinarum*) on the soil OM cycling and processes. These authors concluded that the land use change not only reduced total OM contents but also significantly increased the aliphatic fractions while lignin fractions were almost completely exhausted. In another study, Campo et al. [82] by means of Curie-point pyrolysis with tetramethylammonium hydroxide assessed the effects of afforestation on the soil lignin content (based on the vanillyl, syringyl, and cinnamyl contents). According to them, afforestation with *Pinus nigra* was the best practice for increasing the soil organic carbon stock and the lignin content in soil.

Py–GC–MS using two ionization systems, electron-ionization (EI) and photon-ionization (PI), with different mass selective analyzers (quadrupole- and a time-of-fight, TOF-MS) has been performed for the analysis of DOM in water [84]. In this research, resonance-enhanced-multi-photon-ionization (REMPI) provided information on the molecular weight and a high sensitivity and selectivity for aromatic hydrocarbons. Consequently, the characterization of natural samples by EI-quadrupole/MS and their aromatic fingerprint (REMPI-TOF-MS) were accomplished (Fig. 6). In general, there are few studies incorporating the recent developments of GC–MS, possibly because these analyses are complex and time consuming besides needing highly specialized technicians to deal with the high amount of data generated. In order to overcome this difficulty, statistical tests have been applied to improve and schematize the visualization/interpretation of the information obtained. As pyrolyzates can fluctuate among samples in their presence and intensity, a lot of effort is devoted nowadays to develop automated identification and quantification software that can help to process all the information obtained from the pyrographs and to visualize the results [20, 93].

4 Conclusions

Py–GC–MS is recognized as a valuable technique in food and environmental chemistry as proved by the increasing number of studies using it. Because of its versatility, Py–GC–MS offers ample possibilities to be exploited in multiple applications related to food industry, from food authentication to the analysis of composition, quality, and additives, including contaminants as well. It should be kept in mind that pyrolysis is a time-consuming and complex technique, then, it will be used only in those cases where the other chromatographic techniques failed, such as in the characterization of macromolecules. In recent years, it has been applied to the chemical characterization of organic matter in water, soil, and sediment but also,

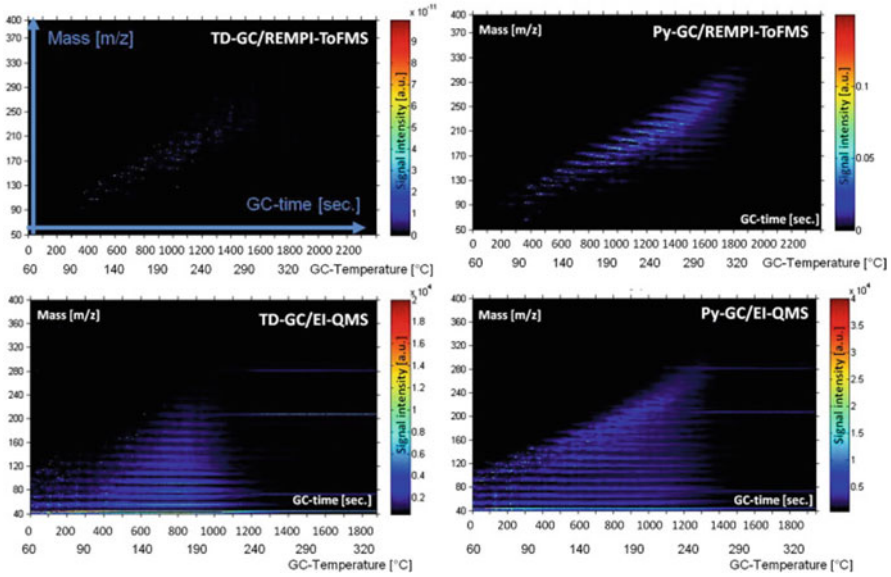


Fig. 6 Direct comparison of the chromatograms resulting from the REMPI-TOFMS (above) and EI-QMS (below) detection for At4 station (Bothnian Bay). The TD step is shown on the left and the pyrolysis step on the right. On the ordinate the GC-time and -temperature are plotted. On the abscissa the mass-to-charge ratio is depicted. Graduated in color are the intensities [84]

to assess the modifications that disturbances as forest fires or land use changes produced. Evaluation of the contamination by MPs and NPs of environmental samples has also become a hot topic addressed by the application of this technique. Py–GC–MS has demonstrated important advantages over others, such as μRaman and μFTIR to evaluate MPs, since it has been the only one able to detect NPs. The use of very small quantities of sample and the lack, in most cases, of pre-treatment have also added value to this technique. However, the large amount of results obtained and the difficulty in interpreting them are constraints that also need to be considered. On the other hand, most of the studies are focused on the robustness of the pyrolyzer and therefore, latest innovations in GC–MS have not been yet introduced in most applications. In this sense, it is expected that Py–GC–MS takes advantage of the great separation power of GCxGC, the speed of rapid GC, and the identification capability that HRMS and HRMS in tandem can provide. Furthermore, environmental analyses can also be enhanced by the application of EGA or shot-cut-EGA that would add a further dimension to multidimensional techniques. Py–GC–MS is a robust, multipurpose, adaptable, and useful technique with a promising future to overcome new challenges as those depicted in this chapter.

Acknowledgements This work has been supported by Grant RTI2018-097158-B-C31 funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe," and project ANTROPOCEN@ (PROMETEO/2018/155) funded by Generalitat Valenciana.

References

1. Obst V, Steinhaus J, Knupp G, Schroeder-Obst D, Fink W, Kusch P (2014) Application of pyrolysis–gas chromatography–mass spectrometry for the identification of polymeric materials. LCGC N Am 32:210–217
2. Picó Y, Barceló D (2020) Pyrolysis gas chromatography-mass spectrometry in environmental analysis: focus on organic matter and microplastics. Trends Anal Chem 130:115964. https://doi.org/10.1016/j.trac.2020.115964
3. McNaught AD, Wilkinson A (eds) (1997) Compendium of chemical terminology.2nd edn. Blackwell Scientific Publications, Oxford
4. Schena T, Farrapeira R, Erle TRB, Krause LC, von Muhlen C, Caramao EB (2019) Fast two-dimensional gas chromatography applied in the characterization of bio-oil from the pyrolysis of coconut fibers. Separation Sci Plus 2(3):89–99. https://doi.org/10.1002/sscp.201800129
5. Zhang Z, Wang JJ, Lyu X, Jiang M, Bhadha J, Wright A (2019) Impacts of land use change on soil organic matter chemistry in the Everglades, Florida – a characterization with pyrolysis-gas chromatography–mass spectrometry. Geoderma 338:393–400. https://doi.org/10.1016/j.geoderma.2018.12.041
6. Basu P (2018) Chapter 5 – pyrolysis. In: Basu P (ed) Biomass gasification, pyrolysis and torrefaction3rd edn. Academic Press, pp 155–187
7. Gómez X, Meredith W, Fernández C, Sánchez-García M, Díez-Antolínez R, Garzón-Santos J et al (2018) Evaluating the effect of biochar addition on the anaerobic digestion of swine manure: application of Py-GC/MS. Environ Sci Pollut Res 25(25):25600–25611. https://doi.org/10.1007/s11356-018-2644-4
8. Laskar DD, Ke J, Zeng J, Gao X, Chen S (2013) Py-GC/MS as a powerful and rapid tool for determining lignin compositional and structural changes in biological processes. Curr Anal Chem 9(3):335–351. https://doi.org/10.2174/1573411011309030003
9. Ma XM, Lu R, Miyakoshi T (2014) Application of pyrolysis gas chromatography/mass spectrometry in lacquer research: a review. Polymers 6(1):132–144. https://doi.org/10.3390/polym6010132
10. Guillou C, Lipp M, Radovic B, Reniero F, Schmidt M, Anklam E (1999) Use of pyrolysis–mass spectrometry in food analysis: applications in the food analysis laboratory of the European Commissions' Joint Research Centre. J Anal Appl Pyrolysis 49(1):329–335. https://doi.org/10.1016/S0165-2370(98)00121-1
11. Jiménez-Morillo NT, Cabrita MJ, Dias CB, González-Vila FJ, González-Pérez JA (2020) Pyrolysis-compound-specific hydrogen isotope analysis (δ2H Py-CSIA) of Mediterranean olive oils. Food Control 110:107023. https://doi.org/10.1016/j.foodcont.2019.107023
12. Lima NK, Lopes AR, Guerrero Jr PG, Yamamoto CI, Hansel FA (2018) Determination of volatile organic compounds in eucalyptus fast pyrolysis bio-oil by full evaporation headspace gas chromatography. Talanta 176:47–51. https://doi.org/10.1016/j.talanta.2017.08.008
13. Llana-Ruiz-Cabello M, Pichardo S, Morillo NJ, Bermudez JM, Aucejo S, Gonzalez-Vila F et al (2015) Fingerprinting (Py–GC/MS) of a bio-film active food package with Origanum vulgare L. essential oil. Toxicol Lett 238(2 Suppl):S72. https://doi.org/10.1016/j.toxlet.2015.08.251
14. Onorevoli B, Machado ME, Polidoro AS, Corbelini VA, Caramao EB, Jacques RA (2017) Pyrolysis of residual tobacco seeds: characterization of nitrogen compounds in bio-oil using comprehensive two-dimensional gas chromatography with mass spectrometry detection. Energy Fuel 31(9):9402–9407. https://doi.org/10.1021/acs.energyfuels.7b00405
15. Fu Y, Xiao Q, Zong S, Wei S (2020) Characterization and quantitation study of ancient lacquer objects by NIR spectroscopy and THM-Py-GC/MS. J Cult Herit 46:95–101. https://doi.org/10.1016/j.culher.2020.06.015
16. Primaz CT, Schena T, Lazzari E, Caramao EB, Jacques RA (2018) Influence of the temperature in the yield and composition of the bio-oil from the pyrolysis of spent coffee grounds: characterization by comprehensive two dimensional gas chromatography. Fuel 232:572–580. https://doi.org/10.1016/j.fuel.2018.05.097

17. Duemichen E, Eisentraut P, Celina M, Braun U (2019) Automated thermal extraction-desorption gas chromatography mass spectrometry: a multifunctional tool for comprehensive characterization of polymers and their degradation products. J Chromatogr A 1592:133–142. https://doi.org/10.1016/j.chroma.2019.01.033
18. Matsui K, Ishimura T, Mattonai M, Iwai I, Watanabe A, Teramae N et al (2020) Identification algorithm for polymer mixtures based on Py-GC/MS and its application for microplastic analysis in environmental samples. J Anal Appl Pyrolysis 149:104834. https://doi.org/10.1016/j.jaap.2020.104834
19. Mao F, Fan H, Wang J (2019) Biogenic oxygenates in lignite pyrolysis tars and their thermal cracking revealed by two-dimensional gas chromatography/time-of-flight mass spectrometry (GC x GC-TOFMS). J Anal Appl Pyrolysis 139:213–223. https://doi.org/10.1016/j.jaap.2019.02.008
20. Almendros G, Hernández Z, Sanz J, Rodríguez-Sánchez S, Jiménez-González MA, González-Pérez JA (2018) Graphical statistical approach to soil organic matter resilience using analytical pyrolysis data. J Chromatogr A 1533:164–173. https://doi.org/10.1016/j.chroma.2017.12.015
21. Campo J, Nierop KGJ, Cammeraat E, Andreu V, Rubio JL (2011) Application of pyrolysis-gas chromatography/mass spectrometry to study changes in the organic matter of macro- and microaggregates of a Mediterranean soil upon heating. J Chromatogr A 1218(30):4817–4827. https://doi.org/10.1016/j.chroma.2011.03.038
22. Faria SR, De la Rosa JM, Knicker H, González-Pérez JA, Keizer JJ (2015) Molecular characterization of wildfire impacts on organic matter in eroded sediments and topsoil in Mediterranean eucalypt stands. Catena 135:29–37. https://doi.org/10.1016/j.catena.2015.07.007
23. Jiménez-Morillo NT, Almendros G, De la Rosa JM, Jordán A, Zavala LM, Granged AJP et al (2020) Effect of a wildfire and of post-fire restoration actions in the organic matter structure in soil fractions. Sci Total Environ 728:138715. https://doi.org/10.1016/j.scitotenv.2020.138715
24. Ma S, Chen Y, Lu X, Wang X (2018) Soil organic matter chemistry: based on pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS). Mini-Rev Org Chem 15(5):389–403. https://doi.org/10.2174/1570193X15666180108152845
25. Santoiemma G (2018) Recent methodologies for studying the soil organic matter. Appl Soil Ecol 123:546–550. https://doi.org/10.1016/j.apsoil.2017.09.011
26. Yamamoto S, Yoshioka H, Ishiwatari R (2007) Pyrolysis- and chemical degradation-GC/MS analyses of environmental kerogen and humic substances and their applications to geochemistry. Bunseki Kagaku 56(2):71–91. https://doi.org/10.2116/bunsekikagaku.56.71
27. Allouzi MMA, Tang DYY, Chew KW, Rinklebe J, Bolan N, Allouzi SMA et al (2021) Micro (nano) plastic pollution: the ecological influence on soil-plant system and human health. Sci Total Environ 788:147815. https://doi.org/10.1016/j.scitotenv.2021.147815
28. Barcelo D, Pico Y (2019) Microplastics in the global aquatic environment: analysis, effects, remediation and policy solutions. J Environ Chem Eng 7(5). https://doi.org/10.1016/j.jece.2019.103421
29. Fu W, Min J, Jiang W, Li Y, Zhang W (2020) Separation, characterization and identification of microplastics and nanoplastics in the environment. Sci Total Environ 721:137561. https://doi.org/10.1016/j.scitotenv.2020.137561
30. Jiang Y, Yang F, Hassan Kazmi SSU, Zhao Y, Chen M, Wang J (2022) A review of microplastic pollution in seawater, sediments and organisms of the Chinese coastal and marginal seas. Chemosphere 286:131677. https://doi.org/10.1016/j.chemosphere.2021.131677
31. Peñalver R, Arroyo-Manzanares N, López-García I, Hernández-Córdoba M (2020) An overview of microplastics characterization by thermal analysis. Chemosphere 242:125170. https://doi.org/10.1016/j.chemosphere.2019.125170
32. Pico Y, Alfarhan A, Barcelo D (2019) Nano- and microplastic analysis: focus on their occurrence in freshwater ecosystems and remediation technologies. Trac-Trends Anal Chem 113:409–425. https://doi.org/10.1016/j.trac.2018.08.022
33. Shi W, Cui T, Wu H, LeBlanc GA, Wang F, Lihui AN (2021) A proposed nomenclature for microplastic contaminants. Mar Pollut Bull 172:112960. https://doi.org/10.1016/j.marpolbul.2021.112960

34. Vilakati B, Sivasankar V, Nyoni H, Mamba BB, Omine K, Msagati TAM (2021) The Py – GC-TOF-MS analysis and characterization of microplastics (MPs) in a wastewater treatment plant in Gauteng Province. S Afr Ecotoxicol Environ Saf 222:112478. https://doi.org/10.1016/j.ecoenv.2021.112478
35. Yu Z-f, Song S, Xu X-l, Ma Q, Lu Y (2021) Sources, migration, accumulation and influence of microplastics in terrestrial plant communities. Environ Exp Bot 192:104635. https://doi.org/10.1016/j.envexpbot.2021.104635
36. Sobeih KL, Baron M, Gonzalez-Rodriguez J (2008) Recent trends and developments in pyrolysis-gas chromatography. J Chromatogr A 1186(1–2):51–66. https://doi.org/10.1016/j.chroma.2007.10.017
37. Fan X, Wei S, Zhu M, Song J, Peng P (2018) Molecular characterization of primary humic-like substances in fine smoke particles by thermochemolysis–gas chromatography–mass spectrometry. Atmos Environ 180:1–10. https://doi.org/10.1016/j.atmosenv.2018.02.033
38. Zhang Z, Xu G, Wang Q, Cui Z, Wang L (2019) Pyrolysis characteristics, kinetics, and evolved gas determination of chrome-tanned sludge by thermogravimetry–Fourier-transform infrared spectroscopy and pyrolysis gas chromatography-mass spectrometry. Waste Manag 93:130–137. https://doi.org/10.1016/j.wasman.2019.05.034
39. Girona-García A, Badía-Villas D, Jiménez-Morillo NT, González-Pérez JA (2019) Changes in soil organic matter composition after Scots pine afforestation in a native European beech forest revealed by analytical pyrolysis (Py-GC/MS). Sci Total Environ 691:1155–1161. https://doi.org/10.1016/j.scitotenv.2019.07.229
40. Mazzetto JML, Melo VF, Bonfleur EJ, Vidal-Torrado P, Dieckow J (2019) Potential of soil organic matter molecular chemistry determined by pyrolysis-gas chromatography/mass spectrometry for forensic investigations. Sci Justice 59(6):635–642. https://doi.org/10.1016/j.scijus.2019.07.003
41. Wu X, Ba Y, Wang X, Niu M, Fang K (2018) Evolved gas analysis and slow pyrolysis mechanism of bamboo by thermogravimetric analysis, Fourier transform infrared spectroscopy and gas chromatography-mass spectrometry. Bioresour Technol 266:407–412. https://doi.org/10.1016/j.biortech.2018.07.005
42. Zhong L, Ni R, Zhang L, He Z, Zhou H, Li L (2019) Determination of total arsenic in soil by gas chromatography after pyrolysis. Microchem J 146:568–574. https://doi.org/10.1016/j.microc.2019.01.057
43. Pico Y, Barcelo D (2019) Analysis and prevention of microplastics pollution in water: current perspectives and future directions. Acs Omega 4(4):6709–6719. https://doi.org/10.1021/acsomega.9b00222
44. Dierkes G, Lauschke T, Becher S, Schumacher H, Foeldi C, Ternes T (2019) Quantification of microplastics in environmental samples via pressurized liquid extraction and pyrolysis-gas chromatography. Anal Bioanal Chem 411(26):6959–6968. https://doi.org/10.1007/s00216-019-02066-9
45. Gong J-S, Tang C, Peng C-X (2012) Characterization of the chemical differences between solvent extracts from Pu-erh tea and Dian Hong black tea by CP–Py–GC/MS. J Anal Appl Pyrolysis 95:189–197. https://doi.org/10.1016/j.jaap.2012.02.006
46. Tian L, Bayen S, Yaylayan V (2017) Thermal degradation of five veterinary and human pharmaceuticals using pyrolysis-GC/MS. J Anal Appl Pyrolysis 127:120–125. https://doi.org/10.1016/j.jaap.2017.08.016
47. Prieto AI, Guzmán-Guillén R, Jos Á, Cameán AM, de la Rosa JM, González-Pérez JA (2020) Detection of cylindrospermopsin and its decomposition products in raw and cooked fish (Oreochromis niloticus) by analytical pyrolysis (Py-GC/MS). Chemosphere 244:125469. https://doi.org/10.1016/j.chemosphere.2019.125469
48. Wang L, Hosaka A, Watanabe C, Ohtani H, Tsuge S (2004) Development of a novel solid-phase extraction element for thermal desorption gas chromatography analysis. J Chromatogr A 1035(2):277–279. https://doi.org/10.1016/j.chroma.2004.02.059

49. Wang L, Zhang X, Wang Y, Wang W (2006) Simultaneous determination of preservatives in soft drinks, yogurts and sauces by a novel solid-phase extraction element and thermal desorption-gas chromatography. Anal Chim Acta 577(1):62–67. https://doi.org/10.1016/j.aca.2006.06.030
50. Huang Y, Qing X, Wang W, Han G, Wang J (2020) Mini-review on current studies of airborne microplastics: analytical methods, occurrence, sources, fate and potential risk to human beings. Trends Anal Chem 125:115821. https://doi.org/10.1016/j.trac.2020.115821
51. Li J, Liu H, Paul Chen J (2018) Microplastics in freshwater systems: a review on occurrence, environmental effects, and methods for microplastics detection. Water Res 137:362–374. https://doi.org/10.1016/j.watres.2017.12.056
52. Mai L, Bao LJ, Shi L, Wong CS, Zeng EY (2018) A review of methods for measuring microplastics in aquatic environments. Environ Sci Pollut Res 25(12):11319–11332. https://doi.org/10.1007/s11356-018-1692-0
53. Rios Mendoza LM, Balcer M (2019) Microplastics in freshwater environments: a review of quantification assessment. Trends Anal Chem 113:402–408. https://doi.org/10.1016/j.trac.2018.10.020
54. Silva AB, Bastos AS, Justino CIL, da Costa JP, Duarte AC, Rocha-Santos TAP (2018) Microplastics in the environment: challenges in analytical chemistry – a review. Anal Chim Acta 1017:1–19. https://doi.org/10.1016/j.aca.2018.02.043
55. Kusch P (2017) Chapter 7 – application of pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). In: Rocha-Santos TAP, Duarte AC (eds) Comprehensive analytical chemistry, vol 75. Elsevier, pp 169–207
56. David J, Steinmetz Z, Kučerík J, Schaumann GE (2018) Quantitative analysis of poly(ethylene terephthalate) microplastics in soil via thermogravimetry-mass spectrometry. Anal Chem 90(15):8793–8799. https://doi.org/10.1021/acs.analchem.8b00355
57. Steinmetz Z, Kintzi A, Muñoz K, Schaumann GE (2020) A simple method for the selective quantification of polyethylene, polypropylene, and polystyrene plastic debris in soil by pyrolysis-gas chromatography/mass spectrometry. J Anal Appl Pyrolysis. https://doi.org/10.1016/j.jaap.2020.104803
58. Watteau F, Dignac MF, Bouchard A, Revallier A, Houot S (2018) Microplastic detection in soil amended with municipal solid waste composts as revealed by transmission electronic microscopy and pyrolysis/GC/MS. Front Sustain Food Syst 2. https://doi.org/10.3389/fsufs.2018.00081
59. Okoffo ED, Ribeiro F, O'Brien JW, O'Brien S, Tscharke BJ, Gallen M et al (2020) Identification and quantification of selected plastics in biosolids by pressurized liquid extraction combined with double-shot pyrolysis gas chromatography-mass spectrometry. Sci Total Environ 715:136924. https://doi.org/10.1016/j.scitotenv.2020.136924
60. Käppler A, Fischer M, Scholz-Böttcher BM, Oberbeckmann S, Labrenz M, Fischer D et al (2018) Comparison of μ-ATR-FTIR spectroscopy and Py-GCMS as identification tools for microplastic particles and fibers isolated from river sediments. Anal Bioanal Chem 410(21):5313–5327. https://doi.org/10.1007/s00216-018-1185-5
61. Doyen P, Hermabessiere L, Dehaut A, Himber C, Decodts M, Degraeve T et al (2019) Occurrence and identification of microplastics in beach sediments from the Hauts-de-France region. Environ Sci Pollut Res 26(27):28010–28021. https://doi.org/10.1007/s11356-019-06027-8
62. Gomiero A, Øysæd KB, Agustsson T, van Hoytema N, van Thiel T, Grati F (2019) First record of characterization, concentration and distribution of microplastics in coastal sediments of an urban fjord in south West Norway using a thermal degradation method. Chemosphere 227:705–714. https://doi.org/10.1016/j.chemosphere.2019.04.096
63. Fischer M, Scholz-Boettcher BM (2019) Microplastics analysis in environmental samples – recent pyrolysis-gas chromatography-mass spectrometry method improvements to increase the reliability of mass-related data. Anal Methods 11(18):2489–2497. https://doi.org/10.1039/c9ay00600a

64. Funck M, Yildirim A, Nickel C, Schram J, Schmidt TC, Tuerk J (2019) Identification of microplastics in wastewater after cascade filtration using Pyrolysis-GC-MS. MethodsX 7: 100778. https://doi.org/10.1016/j.mex.2019.100778
65. Lv L, He L, Jiang S, Chen J, Zhou C, Qu J et al (2020) In situ surface-enhanced Raman spectroscopy for detecting microplastics and nanoplastics in aquatic environments. Sci Total Environ 728. https://doi.org/10.1016/j.scitotenv.2020.138449
66. Yu J, Wang P, Ni F, Cizdziel J, Wu D, Zhao Q et al (2019) Characterization of microplastics in environment by thermal gravimetric analysis coupled with Fourier transform infrared spectroscopy. Mar Pollut Bull 145:153–160. https://doi.org/10.1016/j.marpolbul.2019.05.037
67. Ravit B, Cooper K, Buckley B, Yang I, Deshpande A (2019) Organic compounds associated with microplastic pollutants in New Jersey, U.S.A. surface waters. AIMS Environ Sci 6(6):445–459. https://doi.org/10.3934/environsci.2019.6.445
68. Hendrickson E, Minor EC, Schreiner K (2018) Microplastic abundance and composition in Western Lake superior as determined via microscopy, Pyr-GC/MS, and FTIR. Environ Sci Technol 52(4):1787–1796. https://doi.org/10.1021/acs.est.7b05829
69. Fischer M, Goßmann I, Scholz-Böttcher BM (2019) Fleur de Sel—an interregional monitor for microplastics mass load and composition in European coastal waters? J Anal Appl Pyrolysis 144. https://doi.org/10.1016/j.jaap.2019.104711
70. Hermabessiere L, Himber C, Boricaud B, Kazour M, Amara R, Cassone AL et al (2018) Optimization, performance, and application of a pyrolysis-GC/MS method for the identification of microplastics. Anal Bioanal Chem 410(25):6663–6676. https://doi.org/10.1007/s00216-018-1279-0
71. Fabbri D, Rombolà AG, Vassura I, Torri C, Franzellitti S, Capolupo M et al (2020) Off-line analytical pyrolysis GC–MS to study the accumulation of polystyrene microparticles in exposed mussels. J Anal Appl Pyrolysis 149:104836. https://doi.org/10.1016/j.jaap.2020.104836
72. Diez-Quijada L, de Oliveira FL, Jos Á, Cameán AM, Aparicio-Ruiz R, Vasconcelos V et al (2020) Alterations in Mediterranean mussel (Mytilus galloprovincialis) composition exposed to cyanotoxins as revealed by analytical pyrolysis. J Anal Appl Pyrolysis 152:104970. https://doi.org/10.1016/j.jaap.2020.104970
73. Dehaut A, Hermabessiere L, Duflos G (2019) Current frontiers and recommendations for the study of microplastics in seafood. Trends Anal Chem 116:346–359. https://doi.org/10.1016/j.trac.2018.11.011
74. Mintenig SM, Bäuerlein PS, Koelmans AA, Dekker SC, Van Wezel AP (2018) Closing the gap between small and smaller: towards a framework to analyse nano- and microplastics in aqueous environmental samples. Environ Sci Nano 5(7):1640–1649. https://doi.org/10.1039/c8en00186c
75. Sullivan GL, Gallardo JD, Jones EW, Hollliman PJ, Watson TM, Sarp S (2020) Detection of trace sub-micron (nano) plastics in water samples using pyrolysis-gas chromatography time of flight mass spectrometry (PY-GCToF). Chemosphere 249:126179. https://doi.org/10.1016/j.chemosphere.2020.126179
76. Zhou X-x, Hao L-t, Wang H-y-z, Li Y-j, Liu J-f (2019) Cloud-point extraction combined with thermal degradation for nanoplastic analysis using pyrolysis gas chromatography-mass spectrometry. Anal Chem 91(3):1785–1790. https://doi.org/10.1021/acs.analchem.8b04729
77. Fischer M, Scholz-Böttcher BM (2017) Simultaneous trace identification and quantification of common types of microplastics in environmental samples by pyrolysis-gas chromatography-mass spectrometry. Environ Sci Technol 51(9):5052–5060. https://doi.org/10.1021/acs.est.6b06362
78. Müller YK, Wernicke T, Pittroff M, Witzig CS, Storck FR, Klinger J et al (2020) Microplastic analysis—are we measuring the same? Results on the first global comparative study for microplastic analysis in a water sample. Anal Bioanal Chem 412(3):555–560. https://doi.org/10.1007/s00216-019-02311-1
79. La Nasa J, Biale G, Fabbri D, Modugno F (2020) A review on challenges and developments of analytical pyrolysis and other thermoanalytical techniques for the quali-quantitative

determination of microplastics. J Anal Appl Pyrolysis 149:104841. https://doi.org/10.1016/j.jaap.2020.104841
80. Akoueson F, Chbib C, Monchy S, Paul-Pont I, Doyen P, Dehaut A et al (2021) Identification and quantification of plastic additives using pyrolysis-GC/MS: a review. Sci Total Environ 773:145073. https://doi.org/10.1016/j.scitotenv.2021.145073
81. Chen H, Rhoades CC, Chow AT (2020) Characteristics of soil organic matter 14 years after a wildfire: a pyrolysis-gas-chromatography mass spectrometry (Py-GC-MS) study. J Anal Appl Pyrolysis 152:104922. https://doi.org/10.1016/j.jaap.2020.104922
82. Campo J, Stijsiger RJ, Nadal-Romero E, Cammeraat ELH (2019) The effects of land abandonment and long-term afforestation practices on the organic carbon stock and lignin content of Mediterranean humid mountain soils. Eur J Soil Sci 70(5):947–959. https://doi.org/10.1111/ejss.12799
83. Banach-Szott M, Debska B, Tobiasova E, Pakula J (2019) Structural investigation of humic acids of forest soils by pyrolysis-gas chromatography. Pol J Environ Stud 28(6):4099–4107. https://doi.org/10.15244/pjoes/97392
84. Otto S, Streibel T, Erdmann S, Klingbeil S, Schulz-Bull D, Zimmermann R (2015) Pyrolysis–gas chromatography–mass spectrometry with electron-ionization or resonance-enhanced-multi-photon-ionization for characterization of polycyclic aromatic hydrocarbons in the Baltic Sea. Mar Pollut Bull 99(1):35–42. https://doi.org/10.1016/j.marpolbul.2015.08.001
85. Park J, Choi M, Cho J, Chon K (2018) Transformation of dissolved organic matter in a constructed wetland: a molecular-level composition analysis using pyrolysis-gas chromatography mass spectrometry. Environ Eng Res 23(4):390–396. https://doi.org/10.4491/eer.2018.043
86. Jiang T, Kaal J, Liang J, Zhang Y, Wei S, Wang D et al (2017) Composition of dissolved organic matter (DOM) from periodically submerged soils in the Three Gorges Reservoir areas as determined by elemental and optical analysis, infrared spectroscopy, pyrolysis-GC–MS and thermally assisted hydrolysis and methylation. Sci Total Environ 603–604:461–471. https://doi.org/10.1016/j.scitotenv.2017.06.114
87. Taube PS, Silva DS, Vasconcelos AA, Rebellato L, Madureira LAS, Hansel FA (2018) Exploratory on-line pyrolysis and thermally assisted hydrolysis and methylation for evaluating non-hydrolyzable organic matter in anthropogenic soil from central Brazilian Amazon. Brazilian. J Anal Chem 5(19):38–53. https://doi.org/10.30744/brjac.2179-3425.2018.5.19.38-53
88. Dorado J, Almendros G, González-Vila FJ (2016) Response of humic acid structure to soil tillage management as revealed by analytical pyrolysis. J Anal Appl Pyrolysis 117:56–63. https://doi.org/10.1016/j.jaap.2015.12.016
89. Jimenez-Gonzalez MA, Alvarez AM, Carral P, Gonzalez-Vila FJ, Almendros G (2017) The diversity of methoxyphenols released by pyrolysis-gas chromatography as predictor of soil carbon storage. J Chromatogr A 1508:130–137. https://doi.org/10.1016/j.chroma.2017.05.068
90. Schellekens J, Almeida-Santos T, Macedo RS, Buurman P, Kuyper TW, Vidal-Torrado P (2017) Molecular composition of several soil organic matter fractions from anthropogenic black soils (Terra Preta de Índio) in Amazonia — a pyrolysis-GC/MS study. Geoderma 288:154–165. https://doi.org/10.1016/j.geoderma.2016.11.001
91. Heslop J, Walter Anthony K, Zhang M (2017) Utilizing pyrolysis GC-MS to characterize organic matter quality in relation to methane production in a thermokarst lake sediment core. Org Geochem 103:43–50. https://doi.org/10.1016/j.orggeochem.2016.10.013
92. Fukushima M, Tu X, Aneksampant A, Tanaka A (2018) Analysis of branched-chain fatty acids in humic substances as indices for compost maturity by pyrolysis-gas chromatography/mass spectrometry with tetramethylammonium hydroxide (TMAH-py-GC/MS). J Mater Cycles Waste Manag 20(1):176–184. https://doi.org/10.1007/s10163-016-0559-z
93. Chen H, Blosser GD, Majidzadeh H, Liu X, Conner WH, Chow AT (2018) Integration of an automated identification-quantification pipeline and statistical techniques for pyrolysis GC/MS tracking of the molecular fingerprints of natural organic matter. J Anal Appl Pyrolysis 134:371–380. https://doi.org/10.1016/j.jaap.2018.07.002

Chiral Analysis with Mass Spectrometry Detection in Food and Environmental Chemistry

Ana Rita L. Ribeiro, Alexandra S. Maia, Cláudia Ribeiro, and Maria Elizabeth Tiritan

Contents

A. R. L. Ribeiro
Laboratory of Separation and Reaction Engineering-Laboratory of Catalysis and Materials (LSRE-LCM), Faculdade de Engenharia, Universidade do Porto, Porto, Portugal
e-mail: ritalado@fe.up.pt

A. S. Maia
TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Gandra, Portugal
e-mail: alexandra.maia@iucs.cespu.pt

C. Ribeiro
TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Gandra, Portugal

Interdisciplinary Center of Marine and Environmental Research (CIIMAR), University of Porto, Edificio do Terminal de Cruzeiros do Porto de Leixões, Matosinhos, Portugal
e-mail: claudia.ribeiro@iucs.cespu.pt

M. E. Tiritan (✉)
TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, CRL, Gandra, Portugal

Interdisciplinary Center of Marine and Environmental Research (CIIMAR), University of Porto, Edificio do Terminal de Cruzeiros do Porto de Leixões, Matosinhos, Portugal

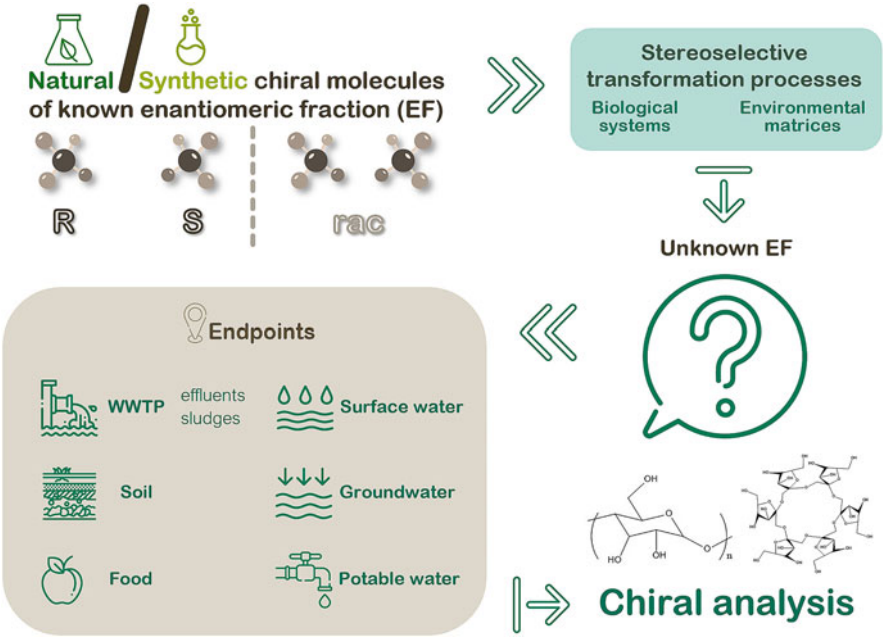
Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal
e-mail: elizabeth.tiritan@iucs.cespu.pt; beth@ff.up.pt

Yolanda Picó and Julian Campo (eds.), *Mass Spectrometry in Food and Environmental Chemistry*, Hdb Env Chem (2023) 119: 249–274, DOI 10.1007/698_2022_889,

Published online: 7 October 2022

Abstract The challenge to develop enantioselective analytical methods to quantify chiral compounds in food and environmental matrices is an actual and imperative issue. It is well known that enantiomers may differ in their biological activities, but the stereochemistry and the discrimination of enantiomers are frequently ignored in analytical workflows. However, the knowledge about their proportion is crucial to guarantee food and environmental safety. In this sense, chiral analysis is a valuable tool to evaluate food quality and genuineness, to determine the geographical origin of a certain sample aiming to find out fraud or adulteration, to investigate toxicity and bioaccumulation of environmental contaminants (e.g., pharmaceuticals and pesticides). This chapter highlights the importance of chiral analysis in food matrices and its relationship with the environmental contamination. The last advances in chiral analysis by liquid chromatography (LC), gas chromatography (GC), and supercritical fluid chromatography (SFC) coupled to mass spectrometry (MS) are presented, with a special remark in the type of chiral column used for chromatography and the proposed workflow in the method development. Some of the most recent applications in chiral analysis of food and beverages, environmental monitoring of surface water, wastewater, soils, biodegradation studies, and contamination of foodstuff are presented and critically discussed.

Graphical Abstract



Keywords Chiral chromatography, Chiral stationary phase, Enantiomers, Pesticides, Pharmaceuticals

1 Introduction

Chirality is a geometric property associated with the asymmetry and three-dimensionality of molecular structures. Asymmetric three-dimensional molecules with the same atomic connection but with different spatial arrangement generate two non-superposable structures called enantiomers [1]. Chirality is often originated by a tetrahedron carbon with four different substituents, or other atoms such as sulfur, phosphorus, and silicon, which generates a stereogenic center that can confer asymmetry to the molecules (Fig. 1). However, beyond the central chirality, other elements of chirality are described such as planar, axial, and helical, found in asymmetric structures without stereogenic centers [1]. Due to their structural likeness, enantiomers possess similar physical and chemical properties, apart from the rotation of the polarized light. The enantiomers that rotate the polarized light to the right (clockwise) are called dextrorotatory (+), and those that present rotation to the left (counter-clockwise) are levorotatory (−). Regarding their nomenclature, the structure configuration is related to the spatial orientation of the substituents of the

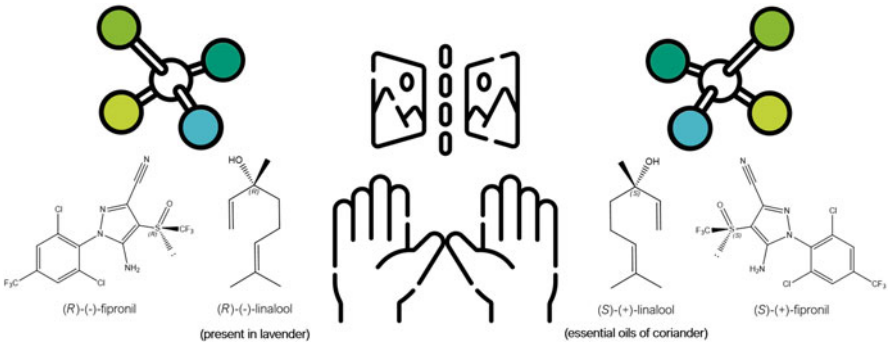


Fig. 1 Example of enantiomers of linalool and fipronil. The (*R*)-(−)-linalool has a lavender or lily-of-the-valley odor and (*S*)- (+)-linalool has a herbaceous and musty green smell [3]. Fipronil is a widely used insecticide, (*S*)-enantiomer is more active as insecticide and safer to mammals, in comparison with (*R*)-fipronil [4]

stereogenic center according to the specific rules established by the International Union of Applied Chemistry (IUPAC), which can be (*R*) from the Latin *rectus*, or (*S*) from the Latin *sinister* (Fig. 1) or L- or D- for amino acids and sugars [2]. The equimolar mixture of both enantiomers is denominated racemate.

Enantiomers are frequently overlooked in analytical methods and measured as a unique molecular entity. Nevertheless, the importance to assess both enantiomers in analytical methods comes from their different behavior in chiral medium, as occurs in the metabolic and regulatory processes mediated by biological systems that are sensitive to stereochemistry [1, 5, 6]. The essential subunits of the biological systems, namely amino acids, peptides, sugar, and nucleic acids are the chiral building blocks of proteins, glycoproteins, and deoxyribonucleic acid, respectively, resulting in the intrinsic chirality of the final arrangement. Therefore, the enantiomers can interact in different ways with enzymes, receptors, transport systems, or other binding chiral molecules displaying different responses (enantioselectivity/enantiospecificity) such as dissimilar taste, aroma or diverse biological activities. Enantiomers may present similar biological response but differ in potency or their biological activity can be completely distinct, as occurs for many pharmaceuticals, which one enantiomer is pharmacologically active, and the other enantiomer can be inactive or toxic. For instance, the biochemical processes of living organisms are based on D-sugars and L-amino acids, though the presence of D-amino acids in human proteins plays a crucial role in human health and the development of diseases [7]. As examples, D-Asp in elastin, β-amyloid, and α-crystalline have been associated with arteriosclerosis, Alzheimer's disease, and cataracts, respectively [7].

Nowadays, liquid chromatography (LC) or gas chromatography (GC) associated with mass spectrometry (MS) are the most applied methodologies in chiral analysis of food and environmental matrices. Nonetheless, most recently supercritical fluid chromatography (SFC) has also become a new trend. As enantiomers possess the same molecular mass and fragmentation pattern in MS, the enantioseparation by

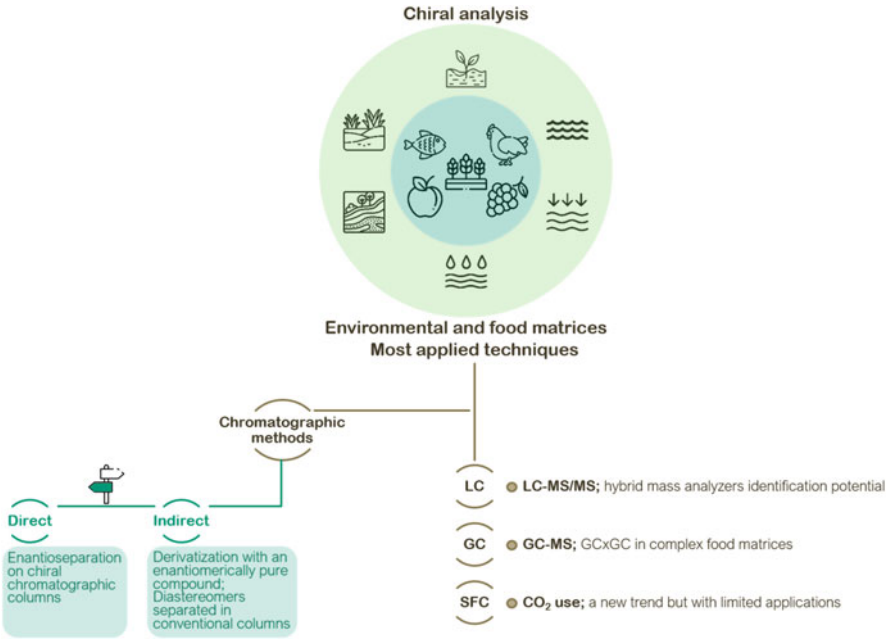


Fig. 2 Representation of the main analytical approaches for chiral analysis in environmental and food matrices

chromatographic methods is indispensable for their identification and quantification. There are two ways to separate enantiomers by chromatography: the direct and indirect methods (Fig. 2). The indirect methods require derivatization toward the formation of diastereomers that have different physico-chemical properties and can be separated in conventional chromatography columns. However, this approach is only possible if the racemate has at least one suitable functional group to react. On the other hand, direct methods do not require previous derivatization due to the presence of a chiral selector in the stationary phase or an additive in the mobile phase. The enantioseparation by direct methods occurs by the continuous formation of transient diastereomeric complexes between the target enantiomers and the chiral selector, resulting in different retention times in the chromatographic methods, i.e., enantioseparation. The use of chiral selectors as additives in the mobile phase is a more complex approach because the selectors can interfere with the detection mode. Moreover, the considerable amount of chiral selector required is difficult to recover, resulting in production of waste at large scale.

The chiral selectors in the stationary phase are adsorbed or covalently bonded to a solid chromatographic support. Most reported methods for chiral analysis, especially by LC, are performed by direct methods involving the use of chiral stationary phases (CSPs) (Fig. 2). A variety of CSPs are nowadays commercially available. Figure 3 shows the most applied CSPs used for LC and GC. Regarding LC, the majority of

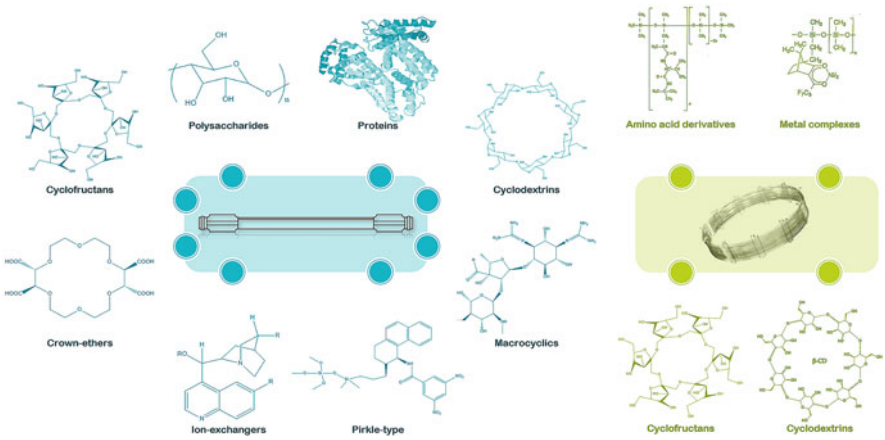


Fig. 3 Most applied CSPs for chromatographic methods

CSPs can be operated in normal, reversed, polar organic, or polar ionic elution modes. The nature of the chiral selector, the mobile phase composition, and the molecular structures of the enantiomers determine the chiral recognition mechanism for the enantiomeric resolution. For more information on models of chiral separations and CSPs, readers can access to some excellent reviews in this field [8–14].

Despite the variety of analytical methodologies to monitor food quality and environmental contamination, only chiral analyses assure a high level of safety and risk assessment since they allow to quantify both enantiomers, which is not possible by conventional analytical methodologies.

The interconnection of environmental pollutants and food safety together with the importance of chiral analysis is schematic represented in Fig. 4. This chapter presents the latest advances in chiral analysis of food and environmental matrices by chromatographic techniques coupled to MS. The proposed workflow in the most innovative methods, including sample preparation, is presented and critically discussed, together with the bottlenecks of the methodologies and some suggestions for further improvements and wider applications.

2 Chiral Analysis in Environmental Chemistry

Despite being well known that enantiomers may differ in their biological activities, the stereochemistry is often ignored in analytical methods applied to environmental studies, which can lead to inaccurate data [15]. However, an increasing interest in enantiomers quantification and evaluation of the enantiomeric fraction (EF) has been observed recently [16]. In fact, several studies have been demonstrating the concerning about environmental occurrence of organic pollutants (e.g., pharmaceuticals and personal care products, illicit drugs, pesticides) [17–19]. The constant

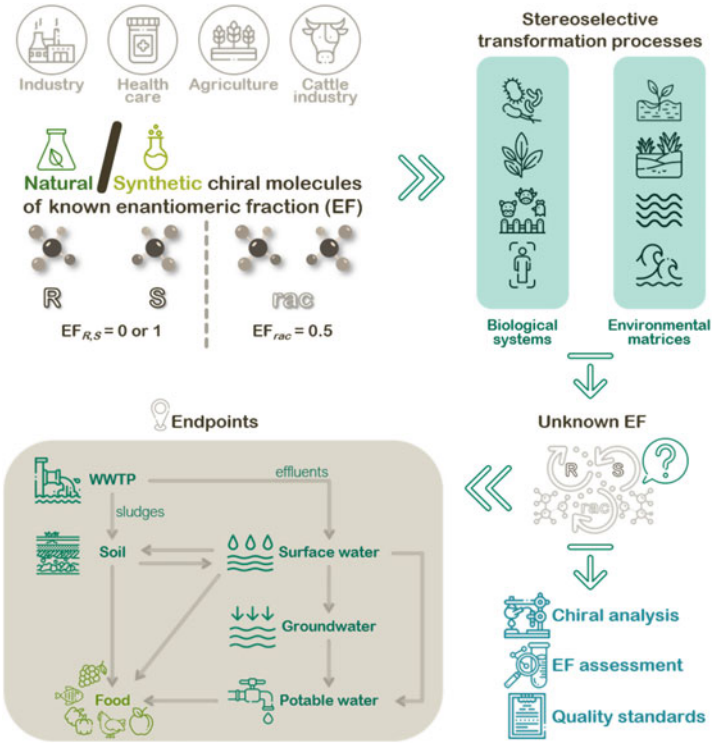


Fig. 4 The interconnection of environmental pollutants and food safety together with the importance of chiral analysis

introduction of an enormous number of pollutants at low concentrations (ng/L to μg/L) in water bodies and soils results from different origins, including domestic, hospital, and industrial wastewater effluents, agriculture, livestock, aquaculture, and landfill leachates (Fig. 4) [17]. Wastewater effluents represent a major pathway of entry of many contaminants as wastewater treatment plants (WWTP) are not designed to remove them. Consequently, the non-degradable or partially eliminated compounds, the metabolites resulting from microbial degradation, and other transformation products resulting from abiotic processes (e.g., hydrolysis and photodegradation) can pseudo-persist in the environment. In the case of suspended solids, sewage sludge or animal manure applied to soils, the sorbed pollutants can desorb and be leached into aquifers with subsequent contamination of water, plants, and food chain [20].

These interconnected pathways together with the recalcitrance and toxicity of most pollutants create a favorable scenario for possible impacts in the ecological status and human health (Fig. 4). Although the research in this topic has evolved massively in the last two decades, much less is known about the chiral signatures of

contaminants in environmental studies, which is a valuable tool for many purposes, namely to:

- get information about the environmental occurrence and fate of chiral pollutants and their metabolites (e.g., wastewater, sewage sludge, surface water, sediments, soils, biota) [21];
- use the data for wastewater-based epidemiological studies (WBE), e.g., to get information on the consumption patterns of pharmaceuticals or illicit drugs [22];
- understand their sources (e.g., to identify the use of banned pesticides, clandestine laboratories) and distinguish between licit and illicit production/usage (e.g., to identify the synthesis pathway or to detect illicit sewage discharges) [22];
- evaluate their (bio)degradation and (bio)adsorption [23];
- assess the formation of metabolites and other transformation products [24];
- distinguish between biotic and abiotic processes [23];
- study their ecotoxicological effects [25] and bioaccumulation [26];
- afford an accurate risk assessment [27];
- support future prioritization exercises and regulations [27];
- provide data for future remediation approaches [27];
- evaluate the uptake contamination by food web [28].

In fact, the development of enantioselective analytical methods to quantify residues of chiral drugs in environmental matrices is a challenging task for analytical chemists. The EF is a key parameter to assess the environmental fate and distribution of chiral pollutants [27] since its variation depends on biological processes like biosorption, biodegradation, biotransformation, uptake, bioaccumulation, and/or has impact on the toxicological effects. In this sense, many efforts have been applied in the topic of enantioselective analysis [29–32]. In the field of chirality in the environment, enantioselective monitoring has been the most studied subfield, with most reports focusing pharmaceuticals on aquatic compartments and pesticides in soils [21, 23, 33–35]. This type of study is useful to show the enantiomeric enrichment of certain drugs [36], to verify time variability [37], or to study the enantioselective degradation patterns [24, 32].

The latest advances in MS coupled to chromatographic methods (LC, GC, SFC, and capillary electrophoresis (CE)) allied with the diversity of CSPs commercially available have been boosting the application of enantioselective methods [16, 38–41]. In general, the most recent analytical methods are driven by automation, miniaturization, reduction/elimination of waste and hazardous substances, reduction of sample amount and usage of sustainable solvents (e.g., ethanol, ionic liquids or supercritical fluids) [27].

LC using CSPs is the most used technique in environmental studies mainly due to the high number of available commercial CSPs and the versatile multimode elution supported by LC [16, 27]. CSPs currently available that are often used in environmental matrices include protein-based [42], antibiotics-based [31, 43], Pirkle type [31], and polysaccharide derivatives [44, 45]. However, the main bottleneck for a wider application of chiral analysis is the lack of universal or complementary chiral selectors and the difficulty in selecting the appropriate CSP, especially considering

that MS narrows the options available for mobile phase. Moreover, the few available sub-2 μm chiral stationary phases (CSPs) is another shortcoming since this type of CSPs are crucial in reducing the chromatographic run time, to improve efficiency gain, resolution and sensitivity, essential for trace analysis [10, 16].

The versatility, selectivity, and sensitivity of LC-MS/MS are the main contributors for its popularity, with triple quadrupole (QqQ) mass analyzers standing out for trace analysis [27]. In turn, high-resolution mass analyzers are precious for structural elucidation [27]. The latest advances on hybrid mass analyzers as the quadrupole time-of-flight (QTOF) with improved sensitivity, selectivity, and ability for structural elucidation, allow the simultaneous clear confirmation of targets and the structural elucidation of non-targets [16, 27].

The first enantioselective method reported to determine anthropogenic markers in septic tank effluents was carried out by LC-MS/MS using a Chirobiotic V2® column that allowed the separation of 16 chiral and achiral anthropogenic indicators under polar organic elution mode [46]. The method reported a high number of chiral compounds with a good enantioresolution, with a relatively short run time for an enantioselective and multi-residue LC-MS/MS method (Fig. 5). In this study, the authors found that amphetamine EF in stream water of rural areas could indicate direct discharge of septic tank wastewater.

Matrix effects may be considered the main drawback of LC-MS/MS for environmental analysis due to the loss of response generated by the co-extracted matrix that can quench the signal of the co-eluting analytes. The first enantioselective method to analyze multi-residue drugs in river sediments reported varying signal suppressions for the 15 target chiral compounds [47], but surprisingly, many selected drugs (e.g., acebutolol, atenolol, citalopram, metoprolol, salbutamol) revealed enantioselective suppression translated by a difference superior to 10% between enantiomers. As example, signal suppression of acebutolol-*E1* and acebutolol-*E2* were −4% and 34%, respectively, showing the need of deuterated surrogates for enantioselective analysis in such complex matrix. However, enantiopure internal standards are scarce and those available are expensive. In turn, another study describing a multi-residue method for the determination of enantiomeric profiles of eight chiral pesticides (diniconazole, metalaxyl, paclobutrazol, epoxiconazole, myclobutanil, hexaconazole, napropamide, and isocarbophos) in environmental soils and sediments by LC-MS/MS after a combined sample pretreatment with matrix solid-phase dispersion and dispersive liquid–liquid microextraction (MSPD-DLLME) highlighted that the matrix effect depended on the physico-chemical characteristics of soil and sediment samples, but in this case, the matrix effects were similar for enantiomer pairs in a given matrix [48]. In fact, matrix effect is the reason why sample preparation is a crucial step in environmental analysis. A pioneering approach of sample preparation was reported [31], which was based on the simultaneous extraction of acidic, basic, and neutral analytes (23 chiral pharmaceuticals, stimulants, and illicit drugs). This sample pretreatment skips the cartridge conditioning step and uses an ecofriendly solvent for elution, before LC-MS/MS analysis under reversed elution mode. Chirobiotic™V and a Pirkle type Whelk-O®1 CSPs were used to enantioseparate basic and acidic compounds, respectively. As

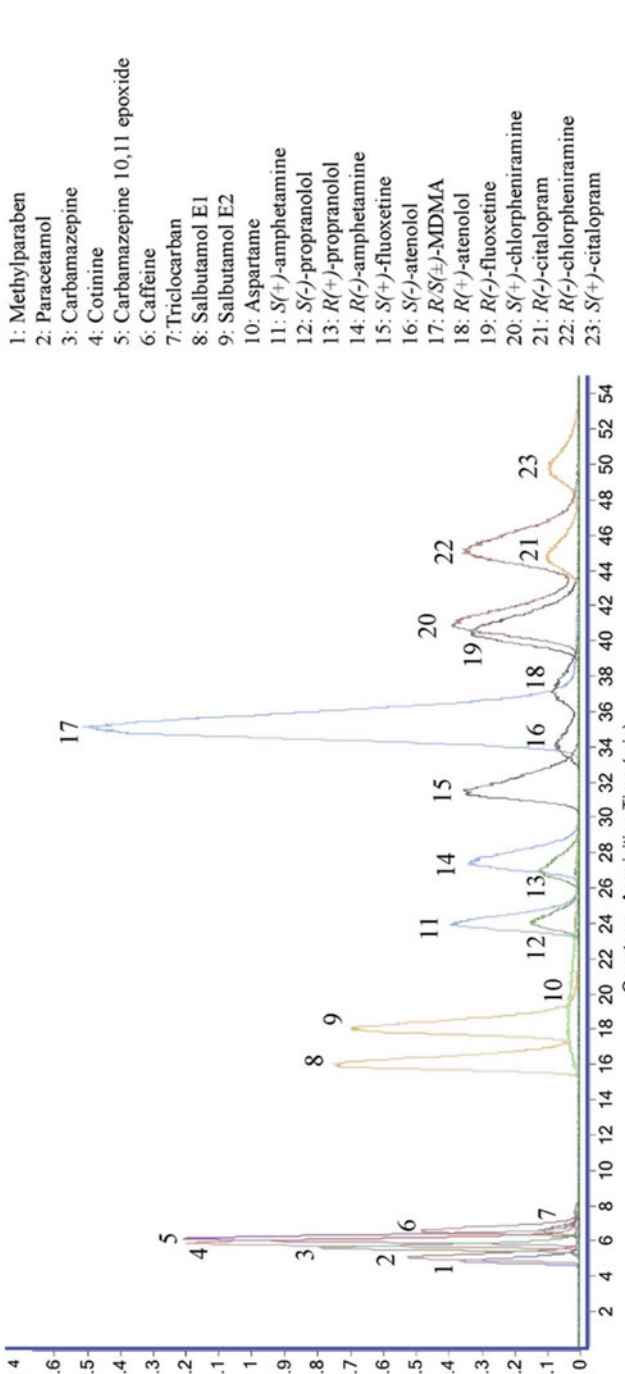


Fig. 5 Multiple reaction monitoring LC-MS/MS chromatograms of spiked anthropogenic markers, showing the enantioresolution of salbutamol (8/9), amphetamine (11/14), propranolol (12/13), fluoxetine (15/19), atenolol (16/18), chlorpheniramine (20/22), and citalopram (21/23) – *reprinted from Chemosphere, 219, Ramage, S., D. Camacho-Muñoz, and B. Petrie, Enantioselective LC-MS/MS for anthropogenic markers of septic tank discharge, 191–201, Copyright (2019), with permission from Elsevier* [46]

advantages, this method has a reduced environmental impact due to less solvent used and plastic wasted (one cartridge instead of two).

A recent study used an acetonitrile/water extraction combined with graphite carbon black solid-phase extraction (GCB-SPE) to extract penconazole, a triazole fungicide frequently used in agricultural, horticultural, and forestry industries. An enantioselective LC-MS/MS method using a chiral Lux Cellulose-2 column was applied to analyze penconazole enantiomers in complex matrices: grape, tea, soil, lotus root, lotus leaf, lotus seed, and hulls [49]. The method was useful to show that (−)-penconazole degraded faster in grape than the (+)-enantiomer, whereas a modest enantioselectivity was detected in soil, being also (−)-penconazole preferentially eliminated.

A sensitive analytical method was established to determine the enantiomers of the broad-spectrum chiral fungicide pydiflumetofen in food and environmental samples by LC-MS/MS using Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) as extraction procedure coupled with octadecylsilane-dispersive SPE [50]. This analytical method was assisted by polarimetry and electronic circular dichroism (ECD) to confirm the elution order of the enantiomers on Lux Cellulose-2, being the first eluted peak assigned to *S*-(−)-pydiflumetofen and the second to the *R*-(+)-enantiomer. The figures of merit were consistent with the requirements of trace analysis for 11 types of matrices, including soil where *R*-(+)-pydiflumetofen dissipated faster, which was the opposite trend to the enantioselective dissipation in pak choi. Another study on the enantioselective behavior of triazole pesticide, *cis*-epoxiconazole in the vegetable-soil-earthworm system, was reported [51], using a modified QuEChERS procedure and LC-QTOF-MS, assisted by ECD to assign the absolute configuration of each enantiomer. The method allowed to highlight that distinct enantioselectivities in diverse vegetables might directly or indirectly expose humans to the enriched enantiomer, which brings uncertainty to the environmental risk assessment since the enriched enantiomer might be riskier to human health.

Gas chromatography-mass spectrometry (GC-MS) for enantiomeric profiling in environmental samples has been used under the indirect method using chiral derivatizing reagents (e.g., applications with some pesticides) or under the direct method using CSPs (e.g., applications with polychlorinated biphenyls and polycyclic aromatic hydrocarbons) [21, 39]. Nonetheless, only a few of CSPs are commercially available, being cyclodextrin-based CSPs the most often used [39, 52].

SFC is a multipurpose option that can be used, for instance, to enantioseparate thermolabile analytes or non-volatiles that cannot be determined by GC, being most CSPs available for LC also useful for SFC [16, 27]. The advantages of SFC include the use of CO_2 as mobile phase, its sustainability, high throughput, reduced waste generated, and ability to reuse CO_2 [27]. Recently, chiral analysis by SFC with MS detection has been increasing due to the use of the same LC CSPs, the simplicity of the mobile phase, and the facility of the multi-column screening systems that are useful to achieve the suitable chiral selector for development of multi-residue methods [53, 54].

An interesting study on ultrafast chromatographic enantioseparation of pharmaceutically-related drugs and intermediates showed sub-minute resolutions

for most compounds by both SFC and reversed phase LC, highlighting a better overall selectivity and peak shape within a shorter analysis time by SFC than LC [55]. The first study using ultrahigh performance SFC coupled to a QqQ mass analyzer (SFC-MS/MS) for multi-residue enantioselective analysis of chiral pharmaceutically active compounds in environmental samples used two coated modified 2.5 μm-polysaccharide-based CSPs: amylose tris-3,5-dimethylphenylcarbamate and cellulose tris-3-chloro-4-methylphenylcarbamate [56]. A baseline enantioresolution was obtained for 13 compounds in less than 10 min of running time, whereas for the other 2 only a partial enantioresolution was reported. Another multi-residue SFC-MS/MS method for river and wastewater analysis was reported, in this case encompassing 140 chiral and non-chiral contaminants, with 81 compounds being fully quantitative, 14 semi-quantitative, and 45 qualitatively analyzed. A range of available SFC columns and optimized chromatographic conditions were used for the development of new, combined non-chiral and chiral-SFC methods capable of separating a range of different chiral and non-chiral analytes [57]. With limits of detection in a range between dozens pg L^{-1} and hundreds of ng L^{-1}, the method proved to give exceptional chromatographic enantioresolution for beta-blockers, benzodiazepines, and antidepressants.

SFC-MS was also used to determine five chiral fungicides in wine and vineyard soils, after sample preparation by SPE and pressurized liquid extraction [58]. The method allowed to reach low limits of quantification (between 0.5 and 2.5 ng mL^{-1}, and from 1.3 to 6.5 ng g^{-1}, for wine and soil, respectively), and it was found that soil dissipation and transfer from vines to wines of azole fungicides were non-enantioselective, whereas the application of metalaxyl to vines was confirmed to occur as racemate and as (*R*)-metalaxyl. Another example, a green ultraperformance convergence chromatography with tandem MS method using CO_2 and ethanol as mobile phase was developed to determine benalaxyl enantiomers in tobacco and soil, after sample preparation by QuEChERS technique [59]. This study reinforced the advantages of SFC as a green, precise (relative standard deviations $<5.0\%$), and rapid methodology, with the baseline separation of benalaxyl enantiomers in less than 5 min.

Another emerging field in environmental analysis is metabolomics. Such studies have been growing in the last years as an effective tool to evaluate biological effects after exposure, to assess the variation of targeted metabolites, or to identify untargeted metabolites and metabolic pathways [25]. However, enantioselectivity has been poorly explored. Recently, LC-TOF/MS-based metabolomics was proposed to understand the enantioselective effects of ibuprofen in adult zebrafish. This untargeted metabolomics study allowed to identify potential biomarkers and related pathways, which was followed by an approach involving targeted metabolomics and oxidative stress analysis [25].

The possible enantioselectivity of biodegradation either in the environmental compartments or in biological treatment of WWTPs has boosted the research on the enantioselective evaluation of biodegradation, with EF being the descriptor most frequently used to depict the relative abundance of enantiomers in biodegradation reports [60]. In the last years, there was a growing application of LC-MS/MS for

environmental monitoring of chiral compounds, which has been also applied to follow enantioselective biodegradation [60]. This is a research area that needs more attention due to the implications of transformation products generated either by biological processes (metabolites) or by abiotic processes. Their potential toxicity and bioaccumulation, either enantioselective or not, urges attention for an accurate environmental risk assessment.

Interestingly, the metabolites detected in the environment can be enriched with the enantiomer with the opposite configuration of the parent compound, as reported elsewhere [61]. Biodegradation can also lead to an enrichment of a certain enantiomer in the environment that is the opposite of that excreted by humans or animals, as reported for fluoxetine (among other compounds) in the same study on WWTPs and receiving waters. Here, the study was monitored by QqQ analyzer assisted by enantioseparation through both protein and antibiotic-based CSPs [61]. Moreover, chiral inversion may also result from microbial metabolism [62]. The enantioselective biodegradation or biotransformation patterns depends greatly on the characteristics of the microbial community [42].

Chiral environmental analysis has also an important role in forensic determinations. LC-MS/MS is the most applied analytical approach for enantioselective determination of chiral compounds in environmental matrices, as occurs in the case of environmental monitoring [22]. Enantiomeric profile is a useful tool to differentiate direct disposal from consumption, to get insights about the synthetic pathway, and to distinguish licit and illicit consumption of certain drugs [63]. For example, WBE and enantioselective analysis were used to estimate consumption patterns of illicit drugs in eight European cities, by using LC-MS/MS with QqQ and a Chiralpak® CBH [64]. Another report using a LC-MS/MS with a QTrap and a Chirobiotic V2 CSP for enantioseparation was focused on the enantiomeric profile of amphetamine and methamphetamine in wastewater in regional and urban areas of Queensland (Australia) to find evidence on consumption in each area and the potential synthetic pathways [65]. Although LC-MS/MS is the most frequent analytical methodology used for forensic environmental studies, GC-MS is preferred for some classes of compounds (e.g., amphetamine related substances) [30]. Despite the potential of two-dimensional GC (2D-GC) for forensic applications [66] due to its advantages (robustness even for complex matrices, selectivity, sensitivity), forensic environmental studies using this technique are missing in the literature. The same occurs for analytical methodologies such as SFC and CE, which are mostly used for biological samples in forensic studies [22].

3 Chiral Analysis in Food Chemistry

3.1 Evaluation of Product Authenticity and Adulteration

Chirality is inherent to natural products and the evaluation of the enantiomeric ratio (e.r.) is of great importance to assess authenticity of essential oils and fruit flavors, to determine the biosynthetic pathway, to establish food origin (natural or synthetic), and to control the storage conditions and the microbial contamination. In fact, chiral analysis has been used for food quality control in the evaluation of genuineness, to determine the geographical origin for finding out the possible fraud or adulteration [67]. Several recent reviews have reunited the representative applications of chiral analysis in food science. GC, LC, and SFC with MS detection using CSPs for enantioseparation are the most applied methodologies [39, 67–69]. In the case of complex matrices, adequate sample preparation process and enantioselective multidimensional methods such as 2D-GC and 2D-LC are of great relevance. The appropriate selection of an achiral column in the first dimension and a chiral column in the second dimension is an important step. The unresolved target compounds are heart-cut from the primary column and delivered to the chiral column for enantioseparation [68, 69].

Chiral GC plays a key role in analysis of flavor and odor, which are imperative indicators in the evaluation of the quality of many food products. Given the volatility of these substances, many GC methods with MS detection have been developed for the analysis of essential oils, milk, herbal infusions, meat, fish, alcoholic beverages (wine and beer), fruit flavoring, vegetables, and food supplements [39]. For instance, the incorporation of a flow-modulated comprehensive 2D-GC provides a possibility to improve the quality of the results due to the enhancement of separation capacity and resolution. As example, the influence of winemaking technology on specific features of botrytized wines was analyzed by 2D-GC [70]. Interestingly, the pleasant honey-like taste and unique fruit flavor of botrytized (noble rot) wines are the result of a specific winemaking technology, which includes overripe grapes infected by *Botrytis cinerea.* A CSP based on 2,3-di-*O*-ethyl-6-*O*-tert-butyl dimethylsilyl-β-cyclodextrin was used in the first dimension and a polar INNOWax column was selected for the second dimension to separate analytes according to their polarity. The results showed a significant difference between wine categories. The prevalence of *S*-(−)-ethyl lactate in the botrytized wines was supposed to be a result of malolactic fermentation supported by *Botrytis cinerea* colonization on the grape berry. It was also found that the enantiomeric distribution of the target analytes changes with the time range of fermentation with grape skins. Increased e.r. of (*R*)-ethyl lactate and a reduction in a *cis/trans* ratio of whiskey lactone were observed with the extension of the fermentation period [70]. Whiskey lactone molecule has four stereoisomers, but only *trans*-(3*S*,4*R*)-and *cis*-(3*S*,4*S*)-whiskey lactones are naturally occurring. The *cis*-stereoisomer has a lower odor threshold, whereas both *cis*- and *trans*-whiskey lactones contribute to fresh wood and coconut aroma of

wines. Moreover, *cis/trans* ratio of whiskey lactone has been suggested as a parameter to distinguish wines aged in America from French oak barrels [71].

Volatile organic compounds in healthy and botrytized grape berries, as well as highly botrytized wines (Tokaj selection essences) were analyzed by GC-MS with solid phase microextraction (SPME) as sample preparation. More than 95 compounds were detected in uninfected grape berries, while the number of detected compounds in Hungarian Tokaj selection essence (206) was doubled when compared to Slovak ones. Limonene, α-terpineol, hotrienol, diethyl malate, 2,3-butanediol, and whiskey lactones were selected for further chiral separation. Enantiomeric separations were performed by heart-cut 2D-GC system, isothermally, with two independent GC ovens using different chiral columns: Chirasil-β-Dex at 50°C (limonene) or 75°C (α-terpineol and hotrienol), and on the MEGA–DEX DMT–Beta at 50°C (2,3-butanediol) or 100°C (diethyl malate or whiskey lactones). Botrytization influenced the e.r. of studied terpenes in grapes of all varieties. The enantiomer distribution of α-terpineol was influenced by the wine processing technology used [72].

The terpenes and terpenoid compounds, responsible for the typical and unique smell of the cannabis plant from different hemp cultivars (Kompolti, Futura 75, Carmagnola, Felina 32, and Finola) were analyzed by GC-MS and flame ionization detectors. The compounds were obtained by microwave distillation and GC-MS using a cyclodextrin-based capillary chiral column, beta-DEX™, was used for enantioseparation. The e.r. is an analytical parameter commonly utilized for establishing the authenticity of suspicious samples. The enantiomeric distribution of 10 enantiomeric pairs was well-defined, the method confirmed the predominance of (−)-caryophyllene oxide in *Cannabis sativa* plant and low levels of (+)-caryophyllene oxide [73]. Another recent work described terpenoid enantiomers proportion in essential oils of fifteen cultivars of *Mentha arvensis* and *M. x piperita*, collected from an experimental farm, hydrodistilled using a Clevenger-type apparatus to obtain essential oils which were analyzed by different cyclodextrin coated stationary phases. Among the 14 enantiomeric pairs studied, PM-β-CD CSP resolved 12 pairs of enantiomers, 10 pairs were enantioseparated in PM-γ-CD and TBDA-γ-CD, while TBDA-β-CD CSP resolved only eight enantiomeric pairs. TBDE-β-CD resolved all 14 enantiomeric pairs present in *M. arvensis*, and *M. x piperita* essential oils. Limonene, menthone, menthol, and menthyl acetate were identified as pure (−)-enantiomers whereas isomenthone, neomenthol, pulegone, and piperitone as pure (+)-enantiomers in menthol mint, and peppermint oils, respectively. The results obtained with this analytical approach may facilitate the origin authentication studies of mint oils as menthol biosynthesis revealed that only (−)-menthol is present in the essential oils of Mentha species [74].

Regarding LC with MS detection, many works report analysis of amino acids. D-amino acids have been identified in various foods, particularly in fermentation systems, such as vinegar, beer, and wine. Food processing under conditions of fermentation, high temperature, strong acid or alkali treatments, or adulteration can also induce the racemization of amino acids [75]. For instance, a sensitive, robust, high-throughput method for D-amino acid profiling was developed with

combination of enantioseparation by chiral columns and TOF/MS. The enantioseparations were performed in CROWNPAK CR-I(+) and CR-I(−) columns, with more than 100 targets, including non-proteinogenic amino acids and amines. The CROWNPAK columns contain a chiral crown ether as a chiral selector, are mainly used to separate chiral amino acids and other small molecules with primary amine groups. The elution order of the enantiomers can be reversed when necessary. Trace of D-amino acids in three kinds of foods (black vinegar, kimchi, and yogurt) were determined [76].

Natural cheese may also contain several D-amino acids originating from microorganisms. A LC-MS/MS high-throughput method combining two types of chiral columns was applied for the analysis of 115 chiral and non-chiral metabolites in cheese in order to know the metabolic profiling of chiral amino acids and related metabolites. CROWNPAK CR-I (+) column was mainly used for the simultaneous separation of targeted metabolites while the identification of D-lysine, L-glutamine, D-threonine, D-allo-threonine and D-homoserine; D-isoleucine, D-allo-isoleucine was possible with CR-I (−). Secondary amines such as D-, and L-proline were analyzed using a CHIRALPAK ZWIX (−). The applicability of the LC-MS/MS method was shown in commercial natural cheeses with different ripening periods (6, 18, and 26 months). The taste and quality of cheese highly depends on metabolites produced during the manufacturing process, and the ripening process especially affects the amount and variety of metabolites. The profiling revealed that the ripening period had a significant impact on the production of each metabolite [77].

Herbal leaves may contain a substantial amount of free amino acids, which contribute to the physiological and pharmacological properties and to the sensory characteristics of many types of herbal infusions. For example, glutamic acid and theanine are the main constituents prompting the umami taste of green tea. The composition of amino acids was used for the differentiation of tea types and the contents of amino acids are indeed positively correlated with the quality and price of certain types of herbal infusions. The herbal infusions manufacturing process and storage period may originate racemization converting their L- to D-forms, this hypothesis was verified with a method developed for analysis of the enantiomers in herbal leaves. The target enantiomers were separated using the CHIROBIOTIC T® chiral column. The identification was performed by high-resolution Q-TOF/MS (Fig. 6). The MS and MS/MS acquisitions, quantitative and identification were combined in the same experiment. Only L-forms of amino acids were found in fresh tea leaves. A total of 11 D-amino acids were determined in 19 tea samples, ranging from residual level to 43 μg g^{-1}. Figure 6 shows the results of the spiking experiment, MS and MS/MS scans, and the mass spectra of D-phenylalanine in a tea infusion. The results indicated that the enantioisomerization of amino acids occurred in post-harvest tea leaves affected by process conditions and storage time [78].

Amino acids can also be an important tool to identify the origin of products. Milk contains free amino acids that are crucial for the growth and health of infants. LC coupled to ion-mobility high-resolution MS (IM-HRMS) was applied for the characterization of 18 pairs of amino acids in different types of milk (human, cow, yak,

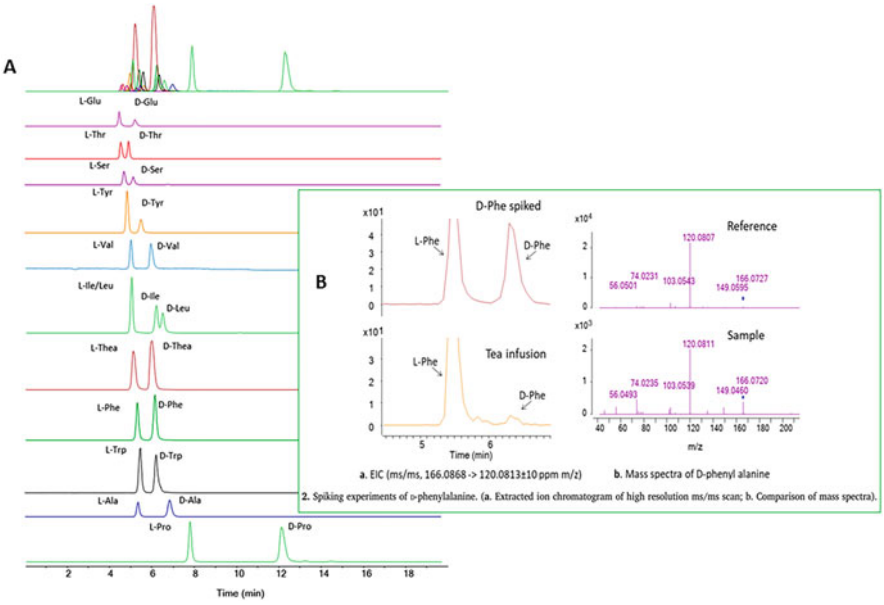


Fig. 6 Enantiosepation and Q-TOF/MS identification of amino acids (**a**); Spiking experiment, MS and MS/MS scans, and the mass spectra of D-phenylalanine in a tea infusion (**b**) – *reprinted from Food Chemistry 317 (2020) 126428, Yu Xu, Ziyi Liu, Zhaoye Liu, Zhihui Feng, Liang Zhang, Xiaochun Wan, Xiaogen Yang Ramage Identification of D-amino acids in tea leaves, Copyright (2020), with permission from Elsevier*

buffalo, goat, and camel). The results demonstrated that the origin of a certain milk can be distinguished based on the D-/L-amino acid ratio-based projection scores. A new approach to investigate amino acids metabolic pathways and to get a comprehensive knowledge on the D/L-amino acid ratio underlying the biological functions of diverse animal milks was provided [79]. Ion mobility spectrometry (IMS) has demonstrated to be a powerful tool for the fast separation of isobaric and isomeric compounds. In drift-tube IMS, ions are separated based on their mobility in an electric field through a neutral gas. As the mobility is a function of the ion collision cross section (CCS), which depends on the size and shape of the ion in the gas phase, compounds may be separated by IMS, even if their *m*/*z* ratio is the same. Although ions of enantiomers have identical charge, *m*/*z*, and CCS, their direct separation by IMS was not feasible and thus, the separation of enantiomers by IMS has been achieved by using gas-phase ion complexation with volatile chiral agents, by forming metal-ion noncovalent complexes with reference compounds or by indirect methods, derivatizing with an enantiomeric pure reagent [80–82].

3.2 Evaluation of Chiral Pollutants in Food Web

The evaluation of EF of contaminants such as fungicides, pesticides, and even pharmaceuticals is decisive for studying toxicity and bioaccumulation in food web. LC with MS detection is the most frequently employed technique for the separation of thermolabile and nonvolatile compounds. Methods for food safety, to monitor pesticides and pharmaceuticals enantiomer residues and to assess their risks, are mostly carried out by chiral LC-MS/MS [67], though SFC- MS/MS has been the new trend.

More than 30% of commercial, globally used pesticides, such as pyrethroid insecticides, triazole fungicides and herbicides, are chiral. Due to the challenge and cost of synthesis and chiral purification, most of the pesticides are commercialized as racemates. Nevertheless, the use of enantiopure formulations could reduce pesticide residues released into the environment, by applying the specific enantiomer that is responsible for the efficacy and/or is less toxic for non-target organisms. Triazole pesticides are a class of broad-spectrum fungicides that are useful in preventing several fungal diseases of fruits, vegetables, beans, and food crops. Usually, the inactive enantiomers of this class of fungicides are difficult to be biodegraded by organisms, resulting in the enantiomeric enrichment or accumulation in the ecosystems (water, soil, plants, animals) and even generating chiral carcinogenic intermediates. A LC-MS/MS method for the simultaneous analysis of six chiral triazole (hexaconazole, tebuconazole, triticonazole, flutriafol, diniconazole, paclobutrazol) in six fruits and vegetables was proposed based on a home-made bridged bis(β-cyclodextrin)-bonded CSP. Sample preparation was performed by magnetically assisted QuECHERS and 90 samples were analyzed. The tebuconazole EF in strawberry and cucumber was 0.63 and 0.43, respectively, whereas a value of 0.57 was obtained for flutriafol in tomato. This high-throughput method was a suitable strategy for enantiomeric evaluation of chiral pesticides in fruits and vegetables [83].

A novel multi-residue method by LC-MS/MS for the enantioselective analysis of 22 chiral pesticides in cucumber, tomato, cabbage, grape, mulberry, apple, and pear from the local market (Benxi, China) was reported. Pesticides were efficiently extracted by magnetic SPE based on graphene as sorbent. The enantiomeric separation was achieved on a Chiralpak IG® column, an immobilized CSP with amylose derivatized with phenyl moieties with chloro- and methyl-groups in meta positions [84].

Five triazole fungicides (penconazole, tebuconazole, triadimefon, myclobutanil, and triadimenol) were analyzed in tobacco by SFC-MS/MS. Optimized separation of the enantiomers was achieved in an ACQUITY UPC2 Trefoil AMY 1–2.5 μm amylose *tris*-(3,5-dimethylphenylcarbamate). The Trefoil columns were designed to use in UPC2 SFC systems. The method was applied to 20 fresh tobacco samples and the results indicated that some samples tested positive for tebuconazole, triadimenol, and penconazole. The EF of tebuconazole, triadimenol, and

penconazole ranged from 0.43 to 0.50, 0.49 to 0.51, and 0.50 to 0.53, respectively [85].

Antibiotics are widely used in veterinary medicine with the purposes of inhibiting the growth of microorganisms, preventing, or treating infections, and promoting growth when used at subtherapeutic doses. The public concern about the presence of antibiotic residues in food-producing animals has increased because they can lead to the growth of antibiotic-resistant bacteria, allergic reactions, or even toxic effects. Flumequine, owing to high antimicrobial activity against a wide range of Gram-negative and Gram-positive pathogens, has become an indispensable part of the treatment of infections in food-producing animals such as cattle, turkey, pig, and poultry. The enantioseparation and determination of flumequine enantiomers in milk, yogurt, chicken, beef, egg, and honey samples by LC-MS/MS on a Chiralpak IC column was reported, providing a reliable method for evaluating the potential risk in animal production [86]. Chiralpak IC is the immobilized *tris*-(3,5-dichlorophenylcarbamate) cellulose column.

Understanding the enantioselective fate of chiral neonicotinoid dinotefuran is of vital importance for accurate dietary exposure assessment and food safety regulation. Dinotefuran enantiomers demonstrated high toxicity to honeybee (*Apis mellifera* Linn.) and (*S*)-dinotefuran was 41.1- to 128.4-fold more toxic than (*R*)-dinotefuran [87]. An analytical method was developed by SFC-MS/MS using an amylose *tris*-(3,5-dimethylphenylcarbamate) chiral column to analyze dinotefuran and main metabolites 1-methyl-3-(tetrahydro-3-furylmethyl) urea (UF) and 1-methyl-3-(tetrahydro-3-furylmethyl)guanidium dihydrogen (DN) in tomato. Estimated half-lives of (*S*)-dinotefuran and (*R*)-dinotefuran were 10.3 and 9.1 days, respectively, by foliage uptake pathway, whereas they were 13.3 and 12.6 days, respectively, by root uptake pathway. Changes in the enantiomeric removal and enantioselectivity orientation of dinotefuran and metabolites were evaluated during home canning of tomato paste, including washing, peeling, homogenization, simmering, and sterilization. Peeling played the key role in reducing (*S*)-dinotefuran by 67.3% and (*R*)-dinotefuran by 69.9% with processing factor of 0.313 and 0.287, respectively. Simmering was the most effective way to remove UF enantiomers by elevated temperature. The study sheds light on the chiral profiles of the fate of dinotefuran from cultivation to processing [28].

4 Conclusion and Future Perspectives

Chiral analysis in food and in environmental matrices is clearly interconnected. Regarding the analysis of chiral components of food for authentication and genuineness, multidimensional GC-MS with 2D-GC plays an important role due to the complexity of the sample and the volatility of the substances used as threshold control. LC-MS/MS is the main technique for chiral analysis of contamination in environmental matrices and uptake by food. However, it is noticeable that SFC-MS/MS is gaining a great interest due to the green association with the technique and the

availability of suitable CSPs. 2D-LC and SFC have been showing importance in method development toward metabolic profiling and multi-residue analysis, but there are few reported applications in quality control of foods. In general, innovation in sample preparation protocols with 2D-LC-MS/MS provided a possibility to improve the quality of results, the time of analysis, the size of sample, and the efficiency of the enantioseparation. The applications exemplified that the environmental impact caused by discharged and management of chiral molecules and the relationship between environmental and food analysis. Combined efforts and exchange of experiences are needed to improve the chiral analysis in both fields.

Acknowledgments This work was supported by national funds through the FCT/MCTES (PIDDAC) under the project PTDC/CTA-AMB/6686/2020; by UIDB/04423/2020 and UIDP/04423/2020 (Group of Natural Products and Medicinal Chemistry) and CESPU: CHIRALSINTESE-APSFCT-IINFACTS_2021.

ARR would like to acknowledge: Project POCI-01-0145-FEDER-030521 funded by ERDF funds through COMPETE2020 – POCI and by National Funds (PIDDAC) through FCT/MCTES; the scientific collaboration under Base-UIDB/50020/2020 and Programmatic-UIDP/50020/2020 Funding of LSRE-LCM, funded by national funds through FCT/MCTES (PIDDAC); and FCT funding under DL57/2016 Transitory Norm Programme.

References

1. Tiritan ME, Ribeiro AR, Fernandes C, Pinto MMM (2016) Chiral pharmaceuticals. In: Kirk-Othmer encyclopedia of chemical technology. Wiley, Hoboken, pp 1–28
2. International Union of Pure and Applied Chemistry (IUPAC) (2014) Compendium of chemical terminology – gold book.2nd edn. International Union of Pure and Applied Chemistry, Research Triangle Park
3. Ozek T (2010) Enantiomeric distribution of some linalool containing essential oils and their biological activities. Rec Nat Prod 4:180–192
4. Konwick BJ, Fisk AT, Garrison AW, Avants JK, Black MC (2005) Acute enantioselective toxicity of fipronil and its desulfinyl photoproduct to Ceriodaphnia dubia. Environ Toxicol Chem 24:2350–2355
5. Lin G, You Q, Cheng J (2011) Chiral drugs, chemistry and biological action.1st edn. Wiley, Hoboken
6. Coelho MM, Fernandes C, Remião F, Tiritan ME (2021) Enantioselectivity in drug pharmacokinetics and toxicity: pharmacological relevance and analytical methods. Molecules 26:3113
7. Bastings JJAJ, van Eijk HM, Olde Damink SW, Rensen SS (2019) d-amino acids in health and disease: a focus on cancer. Nutrients 11:2205
8. Fernandes C, Phyo YZ, Silva AS, Tiritan ME, Kijjoa A, Pinto MMM (2018) Chiral stationary phases based on small molecules: an update of the last 17 years. Sep Purif Rev 47:89–123
9. Lämmerhofer M (2010) Chiral recognition by enantioselective liquid chromatography: mechanisms and modern chiral stationary phases. J Chromatogr A 1217:814–856
10. Teixeira J, Tiritan ME, Pinto MMM, Fernandes C (2019) Chiral stationary phases for liquid chromatography: recent developments. Molecules 24:865
11. Shen J, Ikai T, Okamoto Y (2014) Synthesis and application of immobilized polysaccharide-based chiral stationary phases for enantioseparation by high-performance liquid chromatography. J Chromatogr A 1363:51–61

12. Berkecz R, Tanács D, Péter A, Ilisz I (2021) Enantioselective liquid chromatographic separations using macrocyclic glycopeptide-based chiral selectors. Molecules 26:3380
13. Xiao Y, Ng S-C, Tan TTY, Wang Y (2012) Recent development of cyclodextrin chiral stationary phases and their applications in chromatography. J Chromatogr A 1269:52–68
14. Xie S-M, Yuan L-M (2019) Recent development trends for chiral stationary phases based on chitosan derivatives, cyclofructan derivatives and chiral porous materials in high performance liquid chromatography. J Sep Sci 42:6–20
15. Ribeiro AR, Maia AS, Cass QB, Tiritan ME (2014) Enantioseparation of chiral pharmaceuticals in biomedical and environmental analyses by liquid chromatography: an overview. J Chromatogr B 968:8–21
16. Barreiro JC, Tiritan ME, Cass QB (2021) Challenges and innovations in chiral drugs in an environmental and bioanalysis perspective. TrAC Trends Anal Chem 142:116326
17. Barbosa MO, Moreira NFF, Ribeiro AR, Pereira MFR, Silva AMT (2016) Occurrence and removal of organic micropollutants: an overview of the watch list of EU decision 2015/495. Water Res 94:257–279
18. Wang H, Xi H, Xu L, Jin M, Zhao W, Liu H (2021) Ecotoxicological effects, environmental fate and risks of pharmaceutical and personal care products in the water environment: a review. Sci Total Environ 788:147819
19. Singh NS, Sharma R, Singh SK, Singh DK (2021) A comprehensive review of environmental fate and degradation of fipronil and its toxic metabolites. Environ Res 199:111316
20. Ezzariai A, Hafidi M, Khadra A, Aemig Q, El Fels L, Barret M, Merlina G, Patureau D, Pinelli E (2018) Human and veterinary antibiotics during composting of sludge or manure: global perspectives on persistence, degradation, and resistance genes. J Hazard Mater 359:465–481
21. Ribeiro C, Ribeiro AR, Maia AS, Tiritan ME (2017) Occurrence of chiral bioactive compounds in the aquatic environment: a review. Symmetry 9:215
22. Ribeiro C, Santos C, Gonçalves V, Ramos A, Afonso C, Tiritan ME (2018) Chiral drug analysis in forensic chemistry: an overview. Molecules 23:262
23. Maia AS, Ribeiro AR, Castro PML, Tiritan ME (2017) Chiral analysis of pesticides and drugs of environmental concern: biodegradation and enantiomeric fraction. Symmetry 9
24. Maia AS, Castro PML, Tiritan ME (2016) Integrated liquid chromatography method in enantioselective studies: biodegradation of ofloxacin by an activated sludge consortium. J Chromatogr B 1029-1030:174–183
25. Song Y, Chai T, Yin Z, Zhang X, Zhang W, Qian Y, Qiu J (2018) Stereoselective effects of ibuprofen in adult zebrafish (Danio rerio) using UPLC-TOF/MS-based metabolomics. Environ Pollut 241:730–739
26. Ruan Y, Lin H, Zhang X, Wu R, Zhang K, Leung KMY, Lam JCW, Lam PKS (2020) Enantiomer-specific bioaccumulation and distribution of chiral pharmaceuticals in a subtropical marine food web. J Hazard Mater 394:122589
27. Ribeiro ARL, Maia AS, Ribeiro C, Tiritan ME (2020) Analysis of chiral drugs in environmental matrices: current knowledge and trends in environmental, biodegradation and forensic fields. TrAC Trends Anal Chem 124:115783
28. Chen Z, Dong F, Ren X, Wu X, Yuan L, Li L, Li W, Zheng Y (2021) Enantioselective fate of dinotefuran from tomato cultivation to home canning for refining dietary exposure. J Hazard Mater 405:124254
29. Hellinghausen G, Readel ER, Wahab MF, Lee JT, Lopez DA, Weatherly CA, Armstrong DW (2019) Mass spectrometry-compatible enantiomeric separations of 100 pesticides using core–shell chiral stationary phases and evaluation of iterative curve fitting models for overlapping peaks. Chromatographia 82:221–233
30. Gonçalves R, Ribeiro C, Cravo S, Cunha SC, Pereira JA, Fernandes JO, Afonso C, Tiritan ME (2019) Multi-residue method for enantioseparation of psychoactive substances and beta blockers by gas chromatography–mass spectrometry. J Chromatogr B 1125:121731

31. Coelho MM, Lado Ribeiro AR, Sousa JCG, Ribeiro C, Fernandes C, Silva AMT, Tiritan ME (2019) Dual enantioselective LC–MS/MS method to analyse chiral drugs in surface water: monitoring in Douro River estuary. J Pharm Biomed Anal 170:89–101
32. Petrie B, Mrazova J, Kasprzyk-Hordern B, Yates K (2018) Multi-residue analysis of chiral and achiral trace organic contaminants in soil by accelerated solvent extraction and enantioselective liquid chromatography tandem–mass spectrometry. J Chromatogr A 1572:62–71
33. Sanganyado E, Lu Z, Fu Q, Schlenk D, Gan J (2017) Chiral pharmaceuticals: a review on their environmental occurrence and fate processes. Water Res 124:527–542
34. Zhao P, Wang Z, Li K, Guo X, Zhao L (2018) Multi-residue enantiomeric analysis of 18 chiral pesticides in water, soil and river sediment using magnetic solid-phase extraction based on amino modified multiwalled carbon nanotubes and chiral liquid chromatography coupled with tandem mass spectrometry. J Chromatogr A 1568:8–21
35. Petrie B, Camacho Muñoz MD, Martín J (2019) Stereoselective LC–MS/MS methodologies for environmental analysis of chiral pesticides. TrAC Trends Anal Chem 110:249–258
36. Ma R, Qu H, Wang B, Wang F, Yu Y, Yu G (2019) Simultaneous enantiomeric analysis of non-steroidal anti-inflammatory drugs in environment by chiral LC-MS/MS: a pilot study in Beijing, China. Ecotoxicol Environ Saf 174:83–91
37. Petrie B, Proctor K, Youdan J, Barden R, Kasprzyk-Hordern B (2017) Critical evaluation of monitoring strategy for the multi-residue determination of 90 chiral and achiral micropollutants in effluent wastewater. Sci Total Environ 579:569–578
38. Pilařová V, Plachká K, Khalikova MA, Svec F, Nováková L (2019) Recent developments in supercritical fluid chromatography – mass spectrometry: is it a viable option for analysis of complex samples? TrAC Trends Anal Chem 112:212–225
39. Ribeiro C, Gonçalves R, Tiritan ME (2020) Separation of enantiomers using gas chromatography: application in forensic toxicology, food and environmental analysis. Crit Rev Anal Chem:1–25
40. Cifuentes A, Fanali S (2020) 21st century chiral analysis. TrAC Trends Anal Chem 127:115911
41. Yu X, Yao Z-P (2017) Chiral recognition and determination of enantiomeric excess by mass spectrometry: a review. Anal Chim Acta 968:1–20
42. Camacho-Muñoz D, Petrie B, Lopardo L, Proctor K, Rice J, Youdan J, Barden R, Kasprzyk-Hordern B (2019) Stereoisomeric profiling of chiral pharmaceutically active compounds in wastewaters and the receiving environment – a catchment-scale and a laboratory study. Environ Int:558–572
43. Ribeiro AR, Santos LHMLM, Maia AS, Delerue-Matos C, Castro PML, Tiritan ME (2014) Enantiomeric fraction evaluation of pharmaceuticals in environmental matrices by liquid chromatography-tandem mass spectrometry. J Chromatogr A 1363:226–235
44. Jing X, Yao G, Liu D, Liang Y, Luo M, Zhou Z, Wang P (2017) Effects of wastewater irrigation and sewage sludge application on soil residues of chiral fungicide benalaxyl. Environ Pollut 224:1–6
45. Silva C, Ribeiro C, Maia AS, Gonçalves V, Tiritan ME, Afonso C (2017) Enantiomeric separation of tramadol and its metabolites: method validation and application to environmental samples. Symmetry 9:170
46. Ramage S, Camacho-Muñoz D, Petrie B (2019) Enantioselective LC-MS/MS for anthropogenic markers of septic tank discharge. Chemosphere 219:191–201
47. Eaglesham A, Scott A, Petrie B (2020) Multi-residue enantioselective analysis of chiral drugs in freshwater sediments. Environ Chem Lett 18:2119–2126
48. Zhao P, Zhao J, Lei S, Guo X, Zhao L (2018) Simultaneous enantiomeric analysis of eight pesticides in soils and river sediments by chiral liquid chromatography-tandem mass spectrometry. Chemosphere 204:210–219
49. Zhang X, Wang X, Luo F, Sheng H, Zhou L, Zhong Q, Lou Z, Sun H, Yang M, Cui X, Chen Z (2019) Application and enantioselective residue determination of chiral pesticide penconazole in grape, tea, aquatic vegetables and soil by ultra performance liquid chromatography-tandem mass spectrometry. Ecotoxicol Environ Saf 172:530–537

50. Wang Z, Liu S, Zhao X, Tian B, Sun X, Zhang J, Gao Y, Shi H, Wang M (2021) Enantioseparation and stereoselective dissipation of the novel chiral fungicide pydiflumetofen by ultra-high-performance liquid chromatography tandem mass spectrometry. Ecotoxicol Environ Saf 207
51. Qi P, Di S, Cang T, Yang X, Wang X, Wang Z, Xu H, Zhao H, Wang X (2020) Enantioselective behaviors of cis-epoxiconazole in vegetables-soil-earthworms system by liquid chromatography-quadrupole-time-of-flight mass spectrometry. Sci Total Environ 706
52. Arenas M, Martín J, Santos JL, Aparicio I, Alonso E (2021) An overview of analytical methods for enantiomeric determination of chiral pollutants in environmental samples and biota. TrAC Trends Anal Chem 143:116370
53. Dogan A, Płotka-Wasylka J, Kempińska-Kupczyk D, Namieśnik J, Kot-Wasik A (2020) Detection, identification and determination of chiral pharmaceutical residues in wastewater: problems and challenges. TrAC Trends Anal Chem 122:115710
54. Freund E, Meyer D, Schneider N, Lozac'h M-A, Schröder H, Cinar C, Schlotterbeck G, Wagner T (2021) Automated chiral method screening – evaluation of generated chromatographic data sets to further optimize screening efficiency. J Chromatogr A 1644:462094
55. Barhate CL, Joyce LA, Makarov AA, Zawatzky K, Bernardoni F, Schafer WA, Armstrong DW, Welch CJ, Regalado EL (2017) Ultrafast chiral separations for high throughput enantiopurity analysis. Chem Commun 53:509–512
56. Camacho-Muñoz D, Kasprzyk-Hordern B, Thomas KV (2016) Enantioselective simultaneous analysis of selected pharmaceuticals in environmental samples by ultrahigh performance supercritical fluid based chromatography tandem mass spectrometry. Anal Chim Acta 934: 239–251
57. Rice J, Lubben A, Kasprzyk-Hordern B (2020) A multi-residue method by supercritical fluid chromatography coupled with tandem mass spectrometry method for the analysis of chiral and non-chiral chemicals of emerging concern in environmental samples. Anal Bioanal Chem 412: 5563–5581
58. Pérez-Mayán L, Ramil M, Cela R, Rodríguez I (2021) Supercritical fluid chromatography-mass spectrometric determination of chiral fungicides in viticulture-related samples. J Chromatogr A 1644:462124
59. Yang F, Tang G, Li Z, Fan Z, Wang Y, Liu S, Bian Z, Deng H (2018) An environmentally friendly method for the enantioseparation and determination of benalaxyl in tobacco and soil by ultra-performance convergence chromatography with tandem mass spectrometry. J Sep Sci 41: 4233–4240
60. Tiritan ME, Fernandes C, Maia AS, Pinto M, Cass QB (2018) Enantiomeric ratios: why so many notations? J Chromatogr A 1569:1–7
61. Evans S, Bagnall J, Kasprzyk-Hordern B (2017) Enantiomeric profiling of a chemically diverse mixture of chiral pharmaceuticals in urban water. Environ Pollut 230:368–377
62. Maia AS, Tiritan ME, Castro PML (2018) Enantioselective degradation of ofloxacin and levofloxacin by the bacterial strains labrys portucalensis F11 and Rhodococcus sp. FP1. Ecotoxicol Environ Saf 155:144–151
63. Langa I, Gonçalves R, Tiritan ME, Ribeiro C (2021) Wastewater analysis of psychoactive drugs: non-enantioselective vs enantioselective methods for estimation of consumption. Forensic Sci Int 325:110873
64. Castrignanò E, Yang Z, Bade R, Baz-Lomba JA, Castiglioni S, Causanilles A, Covaci A, Gracia-Lor E, Hernandez F, Kinyua J, McCall A-K, van Nuijs ALN, Ort C, Plósz BG, Ramin P, Rousis NI, Ryu Y, Thomas KV, de Voogt P, Zuccato E, Kasprzyk-Hordern B (2018) Enantiomeric profiling of chiral illicit drugs in a pan-European study. Water Res 130:151–160
65. Gao J, Xu Z, Li X, O'Brien JW, Culshaw PN, Thomas KV, Tscharke BJ, Mueller JF, Thai PK (2018) Enantiomeric profiling of amphetamine and methamphetamine in wastewater: a 7-year study in regional and urban Queensland, Australia. Sci Total Environ 643:827–834

66. Sampat A, Lopatka M, Sjerps M, Vivo-Truyols G, Schoenmakers P, van Asten A (2016) Forensic potential of comprehensive two-dimensional gas chromatography. TrAC Trends Anal Chem 80:345–363
67. Alvarez-Rivera G, Bueno M, Ballesteros-Vivas D, Cifuentes A (2020) Chiral analysis in food science. TrAC Trends Anal Chem 123:115761
68. D'Orazio G, Fanali C, Asensio-Ramos M, Fanali S (2017) Chiral separations in food analysis. TrAC Trends Anal Chem 96:151–171
69. Fanali C, D'Orazio G, Gentili A, Fanali S (2019) Analysis of enantiomers in products of food interest. Molecules 24:1119
70. Vyviurska O, Koljančić N, Thai HA, Gorovenko R, Špánik I (2021) Classification of botrytized wines based on producing technology using flow-modulated comprehensive two-dimensional gas chromatography. Foods 10:876
71. Alamo-Sanza MD, Nevares I, Martínez-Gil A, Rubio-Bretón P, Garde-Cerdán T (2019) Impact of long bottle aging (10 years) on volatile composition of red wines micro-oxygenated with oak alternatives. LWT 101:395–403
72. Machyňáková A, Khvalbota L, Špánik I (2021) Enantiomer distribution of major chiral volatile organic compounds in botrytized grapes and wines. Eur Food Res Technol 247:2321–2331
73. Micalizzi G, Alibrando F, Vento F, Trovato E, Zoccali M, Guarnaccia P, Dugo P, Mondello L (2021) Development of a novel microwave distillation technique for the isolation of Cannabis sativa L. essential oil and gas chromatography analyses for the comprehensive characterization of terpenes and terpenoids, including their enantio-distribution. Molecules 26:1588
74. Chanotiya CS, Pragadheesh VS, Yadav A, Gupta P, Lal RK (2021) Cyclodextrin-based gas chromatography and GC/MS methods for determination of chiral pair constituents in mint essential oils. J Essent Oil Res 33:23–31
75. Genchi G (2017) An overview on d-amino acids. Amino Acids 49:1521–1533
76. Konya Y, Taniguchi M, Fukusaki E (2017) Novel high-throughput and widely-targeted liquid chromatography–time of flight mass spectrometry method for d-amino acids in foods. J Biosci Bioeng 123:126–133
77. Nakano Y, Taniguchi M, Fukusaki E (2019) High-sensitive liquid chromatography-tandem mass spectrometry-based chiral metabolic profiling focusing on amino acids and related metabolites. J Biosci Bioeng 127:520–527
78. Xu Y, Liu Z, Liu Z, Feng Z, Zhang L, Wan X, Yang X (2020) Identification of d-amino acids in tea leaves. Food Chem 317:126428
79. Tian H, Zheng N, Li S, Zhang Y, Zhao S, Wen F, Wang J (2017) Characterization of chiral amino acids from different milk origins using ultra-performance liquid chromatography coupled to ion-mobility mass spectrometry. Sci Rep 7:46289
80. Han D-Q, Yao Z-P (2020) Chiral mass spectrometry: an overview. TrAC Trends Anal Chem 123:115763
81. Wu Q, Wang J-Y, Han D-Q, Yao Z-P (2020) Recent advances in differentiation of isomers by ion mobility mass spectrometry. TrAC Trends Anal Chem 124:115801
82. Zheng H, Zhen X-T, Chen Y, Zhu S-C, Ye L-H, Yang S-W, Wang Q-Y, Cao J (2021) In situ antioxidation-assisted matrix solid-phase dispersion microextraction and discrimination of chiral flavonoids from citrus fruit via ion mobility quadrupole time-of-flight high-resolution mass spectrometry. Food Chem 343:128422
83. Shuang Y, Zhang T, Zhong H, Li L (2021) Simultaneous enantiomeric determination of multiple triazole fungicides in fruits and vegetables by chiral liquid chromatography/tandem mass spectrometry on a bridged bis(β-cyclodextrin)-bonded chiral stationary phase. Food Chem 345:128842

84. Zhao P, Wang Z, Gao X, Guo X, Zhao L (2019) Simultaneous enantioselective determination of 22 chiral pesticides in fruits and vegetables using chiral liquid chromatography coupled with tandem mass spectrometry. Food Chem 277:298–306
85. Yang F, Zhang X, Shao J, Xiong W, Ji Y, Liu S, Tang G, Deng H, Wang Y (2021) A rapid method for the simultaneous stereoselective determination of the triazole fungicides in tobacco by supercritical fluid chromatography-tandem mass spectrometry combined with pass-through cleanup. J Chromatogr A 1642:462040
86. Li S, Liu B, Xue M, Yu J, Guo X (2019) Enantioseparation and determination of flumequine enantiomers in multiple food matrices with chiral liquid chromatography coupled with tandem mass spectrometry. Chirality 31:968–978
87. Chen Z, Yao X, Dong F, Duan H, Shao X, Chen X, Yang T, Wang G, Zheng Y (2019) Ecological toxicity reduction of dinotefuran to honeybee: new perspective from an enantiomeric level. Environ Int 130:104854

Ambient Ionization Techniques in Food and Environmental Analysis

Markus Himmelsbach, Wolfgang Buchberger, and Christian W. Klampfl

Contents

Abstract The present chapter gives an overview on the use of ambient ionization techniques in mass spectrometry for analyzing environmental- and food-related samples. Ambient ionization techniques discussed include among others direct analysis in real time, desorption electrospray ionization, and rapid evaporative ionization. Developments within the field displayed in the literature published since 2015 are discussed. Strategies for improving sensitivity and selectivity by implementing sample pre-treatment devices into the ambient ionization source are presented, as are approaches to improve the ability for reliable quantitative analysis and further advancement in instrument design. Relevant applications published over the last 5 years are summarized and discussed.

Keywords Desorption electrospray ionization, Direct analysis in real time, Environmental analysis, Food analysis

M. Himmelsbach, W. Buchberger, and C. W. Klampfl (✉)
Institute of Analytical and General Chemistry, Linz, Austria
e-mail: markus.himmelsbach@jku.at; wolfgang.buchberger@jku.at; christian.klampfl@jku.at

Yolanda Picó and Julian Campo (eds.), *Mass Spectrometry in Food and Environmental Chemistry*, Hdb Env Chem (2023) 119: 275–310, DOI 10.1007/698_2022_885,

Published online: 20 October 2022

Abbreviations

AIMS	Ambient ionization mass spectrometry
ASAP	Atmospheric solid analysis probe
CBS	Coated blade spray
DAPCI	Desorption atmospheric pressure chemical ionization
DART	Direct analysis in real time
DBDI	Dielectric barrier discharge ionization
DCBI	Desorption corona beam ionization
DESI	Desorption electrospray ionization
EASI	Easy ambient sonic-spray ionization
EESI	Extractive electrospray ionization
FT-ICR	Fourier transform ion-cyclotron resonance
GC	Gas chromatography
HPLC	High performance liquid chromatography
HR-MS	High-resolution mass spectrometry
HRPS	High-resolution product scan
iEESI	Internal extractive electrospray ionization
ISTD	Internal standard
IT-MS	Ion trap mass spectrometer
LDTD	Laser diode thermal desorption
LIT-MS	Linear ion trap mass spectrometer
LTP	Low-temperature plasma ionization
MAII	Matrix-assisted inlet ionization
MIP	Molecularly imprinted polymer
MPT	Microwave plasma torch
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MSI	Mass spectrometry imaging
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PLS-DA	Partial least squares discriminant analysis
PS	Paper spray
Q-MS	Single quadrupole mass spectrometer
QQQ-MS	Triple quadrupole mass spectrometer
QTOF-MS	Quadrupole time-of-flight mass spectrometer
QuEChERS	Quick easy cheap efficient rugged safe
REIMS	Rapid evaporative ionization mass spectrometry
SBSE	Stir bar sorptive extraction
SPME	Solid-phase micro-extraction
TD-ESI	Thermal desorption electrospray ionization
TLC-MS	Thin-layer chromatography-spray mass spectrometry
TM-DART	Transmission mode direct analysis in real time

TOF-MS Time-of-flight mass spectrometer
WWTP Wastewater treatment plant;

1 Introduction

The development of desorption electrospray ionization (DESI) and direct analysis in real time (DART) in 2004 and 2005, respectively, substantially boosted the interest in ambient ionization techniques. This is reflected by the impressive number of research as well as review papers published on this topic in the following years (for exemplary review papers, see [1–4]). Additionally to these two techniques, which are also available in commercial instrumentation, paper or tissue spray [5] can be seen as the third important representative in this field, when judging the number of publications. Furthermore, there is a plethora of other ambient ionization techniques such as easy ambient sonic-spray ionization (EASI), dielectric barrier discharge ionization (DBDI), matrix-assisted inlet ionization (MAII), coated blade spray (CBS), or desorption atmospheric pressure chemical ionization (DAPCI) (just to name a few) playing a somewhat less dominant role in environmental as well as food analysis. Focusing on a possible categorization of the different techniques summarized under the term "ambient ionization" one can follow the excellent tutorial presented by group of Shiea in 2011 [6]. Thereby the three distinct groups are defined: (1) direct ionization methods, (2) direct ionization/desorption methods, and (3) methods based on a two-step ionization process. Based on this scheme, paper spray (PS) is part of the first group whereas techniques like DESI with a combined desorption and ionization mechanism or DBDI may be assigned to the second group. DART based on either the direct or the indirect (via ionization of solvent molecules with subsequent proton transfer) interaction of excited gas molecules with the analyte belongs to the third group of this scheme.

When browsing the relevant literature, it can be seen that food is one of the major fields of application of ambient ionization mass spectrometry (AIMS) techniques. This is reflected in the substantial number of review papers and book chapters published within the last decade focusing on this topic (for an exemplary selection, see [7–10]). On the other hand, the number of papers dealing with the employment of AIMS in environmental analysis is much lower (for a recent review, see [11]). An explanation for this asymmetry in the published literature may be found in the typical nature of samples best suited for analysis by ambient ionization techniques. In food analysis a straightforward examination of a sample "as it is" is in many cases sufficient to provide the information desired. Additionally, there is substantial interest in food analysis directly at the site of production – so AIMS seems more appropriate for examining this type of samples. A typical example for such a problem might be the question whether a certain pesticide can be detected on the surface of fruits or vegetables. Typical problems in environmental analysis require analysis at the trace or even ultra-trace level. Therefore, to comply with the

requirements regarding ultra-low limits of detection paired with the necessity to provide reliable and accurate quantitative data, sample pre-treatment procedures cannot be avoided. So, pre-concentration and/or clean-up steps are mandatory. Next to off-line pre-treatment, this challenge in many cases requires the use of separation techniques before MS analysis. This might be a possible explanation, why AIMS techniques are less frequently used in environmental analysis.

When comparing different AIMS techniques with respect to their frequency of use in both environmental and food analysis a clear trend can be detected. Searching the Web of Science for one particular technique, for example, in combination with food analysis, a preponderance of the most successfully commercialized AIMS technique, namely DART can be detected. Focusing on the time span since 2015, twice as many publications report the use of DART than that of DESI. Less prevalent commercially available solutions such as atmospheric solid analysis probe (ASAP) show frequencies comparable with those of techniques based on lab-made instrumentation such as DBDI or easy ambient sonic-spray ionization (EASI) just to name a few.

2 Applications of Ambient Ionization Techniques in Environmental Analysis

2.1 Direct Analysis in Real Time (DART) and Related Techniques

A major driving force for employing AIMS techniques is the desire to achieve analytical results faster and with less effort than encountered when using conventional approaches, often involving separation with chromatographic techniques, followed by MS detection. Particularly when large numbers of samples need to be categorized (e.g., as contaminated and non-contaminated) this can pose a substantial obstacle. Therefore, AIMS is perfectly suited for screening large sample sets particularly when there is no need for providing highly accurate quantitative analysis. The approach chosen in these cases is to avoid sample pre-treatment and pre-concentration almost completely and analyze samples as they are, resulting in extremely short analysis times. Unfortunately, in the field of environmental analysis, often just the advantage of fast measurement can be exploited as sensitivity issues do not allow to completely sidestep sample clean-up and pre-concentration. An early example for combining conventional sample pre-treatment with fast measurement using AIMS techniques was the analysis of organic UV filters from sunscreens in recreational waters published in 2010 [12]. Here DART-MS was employed in combination with pre-concentration using stir bar sorptive extraction (SBSE). Thereby limits of detection lower than 40 ngL^{-1} could be achieved for all eight analytes, making the developed method well suited for the screening of real water samples. On the other hand, when quantitative data were required, the method

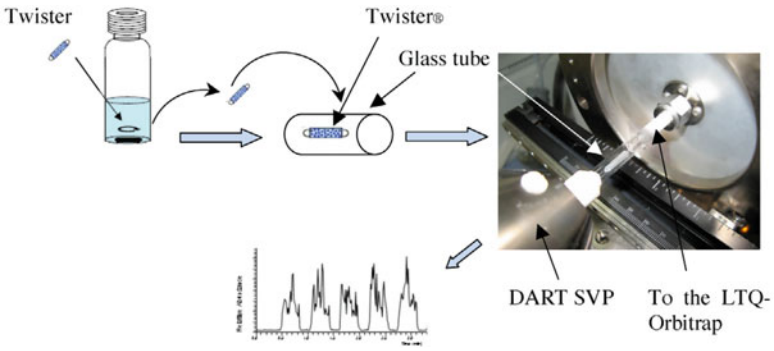


Fig. 1 Coupling of stir bar sorptive extraction (SBSE) with the DART Orbitrap mass analyzer. From [13] with permission

showed its limitations and could only provide semi-quantitative results, although an internal standard (ISTD) (benzyl cinnamate) was used. This was mainly due to fluctuations in signal intensity, something that could in part be attributed to the very basic design of the sample holder used in that work (although the original sample holder had already been replaced by an enhanced custom-made design). On the example of analyzing phosphoric acid esters in water samples Bridoux et al. demonstrated that these issues can be overcome. First, they employed more sophisticated deuterated ISTDs allowing an even better correction of fluctuations in peak intensities during measurement – a promising approach that of course might be hampered by the limited availability of suitable isotopically labeled substances. Second, an improved more reproducible positioning of the stir bars within the DART source (for details on the workflow, see Fig. 1) was achieved by improved instrumental design [13].

The approach just described, namely combining a sample pre-treatment procedure for analyte enrichment, and (to some extent also) clean-up with DART-MS was also followed in a series of more recent papers [14–16]. High-throughput analysis is one of those areas where DART can excel over other techniques – a fact making it well suited for large sample sets. This is also reflected in these papers focusing on the fast analysis of various trace pollutants in water after pre-concentration using sorbent-coated stainless steel bars [14], dispersive magnetic solid-phase extraction [15], and a solid-phase microextraction (SPME) fiber coated with a metal-organic framework [16]. In all these cases, correct positioning of the sample probes in the DART source was accomplished via an automatic sampler ensuring more reproducible results and allowing the consecutive analysis of large sample sets within a short time. Summing up this part it can be stated that a direct combination of DART with devices allowing a sample clean-up and a pre-concentration of the analytes is a promising approach for qualitative and probably also semi-quantitative analysis even at the ultra-trace level. Nevertheless, when accurate quantitative data are required an increased effort must be accepted as demonstrated by several works. This includes highly reproducible (mostly automatic) positioning of the sampling

device in front of the MS orifice and the use of appropriate ISTDs. The latter implies in most cases the need to employ isotopically labeled species of the actual analytes, a fact that might be seen as a substantial obstacle in terms of availability as well as costs.

According to its two-step ionization process, laser diode thermal desorption (LDTD) belongs to the same group of ambient ionization techniques as DART [6]. LDTD was employed by the lab of Roman Grabic for the analysis of pharmaceuticals in soil leachates [17] and in fish brain tissue [18]. Particularly the latter paper is of interest as the analysis of fish brain samples from animals subjected in vivo to environmentally relevant doses of citalopram, a drug commonly found in waste water treatment plant (WWTP) effluents, was reported. Thereby, the biological samples were directly introduced into the AIMS device, a procedure beneficial when only small amounts of sample (as was the cases in this study) are available. The described method allowed a quantitative estimation of the accumulation of this serotonin uptake inhibitor in brain tissue down to the low ng g^{-1} level [18]. For an overview of applications of AIMS in environmental analysis published since 2015, please see Table 1.

2.2 Other Ambient Ionization Techniques

After DART, DESI is the second most widely employed ambient ionization technique. One "unique selling point" of DESI is its ability to be used in an imaging mode allowing to spatially resolve the distribution of the selected analytes on surfaces. This has been demonstrated by the group of Spengler, who screened plant materials (plant stem, leaves) for the presence of systemic and contact pesticides [19]. Other direct desorption ionization techniques (according to the categorization by Shiea) with applications reported from the field of environmental analysis are DBDI [20, 21] and low-temperature plasma [LTP] ionization [22].

PS or tissue spray ionization is a direct ionization technique characterized by its simplicity and ease of operation. Application of PS for soil analysis was reported in several papers. In the first, Liu et al. described the analysis of tetrabromobisphenol in soil, whereby a strong focus was set on achieving accurate quantitative results [23]. The latter was accomplished by implementation of isotopically labeled standards that were added to the soil extracts allowing to correct for signal fluctuations (a strategy already discussed in the context of DART-MS). This resulted in a low relative standard deviation of 5.3% for five replicates at the μg L^{-1} level, and excellent spiking recoveries ranging from 90% to 101%, for analyzing ten soil and sediment samples [23].

Dowling et al. described an approach for the straightforward analysis of soil samples, leaving out any sample treatment steps. Target analytes in this study were hydrolysis products from chemical warfare agents [24]. Thereby, as can be seen from Fig. 2, soil was directly placed into a cartridge holding the paper for spraying followed by adding a combined extraction/spraying solvent.

Table 1 Applications of ambient ionization techniques in environmental analysis

Ambient ionization technique	Mass analyzer	Sample	Analyte(s)/application	Comment	Reference
DART	TOF	Environmental waters	Organic UV filters	Analytes were enriched on the surface of the polydimethylsiloxane-coated stir bars	[12]
DART	Orbitrap	Aqueous samples	Phosphoric acid alkyl esters and mono-butyl phosphate	Stir bar sorptive extraction and quantification using deuterated internal standards	[13]
DART	Orbitrap	Aqueous samples	Phthalic acid esters	Use of sorbent for pre-concentration and solvents for assisting desorption and ionization	[14]
DART	Orbitrap	Environmental waters	Triazine herbicides	Dispersive magnetic solid-phase extraction using magnetic graphene oxide	[15]
DART	Orbitrap	Environmental waters	Triazine herbicides	Use of a laboratory-made solid-phase microextraction device coated with a metal-organic framework fabricated for the on-site enrichment	[16]
LDTD-APCI	QQQ	Soil leachate	Carbamazepine, sulfamethoxazole, irbesartan, fexofenadine	LC-MS/MS and LDTD-APCI-MS/MS method comparison	[17]
LDTD-APCI	QQQ/ Orbitrap	Fish brain tissue	Citalopram	LC-HRPS method in comparison with LDTD-APCI-QqQ-MS/MS and LDTD-APCI-HRPS	[18]
DESI	Orbitrap	Plant stem and leaves	Pyrethrins, rapeseed oil, imidacloprid, methiocarb, dimethoate	Spatially resolved investigation of pesticides both on the surface of leaves and in cross sections of plant stem and leaves using DESI-MSI	[19]
DBDI	Orbitrap	Aqueous samples	Polycyclic aromatic hydrocarbons and polar trace contaminants	Coupling of an active capillary plasma ionization source based on a dielectric barrier discharge directly to solid-phase microextraction	[20]
DBDI	Orbitrap	Aqueous samples	Acenaphthene	The sample solution was nebulized followed by heating and converting into gas-phase analyte molecules prior to DBDI	[21]
MPT and LTP					[22]

(continued)

Table 1 (continued)

Ambient ionization technique	Mass analyzer	Sample	Analyte(s)/application	Comment	Reference
	LIT-TOF	Apple and grape peel extracts	Atrazine, chlorpyrifos, dimethoate, diphenylamine, imazalil, imidacloprid, isoprocarb, and triazophos	Instead of desorption sampling spray-inlet sampling was used	
PSI	LIT	Soils and sediments	Tetrabromobisphenol A	Quantification with a stable isotope internal standard	[23]
PSI	Orbitrap	Soil	Drugs and chemical warfare agent hydrolysis products	To improve quantitative performance an off-line salting out liquid–liquid extraction with internal standardization was also performed	[24]
PSI	LIT	Fish tank water	Non-conjugated steroids	Steroids released in fish water samples were confirmed via tandem mass spectrometry using collision-induced dissociation	[25]
SCWT-ESI	QQQ	Tap and river water	Fluoroquinolone and macrolide antibiotics	The SCWT solid-phase microextraction probe was prepared via silanization and sulfonation	[26]

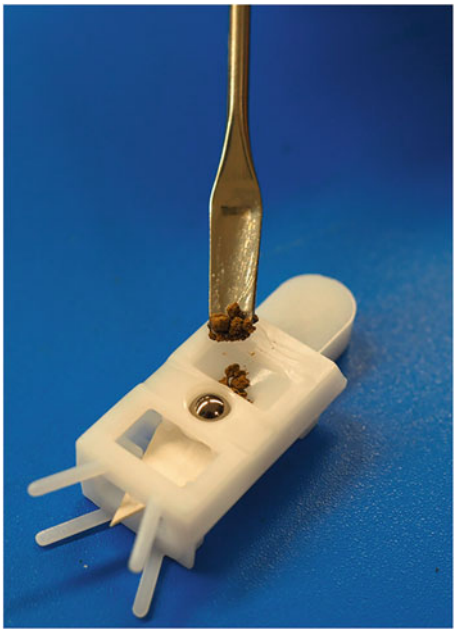


Fig. 2 Prosolia paper spray cartridge; solvent well filled with soil. From [24] with permission

Two further studies presented the use of PS [25], and the quite similar wooden-tip ESI for water analysis [26]. In the latter paper, the strategy of a multi-purpose use of the spraying-tip has been pursued. As can be seen from Fig. 3, wooden sticks were first surface - modified using a multi-step process to allow a selective enrichment of the chosen analyte classes. Subsequently, fluoroquinolones and macrolide antibiotics were extracted from a water sample using this device. Then, by adding a spray solvent and applying high-voltage, an electrospray was induced from the stick and MS analysis was performed. It should be mentioned that such an approach is not limited to wooden sticks but can also be achieved with any other suitable material.

3 Applications of Ambient Ionization Techniques in Food Analysis

3.1 Direct Analysis in Real Time (DART) and Related Techniques

Food analysis is one of the primary fields in analytical chemistry, consequently it also plays an important role when looking for applications of AIMS. The development of DART-MS, together with DESI, may be seen as a starting point for the prosperous development of AIMS techniques in a variety of fields. When searching for “desorption electrospray ionization” and “direct analysis in real time” in

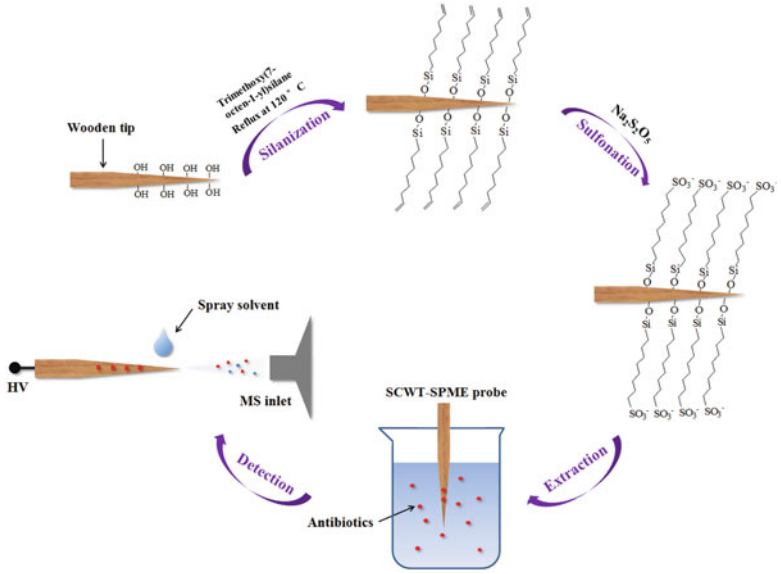


Fig. 3 Schematic diagrams for preparation of a surface-coated wooden-tip electrospray ionization probe and its application to chemical analysis. From [26] with permission

SciFinder, the first shows a distinctly higher number of entries (637 versus 279 – when considering the time span from 2015 till now). The situation is inverted when adding the term "food" to the search algorithm. Here DART-MS definitely plays a more important role (with 177 to 77 entries), revealing the extraordinary success of DART-MS in this field. Food samples have been analyzed with respect to their quality, safety, origin, and their authenticity (just to list some of the most prominent questions asked in the analysis of foods and related products) [27, 28]. For an overview on the use of DART-MS and related techniques in food analysis, see Table 2.

Starting with investigations on the instrumental setup, the importance of reproducibly positioning a sample holder in the DART source in front of the MS orifice was already addressed in the previous section. Now, when coming to food-related applications the question arises how to introduce the sample itself into the DART source – a parameter of utmost relevance when reproducibility of results is an issue. So, throughout the use of DART for a variety of different analytical problems substantial effort towards the development and optimization of sample introduction approaches has been taken. Two papers compared two of the most widely employed modes of sample introduction in DART-MS. This is the classical dip-it glass rod, on the one hand, a device available right from the time when the DART source was launched, and the more recently introduced transmission mode DART, where the sample is applied to a steel mesh [29, 30]. In the first paper, polyphenol profiling for categorization of extra virgin olive oils was done using DART-MS as well as a more conventional HPLC-MS approach. Thereby DART-MS provided comparable results

Table 2 Applications of ambient ionization techniques in food analysis – DART and related techniques

Ambient ionization technique	Mass analyzer	Sample	Analyte(s)/application	Comment	Reference
DART	Orbitrap	Olive oil	Polyphenols	Olive oil was dissolved in hexane and extracted using methanol–water	[29]
DART	QTRAP	Alcoholic drinks	Artificial sweeteners	Samples were only acidified, diluted, and filtered	[30]
DART	LIT	Ginkgo biloba kernels and leaves	Ginkgolic acids	Methanol extracts of Ginkgo biloba kernels or leaves were analyzed	[31]
DART	QQQ	Grape macerates	Linalool, 3-isobutyl-2-methoxypyrazine, 1-hexanol, and β-damascenone	Solid-phase mesh-enhanced sorption from headspace is used	[32]
DART	TOF	Powdered milk	Melamine	Using DART operated with argon gas in combination with acetylacetone and pyridine reagent gases allows to selectively ionize melamine and eliminate the interference from 5-Hydroxymethylfurfural	[33]
LDTD	QQQ	Beverages and liquid dietary supplement products	Caffeine	Samples were diluted with a mixture of methanol, water, and deuterated caffeine internal standard	[34]
DART	Orbitrap	Honey	Geographical origin discrimination	DART-MS combined with multivariate statistical analysis was used for the discrimination of two monofloral (chestnut and acacia) honeys for their geographical origins	[35]
DART	Q	Honey	5-Hydroxymethylfurfural	The influence of instrumental parameters on the composition of the DART mass spectra for 5-Hydroxymethylfurfural and partially degraded carbohydrates was investigated	[36]
DART	QTRAP	Honey	Chloramphenicol		[37]

(continued)

Table 2 (continued)

Ambient ionization technique	Mass analyzer	Sample	Analyte(s)/application	Comment	Reference
				The accuracy was evaluated by comparison with data generated by a validated HPLC-MS/MS method	
DART	Orbitrap	Salmon	Assessing fish authenticity	Discrimination between wildtype and farmed salmon using DART and multivariate analysis	[38]
DART	Orbitrap	Wheat varieties	Triticum species authentication	Chemometric evaluation revealed sample clustering according to the wheat species and the presence of 18 significant markers	[39]
DART	Orbitrap	Milk	Authentication of forage-based milk	25 informative biomarkers were identified to authenticate milk samples derived from maize silage, crop silage/hay, and grassland hay based diets	[40]
REIMS/ DART	TOF/ Orbitrap	Chicken breast meat	Determining poultry production systems	REIMS was compared with DART to assess whether either system was capable of correctly identifying poultry raised in one of six distinct production systems	[41]
DART	TOF	11 herbal teas	Evaluation of the quality of herbal teas	Mass spectra were evaluated by multidimensional chemometric methods, such as cluster analysis, linear discriminate analysis, and principal component analysis	[42]
DART	TOF	Cocoa powders and instant cocoa beverages	Evaluation of cocoa products quality and authenticity	Quantitative analysis of theobromine and caffeine was compared with those obtained from HPLC measurements	[43]
DART/ASAP	Q	Oregano	Authenticity screening of oregano	A set of authentic oregano samples and a wide range of adulterant species were investigated	[44]

DART	Orbitrap	Salmon lipid species	Assessing salmon freshness	The evolution of selected fatty acids and triacylglycerols during the refrigeration of salmon meat was monitored	[45]
DART	Orbitrap	Frying oil	Oxidized triacylglycerols	Various analytical techniques were applied to determine the impact of vacuum frying on quality of potato crisps and frying oil	[46]
MAII/ DAPCI/TM-DART/CBS	TOF/ Orbitrap/ Q/QQQ	Various food samples	Evaluation of ambient ionization techniques for portable mass spectrometry	Four different ambient ionization techniques were investigated for their potential in simplified testing of selected food contaminants such as pesticides, veterinary drugs, and natural toxins	[47]
DART	QQQ	Beef tissue	98 veterinary drugs	Sampling was done with solid-phase microextraction	[48]
DART	TOF	Baby formula	Five drugs of abuse	Comparison of a solid-phase microextraction DART-MS method to a traditional DART-MS method	[49]
DART	QQQ/ Orbitrap	Surface water, milk, grape juice, orange juice	19 pesticides	Sampling was done with the solid-phase micro-extraction−transmission mode technology	[50]
DART	QQQ/ TOF	Wine	31 pesticides	Comparison of different DART methods and different mass analyzers	[51]
DART	Orbitrap/ TOF	Lettuce and celery	Amitrole, cyromazine, propamocarb, melamine, diethanolamine, triethanolamine, and 1,2,4-triazole	A quick polar pesticides extraction (QuPPe) method was used and two accessories for sample introduction were evaluated	[52]
DART	LIT	Herbal and food samples	Six pyrrolizidine alkaloids	Comparison of DART-MS and HPLC-MS methods	[53]
DART	Orbitrap	Foods and surface waters	Cationic dyes	Two temperature steps were employed and results were compared to a HPLC-MS/MS method	[54]

but excelled (as might be expected) through much shorter analysis times [29]. The second study employed DART-MS for the screening of alcoholic drinks with respect to the presence of artificial sweeteners [30]. Coming to the outcome from comparing the two sample introduction modes, the two papers come to quite different results. This finding might be explainable by the quite diverse nature of the samples investigated. In the work on olive oil analysis (whereby oils represent analytes with high viscosities and high boiling points), transmission mode DART provided approximately two times higher signal intensities than the dip-it sampler [29]. In contrast, when analyzing sweeteners in beverages – samples primarily consisting of water – signals obtained with the steel mesh were small and affected by noise. So, in this case the dip-it approach was considered more suitable [30]. These controversial findings might be understood in a way, that (as for many real-world problems) there is no universally valid suggestion for the use of one or the other sample introduction mode. In fact, for finding the best possible solution both the nature of the sample matrix and that of the analytes therein need to be considered.

Focusing on the quantitation of plant ingredients by DART-MS, two recent studies should be highlighted. Food-related plants investigated were *Ginkgo biloba* [31] and grapes [32]. In both cases, quantitative results obtained by DART-MS were compared to data from GC measurements, whereby no perfect agreement between the two methods could be observed. In the first study, the problem might be more associated with the GC measurement as this was burdened by the use of a simple flame-ionization detector combined with a sub-optimal separation of the analytes. So, overlapping peaks for some of the ginkgolic acids (e.g., 17:1 and 17:2) might be responsible for a bias in quantitative GC measurements. In the second study, GC was used in combination with a highly selective MS/MS detector, avoiding problems encountered in the work discussed above. But, plotting the results from the quantitative determination of linalool in grapes obtained by DART-MS versus those by GC-MS/MS led to a slope of 0.16 – a result substantially deviating from the theoretical value of one. Here the most probable explanation for this divergence might be the presence of isobaric interferences, not resolved by the single-stage MS used in combination with DART. This can be regarded as a principal drawback of any direct MS method – as in such cases separation of interfering substances (e.g., by GC or HPLC) before detection is mandatory, particularly when either multi-stage MS is not available or not capable of resolving these issues. Nevertheless, the unique ionization mechanism in DART can also be turned into a powerful tool for distinguishing isobaric (or almost isobaric) compounds, when these substances show a sufficiently different behavior in the DART source. A notable example for such a strategy is the analysis of melamine in the presence of 5-hydroxymethylfurfural (a problem related to the analysis of contaminated dairy products) by DART-MS. Here, as can be seen from Fig. 4, the required selectivity could be achieved by choosing an alternative gas for ionization (argon instead of the more common helium) [33].

Food ingredients were also analyzed by other AIMS techniques with an ionization mechanism belonging to the same category as DART, such as laser diode thermal desorption (LDTD) MS. Andersen et al. reported the determination of

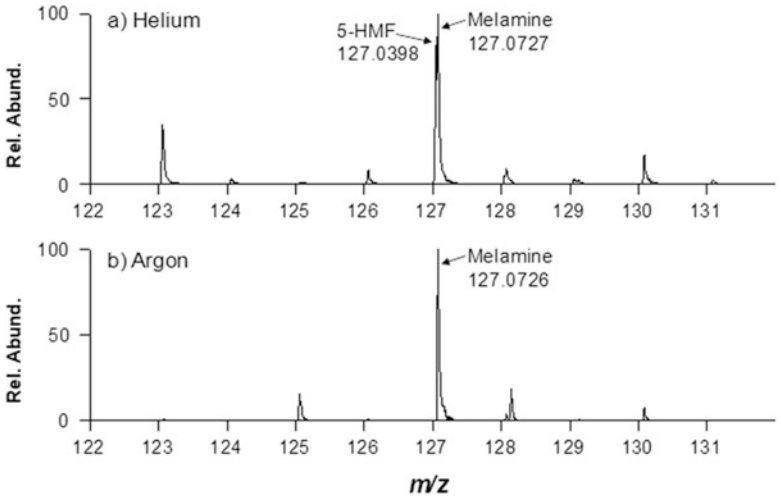


Fig. 4 The helium DART mass spectrum for 10 ppm of melamine in powdered milk (**a**) and the argon DART mass spectrum for the same sample (**b**) over the same sampling time interval. From [33] with permission

caffeine in beverages, whereby the developed procedure allowed the quantitation of caffeine in a range of different samples [34]. A comparison of the results with the official method from AOAC (using HPLC) showed differences of <4%. A potential reason for this good agreement might be the use of caffeine-d_3 as internal standard (ISTD) in LDTD MS.

Switching to investigations on food authenticity and origin, DART-MS again plays an important role [27]. A typical example for such an application is the discrimination of monofloral honeys with respect to geographical origin by DART coupled to high-resolution (HR) MS [35]. As distinction between samples in food authenticity and origin studies is rarely possible by evaluating a handful of specific markers, also in this case the obtained MS spectra had to be evaluated using chemometric tools. Thereby testing three supervised statistical approaches (linear discriminant analysis, partial least squares discriminant analysis (PLS-DA), and k-nearest neighbors) combined with the high spectral resolution of HR-MS allowed to determine the origin of chestnut and acacia honey with sufficient certainty. The use of DART for honey analysis was also reported by other groups [36]. One possible reason might be the high sugar content of this natural product, requiring extensive sample preparation when using chromatographic techniques. On the other hand, when using DART for honey analysis, reaction products from sugars formed at the elevated temperature present in the ion source need to be considered as they may substantially complicate the evaluation of the spectra obtained. Furthermore, DART-MS was employed for the analysis of chloramphenicol in honey [37].

Particularly when using direct MS methods in combination with HR-MS instruments, data processing is an important issue due to the complex spectra and the large

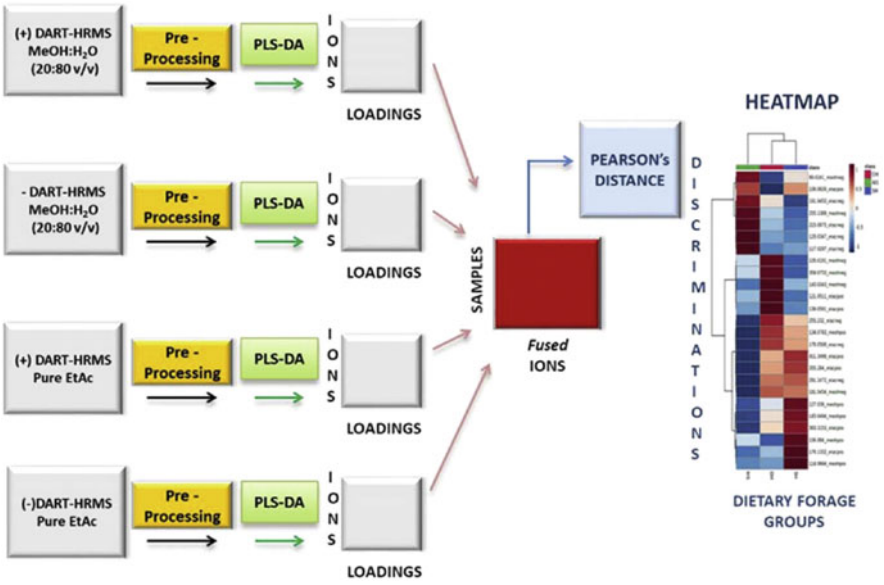


Fig. 5 Flowchart of the detection of the most informative ions from a sample. After pre-processing and Pareto scaling normalization (yellow boxes), preliminary PLS-DA was performed (green boxes); the ions having a loading score > 0.3 (absolute value) were selected (gray boxes) and submitted to mid-level data fusion (red box). Hierarchical cluster analysis (HCA) was performed on the fused ions by the Pearson correlation distance (1 – absolute Pearson correlation coefficient) (blue box). A correlation matrix was generated between the 25 selected ions and the dietary roughage group (heatmap) providing also a clusterization of forage types (among columns) and selected ions (among rows). Red (positive) and blue (negative) color intensities indicate the degree of correlation. From [40] with permission

amount of signals therein. So, also in the case of DART-HR-MS the use of chemometric tools for evaluating the spectra obtained may be beneficial. This combination was employed, for example, for assessing fish authenticity [38], authentication of *Triticum* (a traditional type of wheat) species [39], forage-based milk authentication [40], and for investigating poultry production system history [41]. Fatty acid profiles can be helpful for distinguishing different types of meat. Wild type salmon and animals raised in fish farms were investigated by Fiorino et al. whereby they selected an analyte set comprising of 30 fatty acids (from C_8 to C_{22}) [38]. Signals obtained for these compounds in DART-HR-MS spectra were further evaluated and subjected to principal component analysis (PCA). This procedure allowed the unambiguous differentiation between wild type fish from Canada and farmed salmon from Canada, Norway, and Chile.

Focusing on the study devoted to forage-based milk authentication, DART-HR-MS spectra were recorded from milk samples [40]. The workflow conducted within this study can be seen from Fig. 5. First, two different solvents, a polar and a non-polar one were employed for extraction of the milk. Subsequently, spectra were recorded from the extracts in the positive and in the negative mode to get a

comprehensive overview of components present in these samples. After pre-processing (see yellow boxes) the preliminary PLS data analysis was performed, and a set of fused ions was obtained. Finally, hierarchical cluster analysis (see heatmap on the right) correlating dietary forage groups with the 25 selected ions (from runs with positive and negative ionization) was performed. This strategy allowed differentiating between cow-milks based on the type of feed provided for the animals.

The groups of Hajslova and Elliott [41] compared DART and rapid evaporative ionization MS (REIMS) (applications of REIMS are discussed in more detail in the next section) for differentiating poultry meat. Chicken were sampled from six distinctly different production systems. The ability of REIMS to categorize birds according to the type of feed employed was between 60% and 100%, which was somewhat less than DART, where 100% of the birds were assigned correctly. But, whereas REIMS allowed a direct analysis of the meat samples, some sample preparation was needed for DART-MS measurements – a fact that substantially influenced sample throughput. Steps in DART-MS analysis included meat homogenization and extraction with polar and non-polar solvents; subsequently the two extracts were investigated separately.

Whereas the studies described above involved the use of DART in combination with state-of-the-art HR-MS (Orbitrap) instruments, authenticity studies can also be performed employing MS instruments with somewhat lower spectral resolution, such as common time-of-flight (TOF) or quadrupole time-of-flight (QTOF) spectrometers [42, 43] or even simple quadrupole MS instruments [44]. Particularly the latter study might trigger curiousness as the combination of ambient ionization with low resolution MS is rarely employed. This is understandable as in direct MS all ionizable substances (all analytes and matrix components) will enter the MS at the same time. So, substance discrimination and subsequently (tentative) identification totally relies on the capability of the MS instrument to resolve a maximum of m/z ratios, a qualification commonly strongly associated with mass resolution. This is of particular interest, as that study used a non-targeted approach for detecting fraudulent blending of origanum with olive leaves [44]. In more detail, that study presented the critical evaluation of two AIMS techniques, DART-MS and ASAP-MS revealing distinct advantages for the latter one. One main reason was the fact that for the chosen analytical problem ASAP-MS provided reproducible and diagnostic feature-rich spectra, a fact of distinct importance when employing a non-targeted approach for analysis. One main deficit of the DART source, observed in that study, was its basic layout as a source that is open to the environment. Thereby, a substantial influence of the surrounding atmosphere on the DART-MS spectra was detected. In some cases these signals even interfered with analyte-specific features [44]. This is clearly reflected in Fig. 6, depicting scatter plots for PC1 versus PC2 scores for ten technical replicates of ten oregano samples. Without background subtraction, no acceptable clustering of the sample spectra could be achieved (see left plot). Although the situation could be greatly enhanced by measuring background spectra that were subsequently subtracted from each sample spectrum (result, see right plot), ASAP-MS spectra still excelled over DART when considering the differentiation

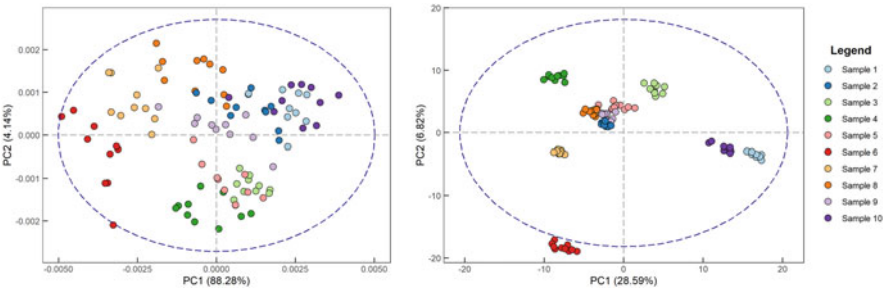


Fig. 6 Scatter plot of PC1 vs PC2 scores obtained from ten technical replicates of ten analyzed oregano samples by applying mathematical pre-processing only (left) or mathematical pre-processing and background subtraction (right). Each sample is marked with a different color. Confidence ellipses (95%) are reported as dashed blue lines. From [44] with permission

between pure and fraudulently blended origanum samples. A further reason for the better performance of the ASAP source might be that ASAP, as an ion source with a closed compartment, resulted in a higher percentage of ions entering the MS instrument, leading to spectra with higher signal intensities – a fact further facilitating the categorization of the investigated samples.

Despite not dealing with authenticity in the strict sense, two more papers might also fit to this group. Fiorino et al. employed DART-MS as a rapid tool for assessing fish freshness [45]. Aim of this study was the influence of storage conditions and period on the concentrations of a number of target analytes. The authors revealed that after 4 days of refrigeration the amount of fatty acids had increased while a reduction of triacylglycerols was observed. The second study investigated potato crisps and the respective frying oils, whereby the main focus was set on a comparison of production processes employed [46]. Thereby DART-MS was used for analyzing the processed potatoes with respect to the formation of acrylamide and the corresponding frying oil for oxidation products after a conventional frying process (165° for 105–135 s) and a low-temperature vacuum frying process (125° for 180–360 s). As might be expected, chemical analysis revealed advantages for the vacuum frying technology, but organoleptic analysis by a panel resulted in advantages for the conventionally produced crisps.

On-site testing is a dominant trend in chemical analysis, a fact that is also reflected when it comes to investigating food and feed. Up to now mainly spectroscopic techniques, assays, or biosensors were used for this purpose (for exemplary review papers, see [55, 56]). Ambient ionization, particularly when combined with a portable MS instrument, may pave the way for MS techniques into this field. Blokland et al. investigated a range of AIMS techniques such as transmission DART, matrix-assisted inlet ionization (MAII), coated blade spray (CBS), and desorption atmospheric pressure chemical ionization (DAPCI) combined with a portable MS system for their suitability in food analysis [47]. Thereby DART and CBS excelled over DAPCI and MAII with respect to sensitivity and subsequently robustness at the concentration level of interest for on-site measurements

[47]. Nevertheless, the study also revealed a more fundamental problem (already discussed above) of the combination tested. Up to date, portable MS instruments are mostly relatively simple devices, neither offering multi-stage MS capabilities nor high resolution. As ambient ionization techniques are direct MS techniques, i.e. everything ionized is transferred into the MS, unambiguous identification of substances is often hampered by these shortcomings. Unfortunately, this fact somewhat diminishes the applicability of the proposed combination with respect to actual on-site analysis of food samples unless more powerful portable MS instruments become available.

Advantages and caveats of combining SPME with DART-MS have already been discussed in the section on environmental analysis. This strategy has, to achieve cleaner samples and to enrich the analytes of interest, also been adopted to food analysis. Typical examples are the determination of pharmaceutical drugs in bovine tissue [48] and that of drugs of abuse in infant formula [49]. The group of Pawliszyn presented a further development of SPME fibers used for sample introduction into DART in 2017 [50]. They modified a mesh employed for transmission DART by coating it with adsorbent particles. In this way, SPME extraction could be easily combined with transmission mode DART-MS. The advantages of this approach were demonstrated by analyzing a range of pesticides in food and environmental matrices, whereby the SPME-mesh led to limits of detection in the low μg L^{-1} range and linearity over almost three orders of magnitude even when complex matrices (such as sugar rich juices) were analyzed. The SPME-mesh allows improving sensitivity and provides cleaner extracts without sacrificing speed. A further advantage of this device is that it can be stored at low temperatures for months allowing retrospective analysis [50].

Yong et al. followed a different approach, namely the direct analysis of wine without any sample pre-treatment [51]. They presented the simultaneous detection of 31 pesticides in wine by DART-MS/MS using a simple customized steel mesh allowing the introduction of a larger sample volume. Thereby sufficient sensitivity was achieved when employing the QQQ in the multiple reaction monitoring (MRM) mode. Repeating the experiment with the same equipment but introducing a preceding QuEChERS extraction step did not improve results while leading to much longer overall analysis times.

Analysis of highly polar pesticides is a quite problematic issue, not only in environmental but also in food samples. Direct (i.e., without derivatization) analysis with GC is often not possible and conventional reversed-phase HPLC methods struggle to resolve the analyte peaks from matrix eluting with the void volume. For this reason DART-MS might be a promising alternative. Based on this assumption, Lara et al. investigated the applicability of combining a quick polar pesticide extraction method with DART coupled either to a time-of-flight (TOF) or an Orbitrap MS for the analysis of these substances in lettuce and celery [52]. By this combination good detection limits (in the μg/kg range) and recovery rates (70–115%) were achieved. Further work reporting the use of DART-MS includes the determination of pyrrolizidine alkaloids (plant toxins) in herbs and food samples [53] and the detection of illegal cationic dyes in various foods [54].

3.2 Techniques Based on Desorption Atmospheric Pressure Chemical Ionization (DAPCI)

Desorption atmospheric pressure chemical ionization (DAPCI), involving corona discharge, is an AIMS method whereby molecules emitted from a sample surface upon impact of a heated gas react with reagent species generated by an atmospheric pressure corona discharge [6]. For an overview on the use of techniques based on a DAPCI process in food analysis, see Table 3.

This technique was employed for evaluating the authenticity of scotch whiskies by Smith et al. [57] and for probing the surface of oranges by Zhang et al. [58]. The latter study compared several AIMS techniques for analyzing the tissue of navel oranges [58]. Part of the comparison were DAPCI (heated and without heating), microwave plasma torch (MPT), and extractive electrospray ionization (EESI). In a similar way as expected for the "conventional" ionization techniques, DAPCI excelled for the less polar components, whereas the ESI-like EESI was better suited for more polar analytes. One outcome of the investigation was a list of substances detected at the surface of the fruits providing information on the performance (signal intensities) of the selected AIMS techniques.

Rapid evaporative ionization mass spectrometry (REIMS) was introduced by the group of Takats in 2009 by interfacing an electrosurgical unit (knife) with an MS instrument [67]. This new AIMS technique was primarily designed for use in medicine, but soon its benefit also for food analysis became obvious. One special feature of this technique is that it also offers the possibility to selectively probe certain spots on a sample surface, thereby allowing some sort of an "imaging" approach. Most investigations in the field of food analysis employing REIMS technology are either directed towards categorizing with respect to quality issues [59–62], or more focused on authenticity [63–66]. Starting with the first group, REIMS was mostly employed for testing different meat products. Using an untargeted approach for analysis, Verplanken et al. managed to identify boar taint (an off-flavor typical for non-castrated male pigs) in 150 samples of fat tissue [59]. Closer inspection of the data revealed that fatty acid and phospholipid profiles provided the highest amount of information suitable for categorization of the meat samples. Combining REIMS with machine learning, Gredell et al. were able to predict meat quality attributes such as tenderness, breed type, or carcass type [60]. Addition of cheap offal products to minced beef is a common strategy in food fraud. REIMS allowed the fast and unambiguous identification of adulterated minced meat containing 1–10% of offals [61]. REIMS can also be used for evaluating food processing methods. Song et al. investigated prawn processed using the more and more common air-frying technique [62]. Thereby a clear connection between frying temperature and the amount of lipid oxidation was revealed. Coming to investigations on food authenticity, two recently published research papers on discriminating fish species based on REIMS measurement should be considered [63, 65]. Main goal of both studies was the detection of mislabeling whereby Song et al. focused on salmon and rainbow trout [65]. Black et al. compared REIMS with

Table 3 Applications of ambient ionization techniques in food analysis – Techniques based on desorption atmospheric pressure chemical ionization

Ambient ionization technique	Mass analyzer	Sample	Analyte(s)/ application	Comment	Reference
DAPCI	QQQ	Scotch whisky	Fingerprinting of authentic and counterfeit samples	Principal component analysis has been applied to dimensionally reduce the data and discriminate between sample groups	[57]
DAPCI/ MPT/ iEESI	LIT	Oranges	Comparative screening analysis	Comparative analysis of navel orange tissue samples with different ambient ionization methods	[58]
REIMS	TOF	Pig neck fat	Boar taint	Classification of tainted and untainted boar samples was achieved	[59]
REIMS	TOF	Beef striploin sections	Quality predictions	Eight different machine learning algorithms were compared to generate predictive models using REIMS data to classify beef quality attributes	[60]
REIMS	TOF	Minced beef samples	Identification of offals within minced beef samples	Offal identifications were obtained for both raw and boiled adulterated samples	[61]
REIMS	TOF	Prawns	Lipid oxidation	The effect of air-frying on the lipid oxidation in prawn at different temperatures was investigated	[62]
REIMS	TOF	Five different white fish species	Metabolomic profiling	478 samples were investigated to detect fish fraud	[63]
REIMS	TOF	Pistachio	Authenticity assessment	Differentiation of pistachio nuts coming from different geographical origins	[64]
REIMS	TOF	Salmon and rainbow trout	Discrimination of fish samples	Lipidomics study for real-time discrimination of salmon and rainbow trout without sample preparation	[65]
REIMS	TOF	Honey	Identification of botanic origin	Classification of honey samples was achieved through principal component analysis-linear discriminant analysis	[66]

polymerase chain reaction (PCR) for fish species identification, testing almost 480 fish samples with five different types of white fish [63]. Thereby REIMS excelled with results comparable to those from PCR, but achieved within a substantially reduced time span (15–20 min versus 24 h). In addition, REIMS has also been successfully employed for authenticity assessment of pistachio [64] and determining the botanical origin of honey [66].

3.3 Paperspray (PS) Ionization and Related Techniques

The group of Ouyang first reported PS ionization in 2010 [68]. Due to its simplicity, it was soon considered as an additional useful technique within the group of AIMS methods [2]. Throughout the years the basic idea of PS was further modified, thereby implementing additional features and leading to PS-related ionization techniques such as thin-layer chromatography-spray mass spectrometry (TLC-MS) [69], thread spray MS [70], spraying from carbon fibers [71], wooden tips [72], molecularly imprinted polymers [72–74], or steel blades [75, 76] (just to name few). All those techniques were also employed in the analysis of foods and beverages. For an overview on the use of these techniques in food analysis, see Table 4.

Starting with conventional PS-MS, the straightforwardness of this method paired with the fact that (chromatography) paper is a cheap and easily accessible material (that can be disposed after single use), made this technique well suited for the analysis of "dirty" samples or for samples where other approaches would have required at least some basic sample pre-treatment (e.g., in order to protect the instrumentation). A convincing example for that is the high-throughput analysis of vitamin E in olive oil, whereby the oil was simply diluted with acetone and directly applied to the paper triangle for spraying [77]. Furthermore, PS-MS was successfully employed for the analysis of relevant ingredients in a range of food and beverage samples. Pepper was investigated with respect to pungency by an approach called imprint PS-MS [78]. Thereby, for direct analyte transfer, the freshly cut pepper was pressed for a fixed time onto triangular paper that was subsequently used for spraying. This allowed evaluating the capsaicinoid ion intensity for a series of peppers, whereby the results agreed well with values of pungency from the Scoville scale. PS coupled to a triple quadrupole (QQQ) MS for improved selectivity was also employed for analyzing resveratrol in wine [79], tyrosol and hydroxytyrosol in extra virgin olive oil [80] whereby, employment of labeled standards and isotope dilution allowed successful quantitation of the selected analytes. Further examples for PS-MS in food analysis are the detection of methylxanthines in cocoa products [81], and that of ginkgo flavonoids [82]. Besides these works focusing on the analysis of selected food ingredients (or groups thereof), studies have been devoted to the determination of pesticides [83, 84], preservatives [85], illegal dyes [86], and antimicrobial residues [87] in foods and beverages employing PS-MS. Combining chemometric tools with PS-MS analysis is a promising approach for studying the authenticity of food- and beverage-related products. Groups from Brazil published

Table 4 Applications of ambient ionization techniques in food analysis – Paper spray ionization and related techniques

Ambient ionization technique	Mass analyzer	Sample	Analyte(s)/application	Comment	Reference
TLC spray ionization	TOF	TLC separations/ sunscreens	Reaction products/UV filters	A direct MS method to analyze spots from TLC plates with aluminum foil backing without the need of an external ion source or devices for analyte transfer from TLC to MS	[69]
Thread spray ionization	LIT/ Orbitrap	Pepper products	Capsaicinoids	Method for sampling the interior of pepper fruits and the analysis of pepper spray residues on clothing	[70]
CFI	TOF	Tomatoes	Pesticides	Qualitative and quantitative analysis of trace pesticide residues from the surface of intact tomatoes	[71]
PSI with MIP	Orbitrap	Apple, banana, and grape methanolic extracts	Diuron and 2,4-dichlorophenoxyacetic acid	MIP was synthesized directly on a cellulose membrane	[73]
MIPCS	QTRAP	Milk	Fluoroquinolone antibiotics	Molecularly imprinted polymer-coated stainless steel sheets were used as ESI emitter	[74]
MIPCWT	FT-ICR	Milk, honey, and water	Macrolide antibiotics	Solid-phase microextraction via the modification of a layer MIP coating on the surface of wooden tips	[72]
CBS	QQQ	Apple, blueberry, grape, and strawberry	Pesticides	Analysis was done through direct-coupling with MS and liquid chromatographic analyses	[75]
CBS	Q/QQQ	Milk	Sulfonamide antibiotics	USB-powered coated blade spray ion source for on-site testing	[76]
PSI	QQQ	Olive oil	Vitamin E	Oil samples were also analyzed with a classical HPLC-FLD method	[77]
PSI	IT	Peppers	Pungency determination	Pepper fruit was pressed onto a triangular paper to imprint and transfer the pepper constituents onto the paper surface	[78]
PSI	QQQ	Red wine	Resveratrol	Quantitative method based on isotope dilution	[79]
PSI	QQQ	Olive oil	Hydroxytyrosol and tyrosol		[80]

(continued)

Table 4 (continued)

Ambient ionization technique	Mass analyzer	Sample	Analyte(s)/application	Comment	Reference
				The free form of tyrosol and hydroxytyrosol and their ester conjugates were assessed	
PSI	QQQ	Cocoa products	Theobromine, theophylline, and caffeine	Samples have been analyzed both by the developed methodology and by using a classical HPLC-UV approach	[81]
PSI	Q	Powder of ginkgo biloba preparations	Ginkgo flavonoids	Paper spray mass spectrometry assisted by microwave-assisted hydrolysis	[82]
PSI	QQQ	Wine	36 pesticides	An off-line QuEChERS treatment and on-line paper adsorption treatment were used	[83]
PSI	IT	Tomatoes	Acephate, chlorpyrifos, and cyazofamid	Pesticide concentrations during pre-harvest intervals were investigated and the fruits were separated into field and stored groups	[84]
PSI	Q	Beverages	Benzoic acid and vitamin C	Deuterated internal standard was used and results were compared to a HPLC method	[85]
PSI	QQQ	Beverages	Six illegal colorants	Samples were diluted in methanol and isotopically-labeled internal standards were used for quantification	[86]
PSI	LIT	Beef, chicken meat, milk, and egg	Nine antimicrobials	Limits of detections were in the μg/kg range	[87]
PSI	IT	Scottish whiskies	Identification of counterfeit products	PSI combined with partial least square discriminant analysis was applied to discriminate authentic and counterfeit samples	[88]
PSI	FT-ICR	Whisky	Quantification of sugarcane spirit addition	19 binary mixtures were analyzed and evaluated by chemometric models	[89]

two papers dealing with investigations on the adulteration of whisky by blending [88, 89]. Focusing on the latter paper [89], the proposed approach is rather straightforward, as solely spectra of untreated liquid samples are measured in positive and negative ionization mode, which are subsequently processed using chemometric methods. Coupling ultra-high-resolution MS (employing a Fourier-transform ion-cyclotron MS instrument) with PS allows highly accurate mass determination, and spectral resolution even of ions with small differences in m/z ratios, a fact that is of substantial significance when measuring spectra from samples directly without any preliminary treatment. This combination and using a multivariate calibration model succeeded in determining the amount of sugar cane spirit added to a series of whisky samples with high accuracy [89].

As already mentioned above, the principle of PS has been further refined by introducing a series of other spraying media, some of them adding further functionalities such as sample pre-concentration, sample cleaning, or rudimental separation of sample constituents [72–76]. An example for such development are spraying devices based on molecularly imprinted polymers (MIPs) [72–74]. Thereby devices including MIPs were first employed to extract agrochemicals from foodstuff [73], fluoroquinolone antibiotics from milk [74], and macrolide antibiotics from milk and honey [72], and subsequently for generating a spray introducing the sample-ions into the MS instrument. In one later paper, coating a wooden tip (subsequently employed for spraying) with a MIP moiety provided enrichment factors between ten (erythromycin from milk) and more than 1,500 (roxithromycin from water) [72].

Coated blade spray (CBS) was first reported by the group of Pawliszyn in 2014 [90]. Coating a metal blade with an appropriate SPME material allows the use of a single device for extracting the analyte from a matrix (ideally providing a cleaning and a pre-concentration effect), and subsequently spraying it into the MS. This was demonstrated on the example of multiresidue pesticide quantitation in a range of fruit matrices by Kasperkiewicz and Pawliszyn [75]. They presented a device particularly suitable for screening large sample sets (one of the major domains of AIMS techniques) allowing the handling of up to 96 samples at a time. Figure 7 provides an overview of the different steps included in the developed workflow.

A major goal pursued by many developments in AIMS is to bring instrumental analysis closer to the sample, i.e. to the field, allowing analyzing food directly at the site of cultivation. An important step towards the realization of this objective has been achieved by Jager et al. when presenting a coated blade spray (CBS) device where the voltage needed for spraying is solely provided by a micro USB connection [76]. Although (unfortunately) not presented in that study, combining USB-powered CBS with a fieldable MS instrument (for possible instruments, see, for example, [91]) would be an excellent solution for real on-site analysis.

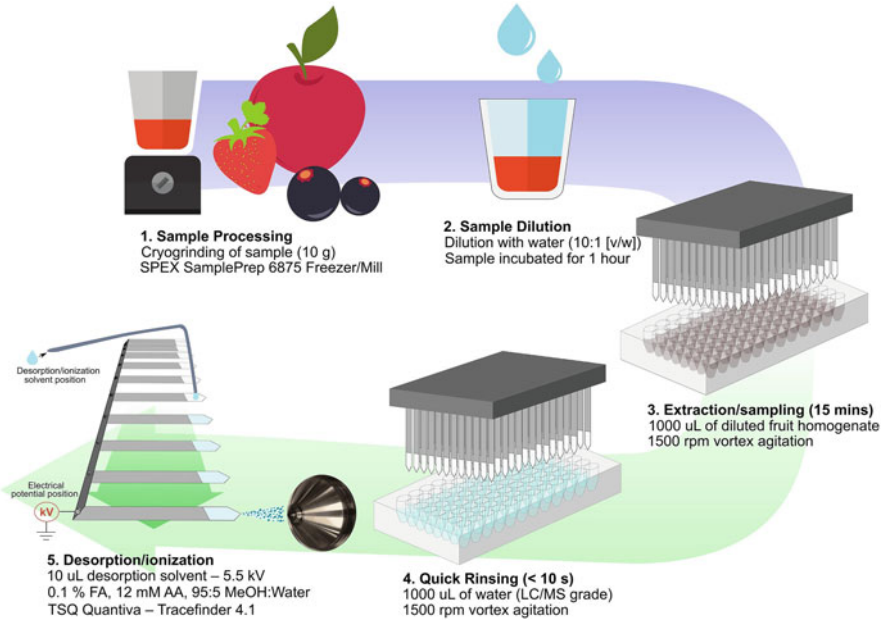


Fig. 7 Workflow for the analysis of pesticides in fruit matrices by coated blade spray MS/MS. From [75] with permission

3.4 Desorption Electrospray Ionization (DESI) and Related Techniques

DESI [92, 93] and techniques with a related ionization mechanism such as desorption corona beam ionization (DCBI) [94], EASI [95], LTP [96], and DBDI [97] have successfully been employed in the analysis of food. As already stated in Sect. 2.2, a major asset of DESI is the possibility to scan surfaces and thereby create an image depicting the spatial distribution of selected analytes. For an overview on the use of DESI and related techniques in food analysis, see Table 5.

Mainero Rocca et al. [92] employed DESI for probing the surfaces of vine and olive leaves for the presence of pesticides. Thereby they did not take advantage of the "imaging" abilities of DESI (provided a x,y,z-movable stage for sample positioning is available) but punched out certain areas of the leaves. The group of Spengler investigated the possibility to use DESI for qualitative and (at least semi-) quantitative analysis of pesticides on fruit surfaces [93]. The developed method was seen as a useful tool for fast (qualitative) screening of large sample sets as quantitative analysis (despite using an isotopically labeled ISTD) suffered from a high scatter of calibration curves when compared to routine methods. From the results presented in these works it can be seen that direct quantitation by DESI from real samples is still affected with a series of problems. A fundamental shortcoming of

Table 5 Applications of ambient ionization techniques in food analysis – DESI and related techniques

Ambient ionization technique	Mass analyzer	Sample	Analyte(s)/ application	Comment	Reference
DESI	QTRAP	Olive and vine leaves	Dimethoate, tebuconazole, and trifloxystrobin	Workers exposure assessment during field re-entry operations since evidence suggests an association between chronic occupational exposure to some agrochemicals and severe adverse effects	[92]
DESI	Orbitrap	Fruit surfaces and extracts	32 pesticides	Quantification of compounds was carried out from QuEChERS extracts and food surfaces using only one isotope-labeled ISTD for normalization	[93]
DCBI	IT	Pork and fish	10 antimicrobials	Analytes were detected in the μg/kg range	[94]
EASI	Orbitrap	Salmon species	Triacylglycerols	Investigations on the impact of the raising regime of salmon species on their triacylglycerol composition	[95]
LTP	LCQ	Tequila and mezcal	Chemical fingerprinting	Method permits the discrimination between tequila and mezcal products	[96]
DBDI	Orbitrap	Grape juice	24 pesticides	Solid-phase microextraction was used for sample preparation	[97]
LEP	Portable mass spectrometer Mini 11	Pepper peel, fish, and coffee	Pesticides, plasticizers, and drugs	Food authentication via metabolomic fingerprinting and multivariate statistics is demonstrated for the analysis of fish fillets and coffee	[98]
TD-ESI	QQQ	Processed vegetables	Basic colorants	Detecting adulterants in processed vegetables	[99]
TD-CFI	QQQ		Capsaicin		[100]

(continued)

Table 5 (continued)

Ambient ionization technique	Mass analyzer	Sample	Analyte(s)/ application	Comment	Reference
		Ethanolic extracts of cookies, chips, and oil		A metal ceramics heater was used for analyte desorption	
TD-ESI	QQQ	Fruits and vegetables	308 pesticides	The residual pesticides on fruits and vegetables were collected by sweeping a metallic probe across the sample surface	[101]
EESI	Orbitrap	Nectars and honeys from citrus	12 polyphenols and 8 amino acids	Nectar and its corresponding honey were chemically differentiated using principal component analysis	[102]

all surface-sampling methods is, that the surface texture substantially influences ionization efficiency, a fact making quantitative analysis highly problematic.

DCBI and EASI are both ambient ionization techniques categorized as direct desorption ionization techniques according to Huang et al. [6]. They have been successfully employed for analysis of meat slices (with respect to antimicrobials) [94], and fish with respect to product quality [95]. In the latter study, triacylglycerol profiles were determined in order to draw conclusions on the raising regime of the respective salmon species. Following a thermal imprinting process for extraction, EASI proves a simple, rapid, and effective technique for distinguishing tissue from fish raised upon different diets [95]. Similar as already discussed in the context with DART and PS, SPME has been interfaced to DBDI, an ambient ionization technique belonging to the same group as DESI [97]. This combination allowed a high-throughput analysis of grape juices for the presence of pesticides. The group of Spengler constructed a handheld liquid extraction pen for on-site MS analysis comparable to the iknife employed for REIMS (when looking at the handling of the device) but based on a DESI mechanism for ionization [98]. Figure 8 depicts the measurement procedure when analyzing pepper peel for pesticides using this device. The substantial error bars recognizable in Fig. 8d are typical for surface-sampling methods (as already discussed above) as surface texture severely influences the ionization process. Nevertheless, coupled to a portable MS instrument the technique developed can be regarded as an ideal tool for on-site analysis, allowing the accurate categorization of samples.

Further AIMS techniques based on a two-step ionization mechanism and employed in the field of food analysis are thermal desorption electrospray ionization (TD-ESI) and EESI. In TD-ESI the sample is placed on a heated ceramic part and

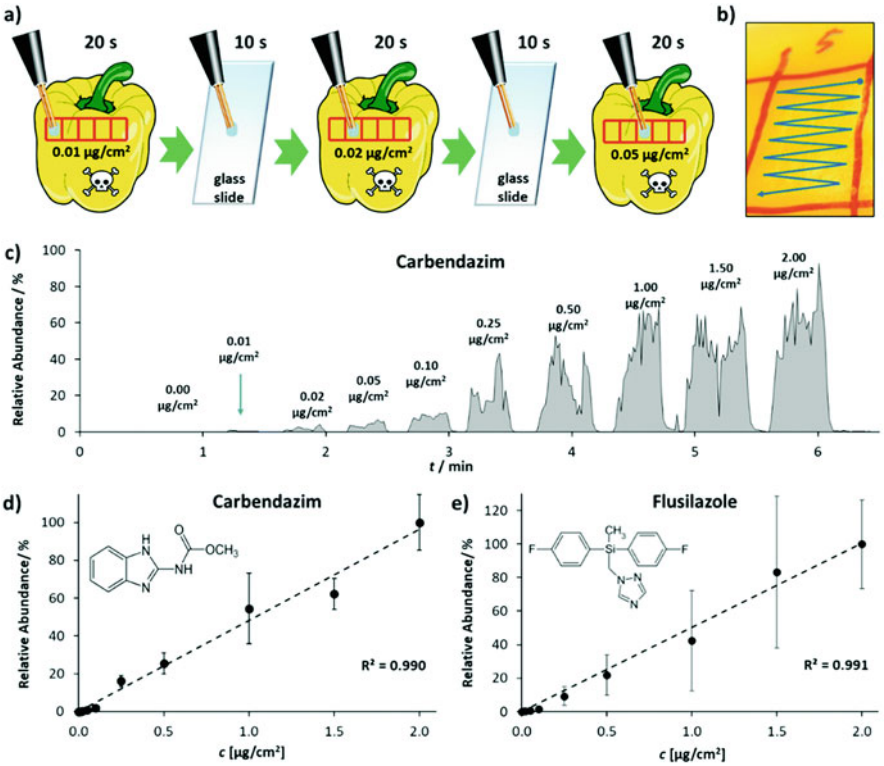


Fig. 8 Quantitative analysis of pesticides from pepper peel, using the self-sustaining and portable LEP source attached to an orbital-trapping mass spectrometer. (**a**) Sampling procedure for pesticide quantification from pepper peel. (**b**) Sampling across the spiked sampling spot. (**c**) Ion intensity chromatogram for different Carbendazim concentrations, detected from pepper peel. (**d**) and (**e**) show calibration curves, obtained for the two pesticides carbendazim and flusilazole. Data points represent averages of three technical replicates. Error bars indicate standard deviation. From [98] with permission

evolving vapors are ionized by an ESI process. This technique was used for analyzing food in several cases [99–101], comprising the analysis of basic colorants in vegetables [99], capsaicin in various food samples [100], and pesticides on surfaces of fruit and vegetables [101]. In EESI a sample solution is nebulized and the resulting spray is subsequently mixed with an electrospray generated from a solvent. An example for the use of this AIMS technique in food analysis was provided by Gao et al. who compared the chemical composition of various nectars and honeys [102].

4 Conclusions and Perspectives

Over the recent decade, AIMS techniques have shown substantial potential in both environmental and food analysis, whereby the latter distinctly dominates when retrieving the number of publications from the relevant databases. Focusing on trends and developments, one thing that can be observed is that frequently devices for sample preparation (clean-up and/or pre-concentration) are implemented in AIMS techniques (for typical examples, see [26, 72, 74, 75, 89]). Such combinations seem sensible as reducing sample complexity and increasing analyte concentrations to meet the requirements for analysis are of utmost importance in all kinds of AIMS techniques. This is the case for applications in the fields of food analysis but even more pronounced in environmental analysis, where (ultra-) trace analysis is the norm.

Right from the moment of their appearance, AIMS techniques were considered as highly suitable for the analysis of large sample sets – mostly for qualitative screening sometimes providing additional (at most) semi-quantitative information. Particularly the latter issue was addressed in a range of reports over the last decade. Common approaches are the use of isotopically labeled standards, ideally for every analyte of interest – a situation that often cannot be reached, as suitable substances are either too costly or not available at all. In addition, exact positioning of the sample within the device for ionization is mandatory, when reproducible (semi-)quantitative results are required – a fact boosting the use of automated sample positioning systems. What also needs to be mentioned is, that the use of AIMS techniques in combination with complex samples unavoidably requires the application of chemometric tools for data treatment. Also in this field substantial development could be observed over the last decade.

A major trend in environmental analysis but even more in the analysis of food and related products is on-site analysis. Due to their ease of use and the possibility to probe samples "as they are," AIMS techniques are well suited to meet the challenges related to on-site analysis. Ideally all parts of the equipment (i.e., the ion source as well as the MS instrument with all its peripheral devices) should be portable. A good example for a fieldable ion source can be found in the publication of Jager et al. [76]. Despite this development, the introduction of truly portable MS instruments for combination with ambient ionization is affected with several obstacles. One main deficit can be found in the fact that instrumentation described so far includes mainly very simple devices with low resolution and no MS/MS capabilities. As AIMS techniques tend to deal with the complete sample, a situation affected with a high probability of matrix interferences, HR-MS or multi-stage MS is often required in order to achieve useful results.

Finally, instrumentation primarily developed for use in a medical (clinical) environment has found its way into the field discussed in this chapter. A typical example is the REIMS technology with the iknife that was successfully employed in food analysis [60–66].

References

1. Monge ME, Fernandez FM (2015) An introduction to ambient ionization mass spectrometry. In: Domin M, Cody R (eds) Ambient ionization mass spectrometry. Royal Society of Chemistry, Cambridge, pp 1–22
2. Klampfl CW, Himmelsbach M (2015) Direct ionization methods in mass spectrometry: an overview. Anal Chim Acta 890:44–59
3. Kuo T-H, Dutkiewicz EP, Pei J, Hsu C-C (2020) Ambient ionization mass spectrometry today and tomorrow: embracing challenges and opportunities. Anal Chem 92:2353–2363
4. Feider CL, Krieger A, DeHoog RJ, Eberlin LS (2020) Ambient ionization mass spectrometry: recent developments and applications. Anal Chem 91:4266–4290
5. Frey BS, Damon DE, Badu-Tawiah AK (2020) Emerging trends in paper spray mass spectrometry: microsampling, storage, direct analysis, and applications. Mass Spectrom Rev 39: 336–370
6. Huang M-Z, Cheng S-C, Cho Y-T, Shiea J (2011) Ambient ionization mass spectrometry: a tutorial. Anal Chim Acta 702:1–15
7. Beneito-Cambra M, Gilbert-López B, Moreno-González D, Bouza M, Franzke J, García-Reyes JF, Molina-Díaz A (2020) Ambient (desorption/ionization) mass spectrometry methods for pesticide testing in food: a review. Anal Methods 12:4831–4852
8. Lu H, Zhang H, Chingin K, Xiong J, Fang X, Chen H (2018) Ambient mass spectrometry for food science and industry. TrAC Trends Anal Chem 107:99–115
9. Klampfl CW (2018) Ambient mass spectrometry in foodomics studies. Curr Opin Food Sci 22: 137–144
10. Black C, Chevallier OP, Elliott CT (2016) The current and potential applications of ambient mass spectrometry in detecting food fraud. TrAC Trends Anal Chem 82:268–278
11. Chen R, Deng J, Fang L, Yao Y, Chen B, Wang X, Luan T (2017) Recent applications of ambient ionization mass spectrometry in environmental analysis. Trends Environ Anal Chem 15:1–11
12. Haunschmidt M, Klampfl CW, Buchberger W, Hertsens R (2010) Determination of organic UV-filters in water by stir bar sorptive extraction and direct analysis in real time mass spectrometry. Anal Bioanal Chem 397:269–275
13. Bridoux MC, Malandain H, Leprince F, Progent F, Machuron-Mandard X (2015) Quantitative analysis of phosphoric acid esters in aqueous samples by isotope dilution stir-bar sorptive extraction combined with direct analysis in real time (DART)-orbitrap mass spectrometry. Anal Chim Acta 869:1–10
14. Jing W, Zhou Y, Wang J, Zhu Y, Lv Y, Bi W, Chen DDY (2020) Sorbent and solvent co-enhanced direct analysis in real time-mass spectrometry for high-throughput determination of trace pollutants in water. Talanta 208:120378
15. Jing W, Zhou Y, Wang J, Ni M, Bi W, Chen DDY (2019) Dispersive magnetic solid-phase extraction coupled to direct analysis in real time mass spectrometry for high-throughput analysis of trace environmental contaminants. Anal Chem 91:11240–11246
16. Wang J, Du Q, You X, Lv Y, Bi W, Li H, Chen DDY (2019) Solvent-free high-throughput analysis of herbicides in environmental water. Anal Chim Acta 1071:8–16
17. Borik A, Vojs Stanova A, Kodesova R, Brooks BW, Grabicova K, Novakova P, Grabic R (2020) Ultrafast laser diode thermal desorption method for analysis of representative pharmaceuticals in soil leachate samples. Talanta 208:120382
18. Borik A, Staňova AV, Brooks BW, Grabicova K, Randak T, Grabic R (2020) Determination of citalopram in fish brain tissue: benefits of coupling laser diode thermal desorption with low- and high-resolution mass spectrometry. Anal Bioanal Chem 412:4353–4361
19. Gerbig S, Brunn HE, Spengler B, Schulz S (2015) Spatially resolved investigation of systemic and contact pesticides in plant material by desorption electrospray ionization mass spectrometry imaging (DESI-MSI). Anal Bioanal Chem 407:7379–7389

20. Huba AK, Mirabelli MF, Zenobi R (2018) High-throughput screening of PAHs and polar trace contaminants in water matrices by direct solid-phase microextraction coupled to a dielectric barrier discharge ionization source. Anal Chim Acta 1030:125–132
21. He J, Wang W, Zhang H, Ju Y, Yu K, Zhang X, Jiang J (2021) Nebulization dielectric barrier discharge ionization mass spectrometry: rapid and sensitive analysis of acenaphthene. Talanta 222:121681
22. Wang Y, Xu L, Zhu H, Dong J, Cheng P, Zhou Z (2019) Spray-inlet microwave plasma torch and low temperature plasma ionization for ambient mass spectrometry of agrochemicals. Anal Meth 11:5421–5430
23. Liu H, Gao W, Tian Y, Liu A, Wang Z, Cai Y, Zhao Z (2019) Rapidly detecting tetrabromobisphenol a in soils and sediments by paper spray ionization mass spectrometry combined with isotopic internal standard. Talanta 191:272–276
24. Dowling S, McBride EM, McKenna J, Glaros T, Manicke NE (2020) Direct soil analysis by paper spray mass spectrometry: detection of drugs and chemical warfare agent hydrolysis products. Forensic Chem 17:100206
25. Jjunju FPM, Damon DE, Romero-Perez D, Young IS, Ward RJ, Marshall A, Maher S, Badu-Tawiah AK (2020) Analysis of non-conjugated steroids in water using paper spray mass spectrometry. Sci Rep 10:10698
26. Deng J, Yu T, Yao Y, Peng Q, Luo L, Chen B, Wang X, Yang Y, Luan T (2017) Surface-coated wooden-tip electrospray ionization mass spectrometry for determination of trace fluoroquinolone and macrolide antibiotics in water. Anal Chim Acta 954:52–59
27. Zhang X, Ren X, Chingin K (2021) Applications of direct analysis in real time mass spectrometry in food analysis: a review. Rapid Commun Mass Spectrom 35:9013
28. Beneito-Cambra M, Gilbert-Lopez B, Moreno-Gonzalez D, Bouza M, Franzke J, Garcıa-Reyes JF, Molina-Dıaz A (2020) Ambient (desorption/ionization) mass spectrometry methods for pesticide testing in food: a review. Anal Meth 12:4831–4852
29. Farre M, Pico Y, Barcelo D (2019) Direct analysis in real-time high-resolution mass spectrometry as a valuable tool for polyphenols profiling in olive oil. Anal Meth 11:472–482
30. Li X, Li S, Li H, Wang J, Luo Q, Yin X (2021) Quantification of artificial sweeteners in alcoholic drinks using direct analysis in real-time QTRAP mass spectrometry. Food Chem 342:128331
31. Huang Z, Xu Y, Huang Y, Liu C, Jiang K, Wang L (2017) Rapid determination of ginkgolic acids in Ginkgo biloba kernels and leaves by direct analysis in real time-mass spectrometry. J Sep Sci 40:4857–4864
32. Jastrzembski JA, Bee MY, Sacks GL (2017) Trace-level volatile quantitation by direct analysis in real time mass spectrometry following headspace extraction: optimization and validation in grapes. J Agric Food Chem 65:9353–9359
33. Dane AJ, Cody RB (2010) Selective ionization of melamine in powdered milk by using argon direct analysis in real time (DART) mass spectrometry. Analyst 135:696–699
34. Andersen WC, VanSickle M, Storey J, Sheldon V, Lohne J, Turnipseed SB, Thomas T, Madson M (2019) Fast analysis of caffeinated beverages using laser diode thermal desorption mass spectrometry (LDTD-MS/MS). Food Addit Contam A 36:1616–1625
35. Lippolis V, De Angelis E, Fiorino GM, Di Gioia A, Arlorio M, Logrieco AF, Monaci L (2020) Geographical origin discrimination of monofloral honeys by direct analysis in real time ionization-high resolution mass spectrometry (DART-HRMS). Foods 9:1205
36. Chernetsova ES, Morlock GE (2012) Assessing the capabilities of direct analysis in real time mass spectrometry for 5-hydroxymethylfurfural quantitation in honey. Int J Mass Spectrom 314:22–32
37. Li XQ, Li HM, Xu S, Gao Y, Zhang QH, Zhang Y, Feng MY (2019) Rapid quantification of trace chloramphenicol in honey under ambient conditions using direct analysis via real-time QTRAP mass spectrometry. Food Chem 276:50–56

38. Fiorino GM, Losito I, De Angelis E, Arlorio M, Logrieco AF, Monaci L (2019) Assessing fish authenticity by direct analysis in real time-high resolution mass spectrometry and multivariate analysis: discrimination between wild-type and farmed salmon. Food Res Int 116:1258–1265
39. Miano B, Righetti L, Piro R, Dall'AstaC, Folloni S, Galaverna G, Suman M (2018) Direct analysis real-time–high-resolution mass spectrometry for Triticum species authentication. Food Addit Contam 35:2291–2297
40. Riuzzi G, Tata A, Massaro A, Bisutti V, Lanza I, Contiero B, Bragolusi M, Miano B, Negro A, Gottardo F, Piro R, Segato S (2021) Authentication of forage-based milk by mid-level data fusion of (+/−) DART-HRMS signatures. Int Dairy J 112:104859
41. Birse N, Chevallier O, Hrbek V, Kosek V, Hajŝlova J, Elliott C (2020) Ambient mass spectrometry as a tool to determine poultry production system history: a comparison of rapid evaporative ionisation mass spectrometry (REIMS) and direct analysis in real time (DART) ambient mass spectrometry platforms. Food Control 123:107740
42. Prchalova J, Kovarik F, Rajchl A (2017) Evaluation of the quality of herbal teas by DART/TOF-MS. J Mass Spectrom 52:116–126
43. Rydlova L, Prchalova J, Skorpilova T, Rohlik BA, Cizkova H, Rajchl A (2020) Evaluation of cocoa products quality and authenticity by DART/TOF-MS. Int J Mass Spectrom 454:116358
44. Damiani T, Dreolin N, Stead S, Dall'Asta C (2021) Critical evaluation of ambient mass spectrometry coupled with chemometrics for the early detection of adulteration scenarios in *Origanum vulgare L.* Talanta 227:122116
45. Fiorino GM, Losito I, De Angelis E, Logrieco AF, Monaci L (2018) Direct analysis in real time coupled to high resolution mass spectrometry as a rapid tool to assess salmon (Salmo salar) freshness. J Mass Spectrom 53:781–791
46. Belkova B, Hradecky J, Hurkova K, Forstova V, Vaclavik L, Hajslova J (2018) Impact of vacuum frying on quality of potato crisps and frying oil. Food Chem 241:51–59
47. Blokland MH, Gerssen A, Zoontjes PW, Pawliszyn J, Nielen MWF (2020) Potential of recent ambient ionization techniques for future food contaminant analysis using (trans)portable mass spectrometry. Food Anal Meth 13:706–717
48. Khaled A, Belinato JR, Pawliszyn J (2020) Rapid and high-throughput screening of multi-residue pharmaceutical drugs in bovine tissue using solid phase microextraction and direct analysis in real time-tandem mass spectrometry (SPME-DART-MS/MS). Talanta 217:121095
49. Watt L, Sisco E (2021) Detection of trace drugs of abuse in baby formula using solid-phase microextraction direct analysis in real-time mass spectrometry (SPME-DART-MS). J Forensic Sci 66:172–178
50. Gomez-Rios GA, Gionfriddo E, Poole J, Pawliszyn J (2017) Ultrafast screening and quantitation of pesticides in food and environmental matrices by solid-phase microextraction-transmission mode (SPME-TM) and direct analysis in real time (DART). Anal Chem 89: 7240–7248
51. Yong W, Guo T, Fang P, Liu J, Dong Y, Zhang F (2017) Direct determination of multi-pesticides in wine by ambient mass spectrometry. Int J Mass Spectrom 417:53–57
52. Lara FJ, Chan D, Dickinson M, Lloyd AS, Adams SJ (2017) Evaluation of direct analysis in real time for the determination of highly polar pesticides in lettuce and celery using modified Quick Polar Pesticides Extraction method. J Chromatogr A 1496:37–44
53. Chen Y, Li L, Xiong F, Xie Y, Xiong A, Wang Z, Yang L (2021) Rapid identification and determination of pyrrolizidine alkaloids in herbal and food samples via direct analysis in real-time mass spectrometry. Food Chem 334:127472
54. Wen R, Zeng D, Yang Z, Jiang L, Ma M, Chen B, Van Beek TA (2018) Rapid analysis of illegal cationic dyes in foods and surface waters using high temperature direct analysis in real time high-resolution mass spectrometry. J Agric Food Chem 66:7542–7549
55. Rahman MM, Lee DJ, Jo A, Yun SH, Eun J-B, Im MH, Shim J-H, El-Aty AMA (2021) Onsite/on-field analysis of pesticide and veterinary drug residues by a state-of-art technology: a review. J Sep Sci. https://doi.org/10.1002/jssc.202001105

56. Bwambok DK, Siraj N, Macchi S, Larm NE, Baker GA, Perez RL, Ayala CE, Walgama C, Pollard D, Rodriguez JD, Banerjee S, Elzey B, Warner IS, Fakayode SO (2020) QCM sensor arrays, electroanalytical techniques and NIR spectroscopy coupled to multivariate analysis for quality assessment of food products, raw materials, ingredients and foodborne pathogen detection: challenges and breakthroughs. Sensors 20:6982
57. Smith BL, Hughes DM, Badu-Tawiah AK, Eccles R, Goodall I, Maher S (2019) Rapid scotch whisky analysis and authentication using desorption atmospheric pressure chemical ionisation mass spectrometry. Sci Rep 9:7994
58. Zhang H, Bibi A, Lu H, Han J, Chen H (2017) Comparative study on ambient ionization methods for direct analysis of navel orange tissues by mass spectrometry. J Mass Spectrom 52: 526–533
59. Verplanken K, Stead S, Jandova R, Poucke CV, Claereboudt J, Bussche JV, Saeger SD, Takats Z, Wauters J, Vanhaecke L (2017) Rapid evaporative ionization mass spectrometry for high-throughput screening in food analysis: the case of boar taint. Talanta 169:30–36
60. Gredell DA, Schroeder AR, Belk KE, Broeckling CD, Heuberger AL, Kim SY, King DA, Shackelford SD, Sharp JL, Wheeler TL, Woerner DR, Prenni JE (2019) Comparison of machine learning algorithms for predictive modeling of beef attributes using rapid evaporative ionization mass spectrometry (REIMS) data. Sci Rep 9:5721
61. Black C, Chevallier OP, Cooper KM, Haughey SA, Balog J, Takats Z, Elliott CT, Cavin C (2019) Rapid detection and specific identification of offals within minced beef samples utilising ambient mass spectrometry. Sci Rep 9:6295
62. Song G, Li L, Wang H, Zhang M, Yu X, Wang J, Xue J, Shen Q (2020) Real-time assessing the lipid oxidation of prawn (Litopenaeus vannamei) during air-frying by iKnife coupling rapid evaporative ionization mass spectrometry. Food Control 111:107066
63. Black C, Chevallier OP, Haughey SA, Balog J, Stead S, Pringle SD, Riina MV, Martucci F, Acutis PL, Morris M, Nikolopoulos DS, Takats Z, Elliott CT (2017) A real time metabolomic profiling approach to detecting fish fraud using rapid evaporative ionisation mass spectrometry. Metabolomics 13:153
64. Rigano F, Stead S, Mangraviti D, Jandova R, Petit D, Marino N, Mondello L (2019) Use of an "intelligent knife" (iknife), based on the rapid evaporative ionization mass spectrometry technology, for authenticity assessment of pistachio samples. Food Anal Meth 12:558–568
65. Song G, Zhang M, Zhang Y, Wang H, Li S, Dai Z, Shen Q (2019) In situ method for real-time discriminating salmon and rainbow trout without sample preparation using iKnife and rapid evaporative ionization mass spectrometry-based lipidomics. J Agric Food Chem 67:4679–4688
66. Wang H, Cao X, Han T, Pei H, Ren H, Stead S (2019) A novel methodology for real-time identification of the botanical origins and adulteration of honey by rapid evaporative ionization mass spectrometry. Food Control 106:106753
67. Schaefer KC, Denes J, Albrecht K, Szaniszló T, Balog J, Skoumal R, Katona M, Tóth M, Balogh L, Takats Z (2009) In vivo, in situ tissue analysis using rapid evaporative ionization mass spectrometry. Angew Chem Int Ed 48:8240–8242
68. Wang H, Liu J, Cooks RG, Ouyang Z (2010) Paper spray for direct analysis of complex mixtures using mass spectrometry. Angew Chem Int Ed 49:877–880
69. Himmelsbach M, Klampfl CW, Waser M (2014) Thin layer chromatography spray - mass spectrometry: a rapid method for easy identification and quantification of substances from TLC plates. Anal Bioanal Chem 406:3647–3656
70. Jackson S, Swiner DJ, Capone PC, Badu-Tawiah AK (2018) Thread spray mass spectrometry for direct analysis of capsaicinoids in pepper products. Anal Chim Acta 1023:81–88
71. Wu ML, Wu YC, Chen YC (2019) Detection of pesticide residues on intact tomatoes by carbon fiber ionization mass spectrometry. Anal Bioanal Chem 411:1095–1105
72. Liu Y, Yang Q, Chen X, Song Y, Wu Q, Yang Y, He L (2019) Sensitive analysis of trace macrolide antibiotics in complex food samples by ambient mass spectrometry with molecularly imprinted polymer-coated wooden tips. Talanta 204:238–247

73. Pereira I, Rodrigues MF, Chaves AR, Vaz BG (2018) Molecularly imprinted polymer (MIP) membrane assisted direct spray ionization mass spectrometry for agrochemicals screening in foodstuffs. Talanta 178:507–514
74. Tian H, Liu T, Mu G, Chen F, He M, You S, Yang M, Li Y, Zhang F (2020) Rapid and sensitive determination of trace fluoroquinolone antibiotics in milk by molecularly imprinted polymer-coated stainless steel. Sheet electrospray ionization mass spectrometry. Talanta 219: 121282
75. Kasperkiewicz A, Pawliszyn J (2021) Multiresidue pesticide quantitation in multiple fruit matrices via automated coated blade spray and liquid chromatography coupled to triple quadrupole mass spectrometry. Food Chem 339:127815
76. Jager J, Gerssen A, Pawliszyn J, Sterk SS, Nielen MWF, Blokland MH (2020) USB-powered coated blade spray ion source for on-site testing using transportable mass spectrometry. J Am Soc Mass Spectrom 31:2243–2249
77. Bartella L, Di Donna L, Napoli A, Sindona G, Mazzotti F (2019) High-throughput determination of vitamin E in extra virgin olive oil by paper spray tandem mass spectrometry. Anal Bioanal Chem 411:2885–2890
78. Ramalho RRF, da Silva LC, Maciel LIL, Pereira I, Nascimento AR, Simas RC, Vaz BG (2020) Directly transferring pepper constituents to triangular papers for pungency determination by paper spray ionization mass spectrometry. Anal Bioanal Chem 412:5389–5396
79. Di Donna L, Taverna D, Indelicato S, Napoli A, Sindona G, Mazzotti F (2017) Rapid assay of resveratrol in red wine by paper spray tandem mass spectrometry and isotope dilution. Food Chem 229:354–357
80. Bartella L, Mazzotti F, Sindona G, Napoli A, Di Donna L (2020) Rapid determination of the free and total hydroxytyrosol and tyrosol content in extra virgin olive oil by stable isotope dilution analysis and paper spray tandem mass spectrometry. Food Chem Toxicol 136:111110
81. Bartella L, Di Donna L, Napoli A, Siciliano C, Sindona G, Mazzotti F (2019) A rapid method for the assay of methylxanthines alkaloids: theobromine, theophylline and caffeine, in cocoa products and drugs by paper spray tandem mass spectrometry. Food Chem 278:261–266
82. Zhou W, Yang Z, Huang S, Fang Z, Chen B, Ma M (2019) Rapid quantitative analysis of ginkgo flavonoids using paper spray mass spectrometry. J Pharm Biomed Anal 171:158–163
83. Guo T, Yong W, Dong Y (2019) Automatically high-throughput quantification by paper spray ionization mass spectrometry for multiple pesticides in wine. Food Anal Meth 12:1208–1217
84. Moura ACM, Lago IN, Cardoso CF, Nascimento AR, Pereira I, Vaz BG (2020) Rapid monitoring of pesticides in tomatoes (Solanum lycopersicum L.) during pre-harvest intervals by paper spray ionization mass spectrometry. Food Chem 310:125938
85. Yu M, Wen R, Jiang L, Huang S, Fang Z, Chen B, Wang L (2018) Rapid analysis of benzoic acid and vitamin C in beverages by paper spray mass spectrometry. Food Chem 268:411–415
86. Guo T, Zhang Z, Yannell KE, Dong Y, Cooks RG (2017) Paper spray ionization mass spectrometry for rapid quantification of illegal beverage dyes. Anal Meth 9:6273–6279
87. Su Y, Ma X, Ouyang Z (2018) Rapid screening of multi-class antimicrobial residues in food of animal origin by paper spray mass spectrometry. Int J Mass Spectrom 434:233–239
88. Teodoro JAR, Pereira HV, Sena MM, Piccin E, Zacca JJ, Augusti R (2017) Paper spray mass spectrometry and chemometric tools for a fast and reliable identification of counterfeit blended Scottish whiskies. Food Chem 237:1058–1064
89. Tosato F, Correia RM, Oliveira BG, Fontes AM, Franca HS, Coltro WKT, Filgueiras PR, Romao W (2018) Paper spray ionization mass spectrometry allied to chemometric tools for quantification of whisky adulteration with additions of sugarcane spirit. Anal Meth 10:1952–1960
90. Gomez-Rios GA, Pawliszyn J (2014) Development of coated blade spray ionization mass spectrometry for the quantitation of target analytes present in complex matrices. Angew Chem Int Ed 53:14503–14507

91. Evans-Nguyen K, Stelmack AR, Clowser PC, Holtz JM, Mulligan CC (2020) Fieldable mass spectrometry for forensic science, homeland security, and defense applications. Mass Spectrom Rev. https://doi.org/10.1002/mas.21646
92. Mainero Rocca L, Cecca J, L'Episcopo N, Fabrizi G (2017) Ambient mass spectrometry: direct analysis of dimethoate, tebuconazole, and trifloxystrobin on olive and vine leaves by desorption electrospray ionization interface. J Mass Spectrom 52:709–719
93. Gerbig S, Stern G, Brunn HE, Düring RA, Spengler B, Schulz S (2017) Method development towards qualitative and semi-quantitative analysis of multiple pesticides from food surfaces and extracts by desorption electrospray ionization mass spectrometry as a preselective tool for food control. Anal Bioanal Chem 409:2107–2117
94. Wang H, Li L, Xing R, Zhang Y, Wu T, Chen B, Li Z, Fei Z, Liu Z, Ding H (2019) Screening of antimicrobials in animal-derived foods with desorption corona beam ionization (DCBI) mass spectrometry. Food Chem 272:411–417
95. Maluly HDB, de Melo PA, da Silva Cunha IB, Pacheco MTB, Eberlin MN, Alberici RM (2019) The impacts of the raising regime of Salmon species on their triacylglycerol composition revealed by easy ambient sonic-spray ionization mass spectrometry. Food Res Int 120: 19–25
96. Martinez-Jarquin S, Moreno-Pedraza A, Cazarez-Garcia D, Winkler R (2017) Automated chemical fingerprinting of Mexican spirits derived from: agave (tequila and mezcal) using direct-injection electrospray ionisation (DIESI) and low-temperature plasma (LTP) mass spectrometry. Anal Meth 9:5023–5028
97. Mirabelli MF, Gionfriddo E, Pawliszyn J, Zenobi R (2018) A quantitative approach for pesticide analysis in grape juice by direct interfacing of a matrix compatible SPME phase to dielectric barrier discharge ionization-mass spectrometry. Analyst 143:891–899
98. Lotz F, Baar P, Spengler B, Schulz S (2021) Development of a handheld liquid extraction pen for on-site mass spectrometric analysis of daily goods. Analyst 146:3004–3015
99. Chao YY, Chen YL, Lin HY, Huang YL (2018) Rapid screening of basic colorants in processed vegetables through mass spectrometry using an interchangeable thermal desorption electrospray ionization source. Anal Chim Acta 1010:44–53
100. Zhang Q, Liu X, Li Z, Su Y, Guo Y (2019) Rapid quantitative analysis with low matrix effects of capsaicin in various samples by thermal desorption carbon fiber ionization mass spectrometry. Anal Chim Acta 1048:115–122
101. Cheng SC, Lee RH, Jeng JY, Lee CW, Shiea J (2020) Fast screening of trace multiresidue pesticides on fruit and vegetable surfaces using ambient ionization tandem mass spectrometry. Anal Chim Acta 1102:63–71
102. Gao Y, Xue A, Li X, Huang X, Ning F, Zhang X, Liu T, Chen H, Luo L (2020) Analysis of chemical composition of nectars and honeys from citrus by extractive electrospray ionization high resolution mass spectrometry. LWT 131:109748

Ion Mobility-Mass Spectrometry in Food and Environmental Chemistry

Shon P. Neal and Christopher D. Chouinard

Contents

Abstract Ion mobility-mass spectrometry (IM-MS) has emerged as a powerful analytical technique currently used in numerous fields including food and environmental chemistry. IM-MS can be employed with several ionization methods and coupled with chromatographic separations to provide multidimensional identification of compounds based on the combination of retention time, collision cross section (CCS), and accurate mass. Experimentally obtained CCS values can be used to populate application-specific libraries that further allow identification and quantification in complex mixtures. This chapter will highlight recent advances in IM-MS methods and technology applied to the food chemistry and environmental chemistry fields. First, instances of IM-MS in nutritional analysis, food safety, food fingerprinting, and process control and quality assurance/quality control (QA/QC) will be discussed. Environmental applications including analysis of per- and

S. P. Neal and C. D. Chouinard (✉)
Department of Biomedical and Chemical Engineering and Sciences, Chemistry Program, Florida Institute of Technology, Melbourne, FL, USA
e-mail: cchouinard@fit.edu

Yolanda Picó and Julian Campo (eds.), *Mass Spectrometry in Food and Environmental Chemistry*, Hdb Env Chem (2023) 119: 311–332, DOI 10.1007/698_2022_886,

Published online: 7 October 2022

polyfluoroalkyl substances (PFASs); polycyclic aromatic hydrocarbons (PAHs), benzene/toluene/xylene (BTX), and volatile organic compounds (VOCs); pesticides; and pharmaceutical and personal care products (PPCPs) will also be outlined. Overall, it is expected that IM-MS will continue to grow due to additional information offered in comparison with conventional methods and because of increased commercialization and improved resolution.

Keywords Collision cross section, Environmental chemistry, Food chemistry, Ion mobility spectrometry (IMS), Ion mobility-mass spectrometry (IM-MS), Mass spectrometry (MS)

1 Introduction to Ion Mobility-Mass Spectrometry (IM-MS)

Ion mobility spectrometry (IMS) is an analytical technique that measures the movement of gas-phase ions through a buffer gas environment under the influence of an electric field [1]. As ions move through the buffer gas (typically helium or nitrogen, maintained at pressures ranging from ~1 Torr to atmospheric pressure), they undergo collisions and travel at different velocities based on their size, shape, and charge, such that larger ions experience more collisions and thus travel more slowly [2]. Several different varieties of IMS have been developed which differ in the nature of the electric field applied (Fig. 1) [3], including drift tube (DTIMS), traveling wave IMS (TWIMS) [4], differential mobility spectrometry (DMS)/high field asymmetric waveform IMS (FAIMS) [5], differential ion mobility (DIM)/differential mobility analysis (DMA) [6], and trapped IMS (TIMS) [7]. DTIMS uses a static electric field which causes ions to achieve a constant drift velocity through the separation cell (Fig. 1a), such that the time to traverse the separation cell can be measured as "drift time"; this technique is analogous to the way a time-of-flight mass spectrometer (operated at high vacuum) measures mass-to-charge ratio (m/z). TWIMS, also a time-dispersive technique, applies a dynamic voltage profile such that ions will either "ride the wave" or "roll back" based on their mobility (Fig. 1b). DMS/FAIMS applies an alternating high and low electric field (for short and long durations, respectively) perpendicular to the main ion motion. The resulting sawtooth motion is a result of ions' net motion toward one of the two electrodes. Meanwhile, a compensation field superimposed on one of the electrodes can be scanned to sequentially allow transmission of ions of different mobility (Fig. 1c). DMS/FAIMS is analogous to a quadrupole mass spectrometer in that it operates as an ion filter. DIM/DMA applies a constant electric field which is perpendicular to ion motion propelled by a gas flow. This field can also be scanned to allow for ions of a certain mobility to be transmitted and measured (Fig. 1d). Lastly, TIMS separates ions propelled by a gas flow as they are opposed, or "trapped," by an electric field. This electric field can be scanned to elute ions based on their mobility.

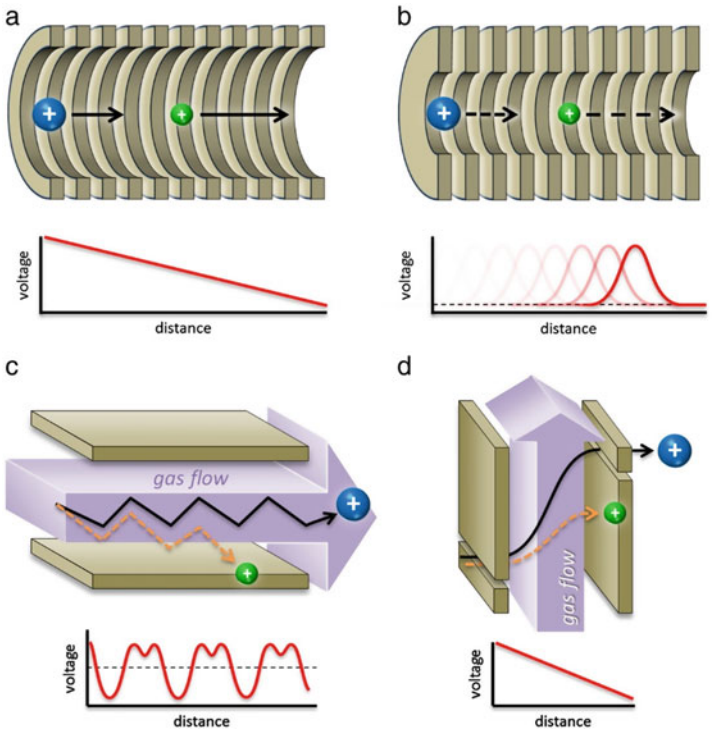


Fig. 1 Basic operational principles of contemporary ion mobility techniques. (**a**) Conventional drift time ion mobility whereby ions are introduced into a gas-filled chamber and separated based upon their differential drift down a continuous declining potential. (**b**) Traveling wave ion mobility which mobility separates ions in a gas-filled chamber using a dynamic traveling potential hill. (**c**) Asymmetric field ion mobility whereby ions are separated based upon their differential migration orthogonal to a sweeping gas flow. (**d**) Differential ion mobility which separates ions based upon their ion mobility-dependent spatial displacement as they traverse an electric field gradient that is applied orthogonal to a constant flow of gas. In all schemes, ion motion is depicted from left to right. Reprinted from "Lipid analysis and lipidomics by structurally selective ion mobility-mass spectrometry," Biochimica et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids, 2011, Volume 1811, Pages 935–945 with permission from Elsevier [3]

Ions studied by DTIMS can be characterized by their mobility (K) which can be calculated by measuring drift velocity ($v_d = L/t_D$ where L = drift tube length and t_D = drift time) as a function of electric field (E), or $K = v_d/E$. Mobility remains constant, with v_d proportional to E, for low electric fields (<100 V/cm). This relationship allows for calculation of an ion's rotationally averaged collision cross section (CCS), or the average two-dimensional area of an ion that undergoes collisions with a given buffer gas. For consistent experimental conditions, especially drift gas composition, temperature, and pressure, CCS is representative of a given ion and thus can be used for identification purposes. As will be seen in later sections of the chapter, CCS databases allow for improved confidence in determining

components of complex mixtures, especially when compared with chemical standards. Furthermore, measurement of CCS provides a means by which to study molecular structure and changes therein [8]. Modern computational modeling methods allow for fairly accurate determination (<2% difference between experimental and theoretical CCS values) of three-dimensional gas-phase structure when used in conjunction with experimentally obtained CCS [9]; these modeling strategies have also demonstrated some predictive capacity and are currently being implemented with machine-learning algorithms to populate massive theoretical CCS libraries [10].

IMS is a fast technique, with time-dispersive methods capable of acquiring a full spectrum in tens of milliseconds or less. Filtering methods such as DMS/FAIMS can operate even faster, with duty cycle approaching 100% for targeted analysis of single or a few analytes. Standalone IMS has seen utility in the defense industry dating back to the 1970s [11] and is still used extensively in modern applications, however its coupling with mass spectrometry (i.e., IM-MS) has expanded its utility for untargeted and biological analyses considerably. Its speed conveniently allows for nesting between chromatographic separations (minutes) and fast acquisition mass spectrometers such as time-of-flight, TOF (hundreds of microseconds). This added separation creates multidimensional techniques (e.g., GC-IM-MS, LC-IM-MS/MS, etc.) that provide multiple measures for a given ion, including retention time, CCS, accurate mass, and fragmentation pattern. Such multidimensional approaches can significantly reduce false positives in analysis (Fig. 2) [9]. Commercialization of IM-MS instrumentation by nearly all major mass spectrometry vendors has caused significant proliferation of its use in structural biology [12], -omics analyses (e.g., metabolomics, lipidomics, proteomics, etc.) [13], and the clinical laboratory [14]. The most recent advances in higher resolution IM-MS instrumentation have included TIMS [7], structures for lossless ion manipulations (SLIM) [15], and cyclic TWIMS [16]; these platforms promise to provide more structural information, better separation of isomers, and more precise CCS measurement. As such, it is expected that the IM-MS footprint in nearly all analytical applications, including in food and environmental chemistry, will continue to grow in coming years.

2 Introduction to IM-MS in Food Chemistry

The use of ion mobility spectrometry (IMS) in the food industry has grown dramatically over the last decade, as evidenced by the rise in publications referencing IMS since 2010 (Fig. 3) [17]. This trend is expected to continue as IMS and associated techniques (e.g., LC-IM-MS, GC-IM-MS, etc.) are further developed. Currently, the food industry utilizes IMS in four broad categories: (A) nutritional analysis, (B) food safety, (C) food fingerprinting, and (D) process control and quality assurance/quality control (QA/QC). Some of these categories are dominated by GC/LC-IM-MS instrument design and development, while others are focused on portable, inexpensive, and fast standalone IMS instrumentation. For example, nutritional analysis

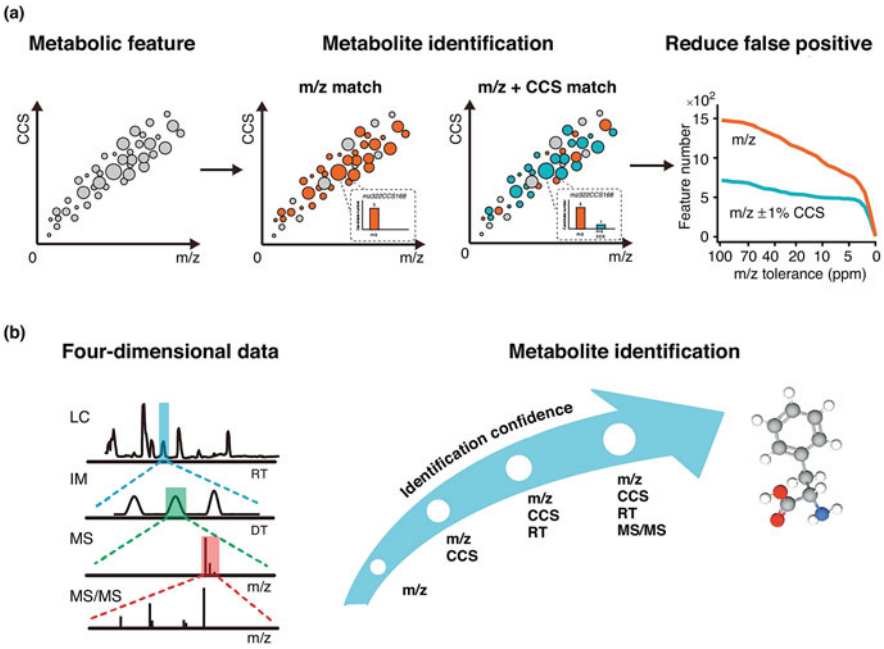


Fig. 2 The use of CCS values to support metabolomics and lipidomics. (**a**) The CCS value is an important criterion to identify metabolites and reduce false positives; (**b**) the integration of *m/z* value, CCS value, retention time, and MS/MS spectra improves the confidence of metabolite identification. Reprinted from "Advancing the large-scale CCS database for metabolomics and lipidomics at the machine-learning era," Current Opinion in Chemical Biology, 2018, Volume 42, Pages 34–41 with permission from Elsevier [9]

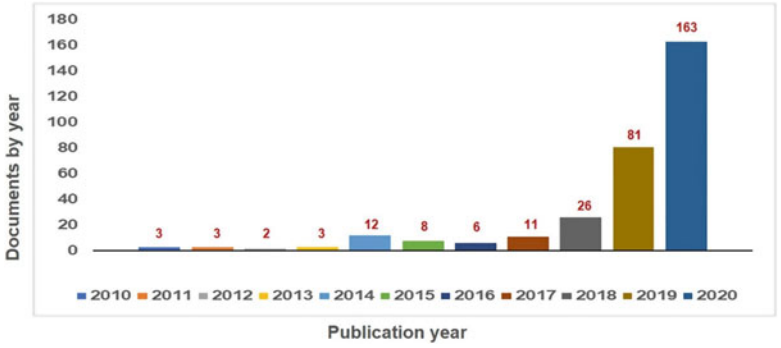


Fig. 3 Search of literature related to GC-IMS in analysis from 2010 to 2020 on Scopus database. The terms "gas chromatography-ion mobility spectrometry (GC-IMS)" have been included in the search topic. Reprinted from "Application and development trends of gas chromatography–ion mobility spectrometry for traditional Chinese medicine, clinical, food and environmental analysis," Microchemical Journal, 2021, Volume 168, Page 106526 with permission from Elsevier [17]

focuses on complete and detailed analysis of complex food matrices, which requires hyphenated systems capable of multiple separation dimensions. Food safety as well should see growth in IM-MS over standalone IMS systems. Governmental regulation drives food safety which generally requires identification capabilities that IMS alone cannot provide [18]. Food fingerprinting and QA/QC, on the other hand, prefer the simplest analytical technique(s) to meet their needs. Lessons learned from more detailed nutritional analysis research (i.e., with more complex hyphenated techniques) eventually translate to more straightforward QA/QC process control methods. This section will discuss the current state of IM-MS in each of the four aforementioned categories.

2.1 Nutritional Analysis

Nutritional analysis is the broad categorization of the food matrix into its base nutritional components. Foodstuffs are complex mixtures containing numerous different compounds, many of which are present at very low concentration. However, the resulting requirements for low limits of detection (LODs) and high sensitivity can be complicated by difficulty in separating the myriad of potential interferences. As such, IM-MS has seen increased use in nutritional analysis due to the added separation ability increasing the confidence of identification. The supplemental separation dimension afforded by IM-MS allows for additional filtering of complex datasets and provides cleaner MS spectra. Furthermore, IM measures collision cross section (CCS) values which can be collected to populate nutrition-specific databases that complement existing libraries of chromatographic retention time, accurate mass, and/or MS/MS fragmentation spectra.

Work by Willems et al. is an excellent example of classifying nutritional components by IM-based methods. In this study, ESI-FAIMS-MS was used to separate and identify chlorogenic acid isomers, specifically mono-caffeoylquinic acids which are commonly found in fruits (e.g., apples, pears, etc.) and are among the most abundant phenolics found in the human diet [19]. The three most common (3-, 4-, and 5-caffeoylquinic acid) are isomers and difficult to differentiate with conventional methods. Combining FAIMS and MS/MS provided a method for separation and identification with an analysis time of <1 min, a considerable improvement over HPLC-based methods that often require more than 1 h.

McCullagh et al. used TW-IMS-MS to study steviol glycosides, which are zero-calorie sweeteners used in many manufactured food products [20]. Steviol, a regulated food additive in Europe with a maximum permitted level, currently consists of seven different chemically defined compounds, each with different flavor profile (i.e., sweetness, bitter aftertaste, etc.). The processing industry aims to maximize use of the sweetener while staying under the regulatory limit. McCullagh et al. used TW-IMS-MS to separate and identify isomeric steviol glycoside pairs based on unique CCS values, which eliminated the need to rely on characteristic fragmentation ratios (Fig. 4). This research also has broader implications for food safety and QA/QC

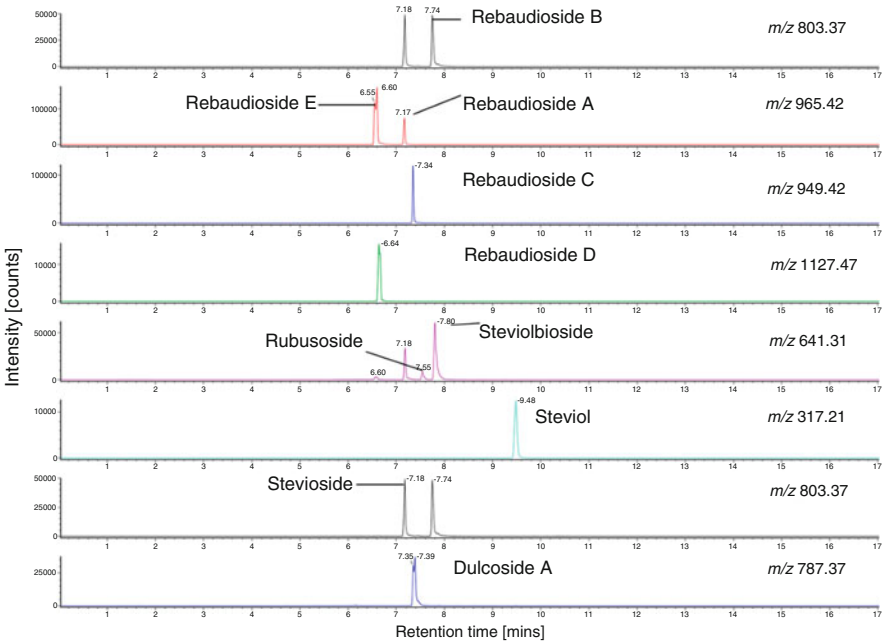


Fig. 4 Extracted mass chromatograms for steviol and profiled steviosides <100 pg/UL spikes into chocolate. Reprinted from "Exploring the Complexity of Steviol Glycosides Analysis Using Ion Mobility Mass Spectrometry," Analytical Chemistry, 2018, Volume 90, Pages 4585–4595 with permission from American Chemical Society [20]

methods, as it presents potential for development of less expensive and more precise methodologies for such markers.

Groups like Chen et al. combine aspects of food and medical research, such as with their IM-MS studies of the digestive properties associated with food consumption [21]. UPLC-IM-QTOF-MS analysis identified anthocyanins from *Aronia melanocarpa* (AAM), which were involved in decreased lipid content and inflammation in 3 T3-L1 cells without cytotoxicity. Wenjie et al. presented another good example of food/supplement analysis in which they also studied anthocyanins [22]. These compounds, of interest in natural supplements as essential molecules in the so-called super fruits, were analyzed using Hadamard transform ion mobility mass spectrometry (HT-IMMS). The authors were able to separate isomers pelargonidin-3,5-diglucoside (found in pomegranate) from cyanidin-3-rutinoside (found in blackberry). This enabled determination of the anthocyanin ratios in many different fruit types (blueberry, blackberry, raspberry, strawberry, and pomegranate), which offers a possible fingerprinting method.

An extensive database for fatty acid methyl esters (FAMEs) was developed using GC with an atmospheric pressure chemical ionization source. The GC-APCI-IMS-TOF-MS method significantly increased selectivity, and thus confidence of identification, of FAMEs in this analysis [23]. This research identified trace amounts of

various compounds while also providing a more detailed characterization of the edible oils present. The significantly increased depth and quality of information could be helpful when applied to detecting food fraud or when used in the quality control of manufacturing processes.

Although numerous analytical methodologies are employed in nutritional analysis, ranging from DNA/protein tests to targeted MS/MS, IM-MS techniques will continue to grow and replace other methods in this category due to their precision, speed, and identification capabilities of individual testing. Another noticeable trend is that work completed in this category will provide actionable markers for food safety, QA/QC, and fingerprinting, to be discussed in the following sections.

2.2 Food Safety

Governmental regulation drives food safety requirements and thus the analytical methods used to determine food safety. This includes analysis of materials that can cause harm if injected, inhaled, or consumed. However, new methods are adopted slowly, even if they demonstrate tangible benefits. Analytical techniques that increase speed and decrease costs are likely to see growth in this field as screening techniques feeding into the more detailed analysis as required. An important consideration in food safety is governmentally mandated acceptable exposure limits, requiring accuracy and sensitivity in the analytical methods used. As such, MS-based techniques will continue to grow and replace current methods in this category. For instance, steviol derivatives differ significantly in their flavor profile and health safety, but for regulation purposes are often grouped together. It is of interest to the food manufacturers to differentiate and quantitate the different steviols, even though it is not currently required [20]. Despite lack of regulation, IM-MS provides significantly more information than traditional methods and could be of considerable value in the future, allowing manufacturers to stay ahead of the game and comply with potentially new laws, while simultaneously improving food quality by increasing the desired steviol derivative.

Another example of food safety application is analysis of the sealants used in the lids of hermetically sealed food cans. These sealants contain a wide variety of compounds, including associated non-intentionally added substances (NIAS) that can potentially migrate to the food. UHPLC-IM-QTOF-MS has been used to identify such compounds by providing characteristic CCS values and high-resolution m/z, increasing the confidence in their identification [24]. Another safety application concerns trade organizations, such as the International Olive Council, which have created certain safety regulations for their products [25]. These organizations stand to benefit significantly from the added separation capabilities of IM-MS techniques for monitoring food safety regulations and could adopt the technology more quickly than government-regulated bodies. Despite the widespread use of standalone IMS, its inability to definitively identify molecular composition of potential safety concerns will like lead to future use of IM-MS and its better identification capabilities.

2.3 *Food Fingerprinting*

Food fingerprinting is the identification of molecular markers that identify a specific food. Unlike nutritional analysis or food safety, which focus on understanding the food makeup or identifying pesticides and toxins, respectively, food fingerprinting is about determining the origin and purity of the product. Identifying molecular markers, such as a particular analyte or ratio of analytes, representing a characteristic food state or condition provides more effective product discrimination, which is needed because of threats to the world's food supply by fraud, adulteration, and counterfeiting. Many different methods exist to identify and exploit specific molecular markers [26, 27], including MS and IMS techniques which are valuable due to their precision and speed, respectively. Coupling of these methods provides the high sensitivity, selectivity, throughput, and multi-analyte capabilities suited to solve food fingerprinting requirements.

One common product in high demand for fingerprinting is olive oil, a frequent target for alteration or imitation. Manufacturers who market and price their food items based on quality, specific location or origin, or processing techniques can use IM-MS to confirm these properties. For example, García-Nicolás et al. demonstrated both IM-MS and standalone IMS applied to determining the quality level of olive oils (Fig. 5) [28]. Additional studies have been shown capable of identifying specific regions of origin that produced the oil [29].

Arroyo-Manzanares et al. also used headspace (HS) sampling coupled with GC-IMS to differentiate authentic Iberian Ham from fraudulent versions [30]. Their group was able to identify markers based on the different feedstock provided to the pigs. The method improved on prior high-resolution MS fingerprinting because it no longer required extensive sample preparation thus reducing the analysis time. HS-GC-IMS has also been used to differentiate honey according to its purity and level of impurities adulterated from sugarcane or corn syrups (Fig. 6) [32]. These methods determined adulteration and identified counterfeit honey based on the source of the nectar.

The current trend of standalone or GC-IMS techniques to monitor previously identified markers may be giving way to IM-MS-based methods given the economic implications of fraud and counterfeiting and the need to stay ahead of future adulteration schemes. An example is how HPLC-IMS-TOFMS has been used to identify markers that confidently characterize and fingerprint wine using a metabolomics-type workflow [33]. This method distinguished wines by type and location and could help reduce counterfeiting by cheaper wines pretending to be from more desired regions.

Fingerprinting will continue as a growth area for IM-MS methods with expected growth in exploratory sample classification to identify novel markers and/or their ratio(s). Such markers would be used to build new databases that identify unique signatures for food fingerprinting.

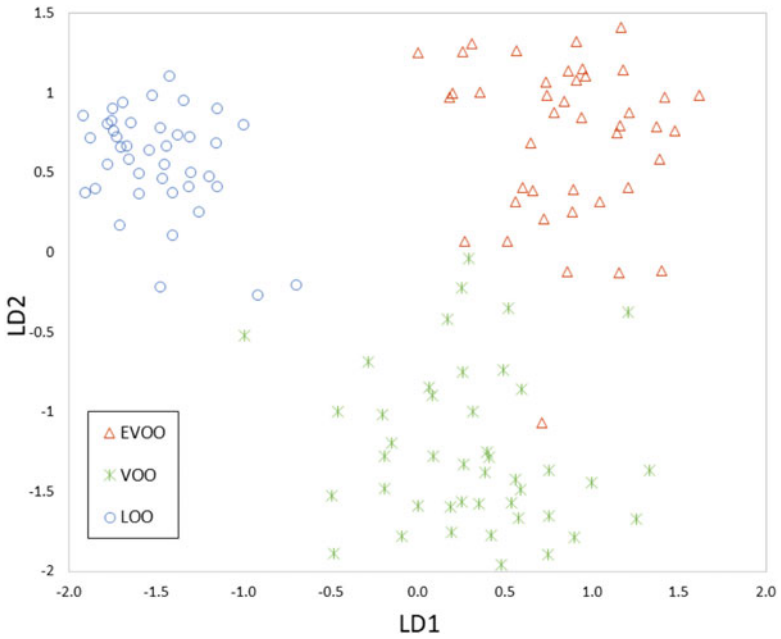


Fig. 5 Principal component analysis–linear discriminant analysis (PCA-LDA) model constructed using the HS-GC-IMS markers. Reprinted from "Headspace Gas Chromatography Coupled to Mass Spectrometry and Ion Mobility Spectrometry: Classification of Virgin Olive Oils as a Study Case," Foods, 2020, Volume 9, Page 1288 with permission from MDPI [28]

2.4 Process Control and Quality Assurance/Quality Control (QA/QC)

Standalone IMS is routinely employed by food companies and researchers for process control due to its low cost, high speed, and ability to test VOCs relating to food quality at the source of production. Such process control and quality assurance/quality control applications, however, can benefit significantly from tools for more sophisticated analysis. A great example of using IMS for process control is how GC-UV-IMS allows for greater precision and shorter hold times for the beer brewing process [34]. Brewing beer requires that diacetyl and 2,3-pentanedione degrade below the odor threshold. Many breweries will allow this process to run multiple days to ensure degradation, but this time-consuming and unnecessary when using an IMS process. IMS can measure diacetyl and 2,3-pentanedione concentration in under 10 min with minimal sample prep. This analysis method decreases current testing costs as well as reducing brewing time while maximizing flavor quality.

QA/QC processes are improved using IMS methods as a rapid and deployable screen tool. A model can discriminate between fresh and spoiled egg products by recording volatile fingerprints (Fig. 7) [35]. The use of IMS offers a low-cost and fast analytical protocol that has also been applied to other food products, such as bread,

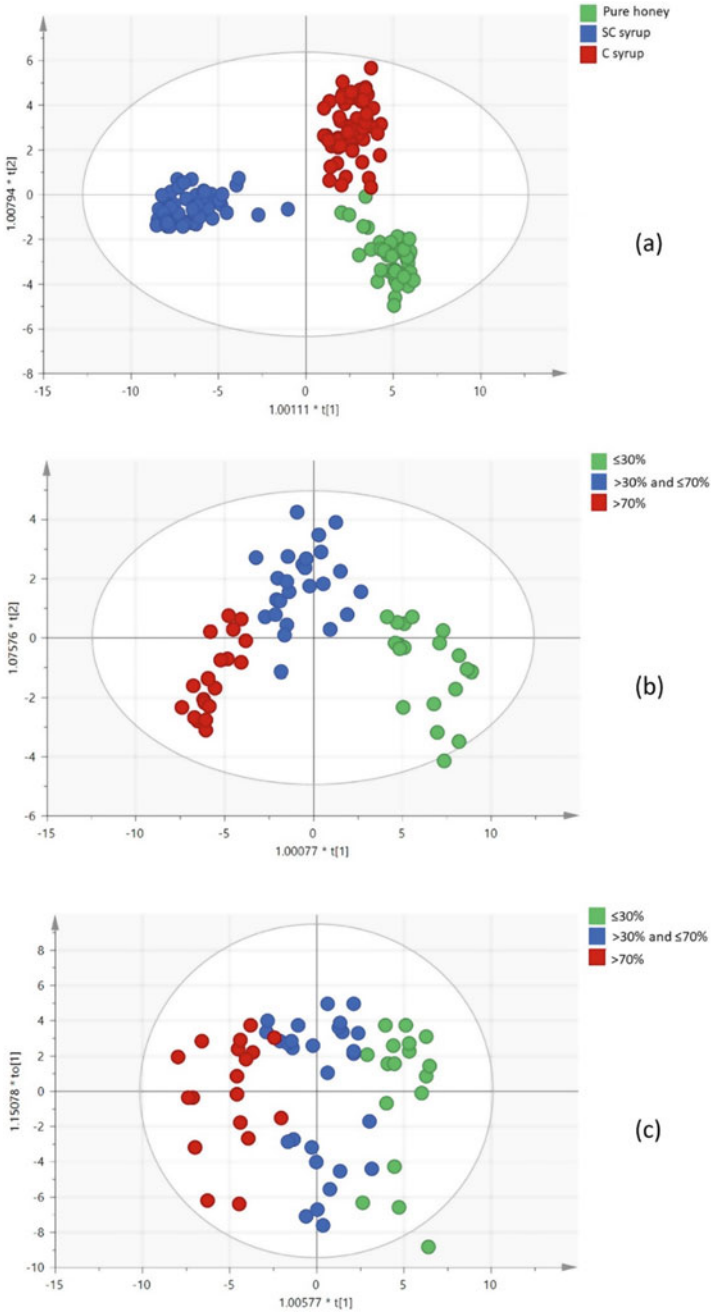


Fig. 6 Ternary OPLS-DA model using normalized data and UV scaling to discriminate between pure and adulterated honey samples. SC syrup: honey samples adulterated with sugarcane syrup: C syrup: honey samples adulterated with corn syrup. Reprinted from "Untargeted headspace gas chromatography – Ion mobility spectrometry analysis for detection of adulterated honey," Talanta, 2019, Volume 205, Page 120123 with permission from Elsevier [31]

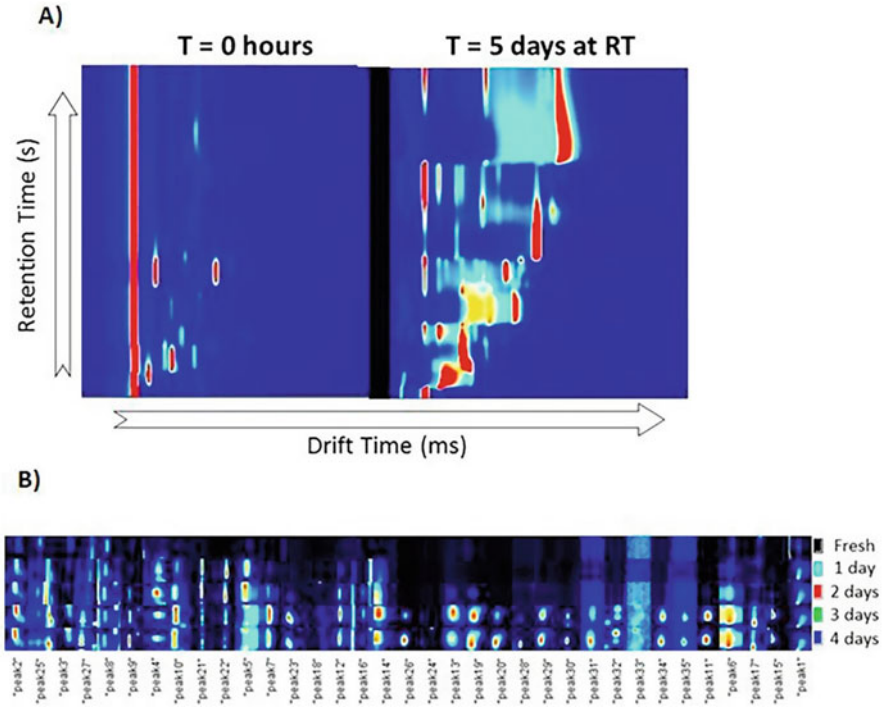


Fig. 7 (**a**) Ion mobility spectrum of an egg product at $T = 0$ h (left) and after 5 days at room temperature (right). The red line indicates the Reaction Ion Peak (RIP) position. (**b**) Global overview of the spots identified in the egg product "Sample_20" at different time points. Reprinted from "Ion mobility spectrometry coupled to gas chromatography: A rapid tool to assess eggs freshness," Food Chemistry, 2019, Volume 271, Pages 691–696 with permission from Elsevier [35]

in which IMS can be used to measure biogenic amines which affect test as a quality control process. There are still many applications that are expected to benefit from IM-MS technology especially as these methods become less expensive and of higher resolution.

3 Introduction to IM-MS in Environmental Analysis

Environmental analysis with MS-based methods primarily focuses on identification and quantification of xenobiotic compounds that pose a toxic threat to ecological health, including both humans and wildlife. These compounds include persistent organic pollutants (POPs) which are often by-products of chemical, manufacturing, and/or agricultural processes. This section will discuss examples of IM-MS in the following environmental applications: (A) per- and polyfluoroalkyl substances (PFASs); (B) polycyclic aromatic hydrocarbons (PAHs), benzene/toluene/xylene

(BTX), and volatile organic compounds (VOCs); (C) pesticides; and (D) pharmaceutical and personal care products (PPCPs).

3.1 Per- and Polyfluoroalkyl Substances (PFASs)

Per- and polyfluoroalkyl substances (PFASs) are an environmentally ubiquitous class of persistent organic pollutants. Used in numerous consumer and industrial products including firefighting foam and fire-retardant coatings, and nonstick cookware, PFAS are of significant concern due to their chemically inert properties and toxic effects causing adverse health outcomes (e.g., developmental/hormonal effects, thyroid disease, decreased liver function, etc.). The manufacturing process results in production of many different species including linear and branched isomers with different toxicological properties. The most commonly used analytical approach for identification and quantification is chromatography-mass spectrometry methods, but identification of the many isomers is challenging due to long analysis times and inability to resolve using MS/MS fragmentation pattern.

The first application of ion mobility to PFAS analysis by Ahmed et al. involved high-resolution differential mobility spectrometry (DMS) to separate PFAS isomers [36]. This demonstrated the capability to resolve various linear, secondary- and tertiary-branched species, and even isomers that differed in the position of a single perfluoromethyl group. Figure 8 illustrates the filtering of several isomers by DMS compensation field, allowing resolution of these four compounds. Dodds et al. later used DTIMS to measure CCS for over 50 PFAS species and demonstrated an LC-IM-MS workflow for study of environmental samples [37]. This work also yielded CCS vs. *m/z* trends for several subclasses such as fluorotelomer sulfonic acids (FTSAs) and perfluoroalkyl sulfonic acids (PFSAs). Measurement of these

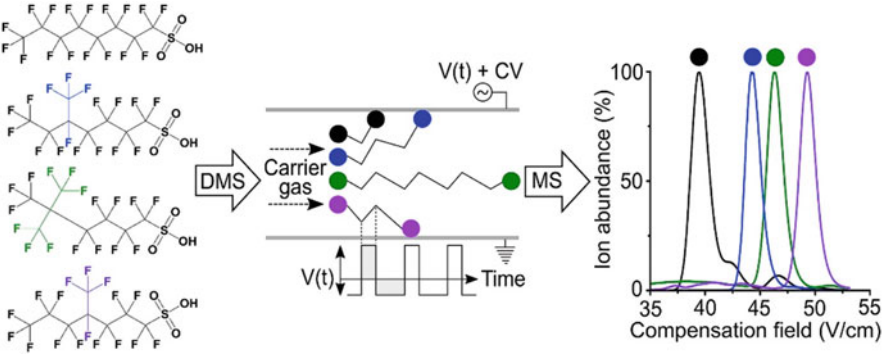


Fig. 8 Four PFAS isomers, one linear and three branched, are separated based on their transmission through a DMS device at different compensation field. Reprinted from "Rapid separation of isomeric perfluoroalkyl substances by high-resolution differential ion mobility mass spectrometry," Analytica Chimica Acta, 2019, Volume 1058, Pages 127–135 with permission from Elsevier [36]

substances in wastewater outfall and lake water samples demonstrated good reproducibility in CCS, differing by <0.5% from analysis of pure standards. Luo et al. later applied the developed LC-IM-MS workflow to rapid untargeted characterization of seven different aqueous film-forming foams (AFFFs) [38]. Interestingly, they identified more than 20 homologous series which had not been reported previously, further demonstrating improved confidence in the analysis of complex mixtures containing unknowns. Yukioka et al. applied a novel IM-MS/MS fragmentation flagging approach for identification of several PFAS isomers in a household fire extinguisher liquid [39]. They later applied data-dependent acquisition (DDA) to exclude unrelated chromatographically coeluting species, allowing for improved identification of more than 40 PFAS species in firefighting foam impacted groundwater samples [40].

3.2 Polycyclic Aromatic Hydrocarbons (PAHs); Benzene, Toluene, and Xylene (BTX); and Other Volatile Organic Compounds (VOCs)

Polychlorinated biphenyls (PCBs) are endocrine disruptors still found in the environment, despite their production having been banned in the USA in the 1970s. Adams et al. demonstrated LC-TIMS-TOF for differentiation of the hydroxylated metabolites of isomeric penta-, hexa-, and hepta-CBs (Fig. 9) in diluted human blood plasma [41]. This study showed impressive IM resolving power ($R_p > 150$) with limits of detection (~10 pg/mL) comparable to other traditional methods. Zheng et al. further characterized the hydroxylated metabolites of PCBs, as well as other PAHs and polybrominated diphenyl ethers (PBDEs) using several ionization methods including electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI) [42]. This allowed rapid distinction of isomers and showed characteristic CCS vs. *m/z* trends for different molecule types. Zheng et al. also used DTIMS to populate a library of over 500 different chemical species, including xenobiotics [43]. Most recently, Olanrewaju et al. demonstrated a novel analytical workflow combining IM with GC and both atmospheric pressure laser ionization (APLI) and electron ionization (EI) [44]. This approach characterized over 100 PAHs in terms of their retention time, mobility, and accurate mass. Determination of gas-phase candidate structures for each was enabled by complementary computational modeling.

Benzene, toluene, and xylene (BTX) are hazardous volatile aromatic hydrocarbons involved in many chemical manufacturing processes. Environmental monitoring of these compounds has been of particular interest in recent decades due to their toxicity, and several recent advances in IMS-MS instrumentation development have focused on BTX detection in air samples. Sabo et al. coupled corona discharge ionization with a homebuilt 8.2 cm IMS attached to an oa-TOF to investigate n-propanol and isopropanol mixtures [45]. This method produced two-dimensional

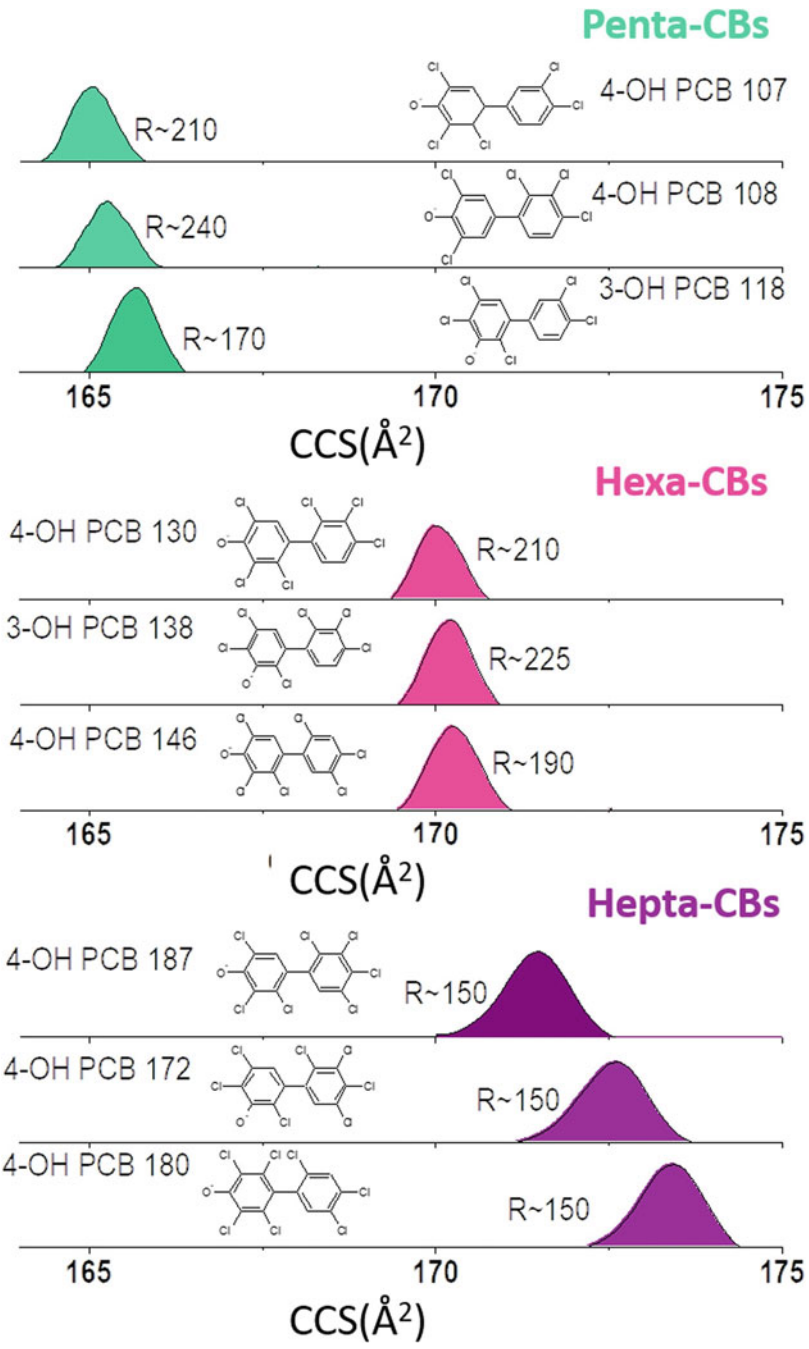


Fig. 9 Typical mobility profiles of single standards of penta-, hexa, and hepta-CBs. Reprinted from "Discovery and targeted monitoring of polychlorinated biphenyl metabolites in blood plasma using LC-TIMS-TOF MS," International Journal of Mass Spectrometry, 2018, Volume 427, Pages 133–140 with permission from Elsevier [41]

maps of IMS drift time vs. TOFMS flight time, allowing for distinction of benzene, toluene, and xylene, as well as their NO^+ adduct ions measured in zero air. They further measured mobility trends for the BTX compounds and small molecule alcohols (methanol, ethanol, etc.) as various ion adducts included hydrated species. Heptner et al. incorporated a ^3H source with atmospheric pressure drift tube to a commercial TOF for measurement of unknown compounds in a gas mixture [46]. The reactive ion products of acetone and BTW compounds could be detected, along with several other small organic molecules such as dimethyl-methylphosphonate (DMMP), ammonia, and tetrachloroethylene. Lastly, Allers et al. developed a high-resolution ambient pressure drift tube capable of achieving $R_P = 100$ and used it for analysis of BTX at low parts per million (ppm) concentrations [47]. This proof-of-concept instrument demonstrated utility in detection of these challenging compounds in air samples. Numerous other volatile organic compounds (VOCs) have also been the focus of IMS-MS studies. For example, Michalczuk et al. analyzed methanol, ethanol, isopropanol, acetone, diethylamino, and ethyl acetate monomers and dimers at different hydration levels using atmospheric pressure chemical ionization (APCI) coupled to a homebuilt IMS-TOF.

3.3 Pesticides

Regueiro et al. used UHPLC-TWIMS-QTOFMS to create a CCS database of more than 200 pesticides, with very high intra- and inter-day repeatability (RSD $<1\%$) [48]. They applied CCS as an additional measure and demonstrated the ability to resolve isomers ipconazole and tebufenpyrad (Fig. 10). This database was later applied for qualitative pesticide screening of several commercial salmon feed samples, revealing that pirimiphos-methyl and ethoxyquin were frequently found in these feeds [49]. Bauer et al. similarly evaluated an IM-QTOF pesticide screening approach using an in-house database of 280 pesticides applied to 20 different plant-derived foods [50]. With a tolerable CCS error of $\pm 2\%$, false assignment of analyte molecules was reduced. Notably, this approach also revealed brodifacoum protomers and spinetoram sodium adducts with unique CCS. The same group then applied this methodology to pesticide metabolites in the *Brassica* plant, identifying phase I and phase II metabolites of eight thiacloprid, 11 azoxystrobin, and three difenoconazole in different plant organs such as leaves, stems, and roots [51]. Goscinny et al. similarly created a pesticide database for 200 compounds, but reported very consistent CCS measurements that differed by no more than 1% from the reference data library [52]. Chen et al. introduced a more rapid method, not involving chromatographic separations to screen for pesticides in various plant samples with total analytical time of only 1.6 min per sample [53]. Limits of detection were reported as ≤ 20 ng/mL for all species, and pyrimethanil was confirmed in celery.

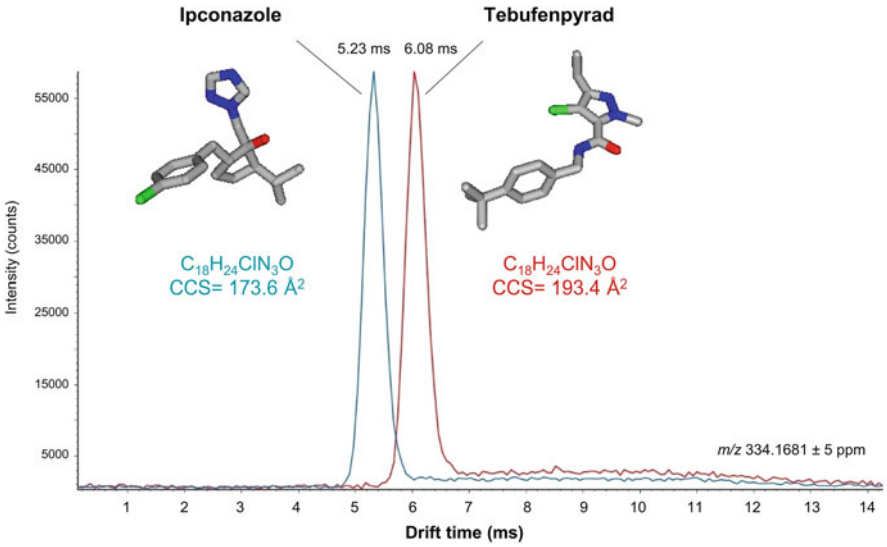


Fig. 10 Extracted ion mobiligrams obtained for the $[M + H]^+$ ions of the structural isomers ipconazole and tebufenpyrad. Conditions: trap bias, 40 V; IMS wave velocity, 250 m/s; IMS wave height, 45 V. Reprinted from "Ion-Mobility-Derived Collision Cross Section as an Additional Identification Point for Multiresidue Screening of Pesticides in Fish Feed," Analytical Chemistry, 2016, Volume 88, Pages 11169–11177 with permission from American Chemical Society [48]

3.4 Pharmaceuticals and Personal Care Products (PPCPs)

Klampfl and coworkers have demonstrated multiple recent instances of IMS-MS for detection of PPCPs. Mlynek et al. used HPLC-IMS-MS to investigate the presence of various xenobiotics and their metabolites in different plant species [54]. Specifically, they studied diclofenac (DCF), a representative non-steroidal anti-inflammatory drug (NSAID), and positively identified 23 previously undetected DCF metabolites using a combination of retention time, drift time, fragment spectra, and accurate mass. Mlynek et al. later investigated phase II metabolites (e.g., glucose, glucuronic acid, and ammonia adducts) of several other NSAIDs (ketoprofen, mefenamic acid, and naproxen) by combining their experimental methods with an in silico database that provided unique identifiers for 41 new metabolites [55]. Similar approaches were undertaken to study uptake of sunscreen ingredients by duckweed [56] and the antihypertensive drug telmisartan [57] and statins and statins by cress [58].

References

1. Eiceman GA, Karpas Z (2005) Ion mobility spectrometry. CRC Press, Boca Raton
2. Gabelica V, Marklund E (2018) Fundamentals of ion mobility spectrometry. Curr Opin Chem Biol 42:51–59. https://doi.org/10.1016/j.cbpa.2017.10.022
3. Kliman M, May JC, McLean JA (2011) Lipid analysis and lipidomics by structurally selective ion mobility-mass spectrometry. Biochim Biophys Acta Mol Cell Biol Lipids 1811:935–945. https://doi.org/10.1016/j.bbalip.2011.05.016
4. Shvartsburg AA, Smith RD (2008) Fundamentals of traveling wave ion mobility spectrometry. Anal Chem 80:9689–9699. https://doi.org/10.1021/ac8016295
5. Shvartsvurg AA (2008) Differential ion mobility spectrometry: nonlinear transport and fundamentals of FAIMS. CRC Press, Boca Raton
6. Winklmayr W, Reischl G, Lindner AO, Berner A (1991) A new electromobility spectrometer for the measurement of aerosol size distributions in the size range from 1 to 1000 nm. J Aerosol Sci 22:289–296
7. Ridgeway ME, Lubeck M, Jordens J, Mann M, Park MA (2018) Trapped ion mobility spectrometry: a short review. Int J Mass Spectrom 425:22–35. https://doi.org/10.1016/j.ijms.2018.01.006
8. Creaser CS, Griffiths JR, Bramwell CJ, Noreen S, Hill CA, Thomas CLP (2004) Ion mobility spectrometry: a review. Part 1. Structural analysis by mobility measurement. Analyst 129:984–994
9. Zhou Z, Tu J, Zhu ZJ (2018) Advancing the large-scale CCS database for metabolomics and lipidomics at the machine-learning era. Curr Opin Chem Biol 42:34–41. https://doi.org/10.1016/j.cbpa.2017.10.033
10. Zhou Z, Xiong X, Zhu ZJ (2017) MetCCS predictor: a web server for predicting collision cross-section values of metabolites in ion mobility-mass spectrometry based metabolomics. Bioinformatics 33:2235–2237
11. Eiceman GA, Stone JA (2004) Ion mobility spectrometers in national defense. Anal Chem 76:390–397
12. Allison TM (2019) Ion mobility in structural biology. In: Comprehensive analytical chemistry. Elsevier, pp 161–195
13. Chouinard CD, Nagy G, Smith RD, Baker ES (2019) Ion mobility-mass spectrometry in metabolomic, lipidomic, and proteomic analyses. Compr Anal Chem 83:123–159. https://doi.org/10.1016/bs.coac.2018.11.001
14. Chouinard CD, Wei MS, Beekman CR, Kemperman RHJ, Yost RA (2016) Ion mobility in clinical analysis: current progress and future perspectives. Clin Chem 62:124–133. https://doi.org/10.1373/clinchem.2015.238840
15. Webb IK, Garimella SVB, Tolmachev AV, Chen TC, Zhang X, Norheim RV, Prost SA, LaMarche B, Anderson GA, Ibrahim YM, Smith RD (2014) Experimental evaluation and optimization of structures for lossless ion manipulations for ion mobility spectrometry with time-of-flight mass spectrometry. Anal Chem 86:9169–9176. https://doi.org/10.1021/ac502055e
16. Giles K, Ujma J, Wildgoose J, Pringle S, Richardson K, Langridge D, Green M (2019) A cyclic ion mobility-mass spectrometry system. Anal Chem 91:8564–8573. https://doi.org/10.1021/acs.analchem.9b01838
17. Yin J, Wu M, Lin R, Li X, Ding H, Han L, Yang W, Song X, Li W, Qu H, Yu H, Li Z (2021) Application and development trends of gas chromatography–ion mobility spectrometry for traditional Chinese medicine, clinical, food and environmental analysis. Microchem J 168:106527. https://doi.org/10.1016/j.microc.2021.106527
18. Food safety and inspection service: USDA Chemistry Laboratory guidebook. https://www.fsis.usda.gov/news-events/publications/chemistry-laboratory-guidebook
19. Willems JL, Khamis MM, Mohammed Saeid W, Purves RW, Katselis G, Low NH, El-Aneed A (2016) Analysis of a series of chlorogenic acid isomers using differential ion mobility and

tandem mass spectrometry. Anal Chim Acta 933:164–174. https://doi.org/10.1016/j.aca.2016.05.041
20. McCullagh M, Douce D, Van Hoeck E, Goscinny S (2018) Exploring the complexity of steviol glycosides analysis using ion mobility mass spectrometry. Anal Chem 90:4585–4595. https://doi.org/10.1021/acs.analchem.7b05002
21. Chen C, Yang X, Liu S, Zhang M, Wang C, Xia X, Lou Y, Xu H (2021) The effect of lipid metabolism regulator anthocyanins from Aronia melanocarpa on 3T3-L1 preadipocytes and C57BL/6 mice via activating AMPK signaling and gut microbiota, vol 12. Food Funct, pp 6254–6270
22. Liu W, Zhang X, Siems WF, Hill HH, Yin D (2015) Rapid profiling and identification of anthocyanins in fruits with Hadamard transform ion mobility mass spectrometry. Food Chem 177:225–232. https://doi.org/10.1016/j.foodchem.2015.01.034
23. Potter CM, Jones GR, Barnes S, Jones DL (2021) Quantitative and qualitative analysis of edible oils using HRAM MS with an atmospheric pressure chemical ionisation (APCI) source. J Food Compos Anal 96:103760. https://doi.org/10.1016/j.jfca.2020.103760
24. Canellas E, Vera P, Nerín C, Dreolin N, Goshawk J (2020) Ion mobility quadrupole time-of-flight high resolution mass spectrometry coupled to ultra-high pressure liquid chromatography for identification of non-intentionally added substances migrating from food cans. J Chromatogr A 1616:460778. https://doi.org/10.1016/j.chroma.2019.460778
25. Liedtke S, Seifert L, Ahlmann N, Hariharan C, Franzke J, Vautz W (2018) Coupling laser desorption with gas chromatography and ion mobility spectrometry for improved olive oil characterisation. Food Chem 255:323–331. https://doi.org/10.1016/j.foodchem.2018.01.193
26. Medina S, Pereira JA, Silva P, Perestrelo R, Câmara JS (2019) Food fingerprints – a valuable tool to monitor food authenticity and safety. Food Chem 278:144–162. https://doi.org/10.1016/j.foodchem.2018.11.046
27. Alikord M, Mohammadi A, Kamankesh M, Shariatifar N (2021) Food safety and quality assessment: comprehensive review and recent trends in the applications of ion mobility spectrometry (IMS). Crit Rev Food Sci Nutr 0:1–34. https://doi.org/10.1080/10408398.2021.1879003
28. García-Nicolás M, Arroyo-Manzanares N, Arce L, Hernández-Córdoba M, Viñas P (2020) Headspace gas chromatography coupled to mass spectrometry and ion mobility spectrometry: classification of virgin olive oils as a study case. Foods 9. https://doi.org/10.3390/foods9091288
29. Gerhardt N, Birkenmeier M, Sanders D, Rohn S, Weller P (2017) Resolution-optimized headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS) for non-targeted olive oil profiling. Anal Bioanal Chem 409:3933–3942. https://doi.org/10.1007/s00216-017-0338-2
30. Arroyo-Manzanares N, Martín-Gómez A, Jurado-Campos N, Garrido-Delgado R, Arce C, Arce L (2018) Target vs spectral fingerprint data analysis of Iberian ham samples for avoiding labelling fraud using headspace – gas chromatography–ion mobility spectrometry. Food Chem 246:65–73. https://doi.org/10.1016/j.foodchem.2017.11.008
31. Arroyo-Manzanares N, García-Nicolás M, Castell A, Campillo N, Viñas P, López-García I, Hernández-Córdoba M (2019) Untargeted headspace gas chromatography – ion mobility spectrometry analysis for detection of adulterated honey. Talanta 205:120123. https://doi.org/10.1016/j.talanta.2019.120123
32. Yan S, Song M, Wang K, Fang X, Peng W, Wu L, Xue X (2021) Detection of acacia honey adulteration with high fructose corn syrup through determination of targeted α-Dicarbonyl compound using ion mobility-mass spectrometry coupled with UHPLC-MS/MS. Food Chem 352:129312. https://doi.org/10.1016/j.foodchem.2021.129312
33. Causon TJ, Ivanova-Petropulos V, Petrusheva D, Bogeva E, Hann S (2019) Fingerprinting of traditionally produced red wines using liquid chromatography combined with drift tube ion mobility-mass spectrometry. Anal Chim Acta 1052:179–189. https://doi.org/10.1016/j.aca.2018.11.040

34. Vautz W, Baumbach JI, Jung J (2004) Continuous monitoring of the fermentation of beer by ion mobility spectrometry. Int J Ion Mobil Spectrom 7:3–5
35. Cavanna D, Zanardi S, Dall'Asta C, Suman M (2019) Ion mobility spectrometry coupled to gas chromatography: a rapid tool to assess eggs freshness. Food Chem 271:691–696. https://doi.org/10.1016/j.foodchem.2018.07.204
36. Ahmed E, Mohibul Kabir KM, Wang H, Xiao D, Fletcher J, Donald WA (2019) Rapid separation of isomeric perfluoroalkyl substances by high-resolution differential ion mobility mass spectrometry. Anal Chim Acta 1058:127–135. https://doi.org/10.1016/j.aca.2019.01.038
37. Dodds JN, Hopkins ZR, Knappe DRU, Baker ES (2020) Rapid characterization of per- and polyfluoroalkyl substances (PFAS) by ion mobility spectrometry-mass spectrometry (IMS-MS). Anal Chem 92:4427–4435. https://doi.org/10.1021/acs.analchem.9b05364
38. Luo YS, Aly NA, McCord J, Strynar MJ, Chiu WA, Dodds JN, Baker ES, Rusyn I (2020) Rapid characterization of emerging per- and polyfluoroalkyl substances in aqueous film-forming foams using ion mobility spectrometry-mass spectrometry. Environ Sci Technol 54:15024–15034. https://doi.org/10.1021/acs.est.0c04798
39. Yukioka S, Tanaka S, Suzuki Y, Fujii S, Echigo S (2020) A new method to search for per- and polyfluoroalkyl substances (PFASs) by linking fragmentation flags with their molecular ions by drift time using ion mobility spectrometry. Chemosphere 239:124644. https://doi.org/10.1016/j.chemosphere.2019.124644
40. Yukioka S, Tanaka S, Suzuki Y, Echigo S, Fujii S (2021) Data-independent acquisition with ion mobility mass spectrometry for suspect screening of per- and polyfluoroalkyl substances in environmental water samples. J Chromatogr A 1638:461899. https://doi.org/10.1016/j.chroma.2021.461899
41. Adams KJ, Smith NF, Ramirez CE, Fernandez-Lima F (2018) Discovery and targeted monitoring of polychlorinated biphenyl metabolites in blood plasma using LC-TIMS-TOF MS. Int J Mass Spectrom 427:133–140. https://doi.org/10.1016/j.ijms.2017.11.009
42. Zheng X, Dupuis KT, Aly NA, Zhou Y, Smith FB, Tang K, Smith RD, Baker ES (2018) Utilizing ion mobility spectrometry and mass spectrometry for the analysis of polycyclic aromatic hydrocarbons, polychlorinated biphenyls, polybrominated diphenyl ethers and their metabolites. Anal Chim Acta 1037:265–273. https://doi.org/10.1016/j.aca.2018.02.054
43. Zheng X, Aly NA, Zhou Y, Dupuis KT, Bilbao A, Paurus VL, Orton DJ, Wilson R, Payne SH, Smith RD, Baker ES (2017) A structural examination and collision cross section database for over 500 metabolites and xenobiotics using drift tube ion mobility spectrometry. Chem Sci 8:7724–7736. https://doi.org/10.1039/c7sc03464d
44. Olanrewaju CA, Ramirez CE, Fernandez-Lima F (2021) Comprehensive screening of polycyclic aromatic hydrocarbons and similar compounds using GC–APLI–TIMS–TOFMS/GC–EI–MS. Anal Chem 93(15):6080–6087. https://doi.org/10.1021/acs.analchem.0c04525
45. Sabo M, Matejčík Š (2012) Corona discharge ion mobility spectrometry with orthogonal acceleration time of flight mass spectrometry for monitoring of volatile organic compounds. Anal Chem 84:5327–5334. https://doi.org/10.1021/ac300722s
46. Heptner A, Reinecke T, Langejuergen J, Zimmermann S (2014) A gated atmospheric pressure drift tube ion mobility spectrometer-time-of-flight mass spectrometer. J Chromatogr A 1356:241–248. https://doi.org/10.1016/j.chroma.2014.06.059
47. Allers M, Timoumi L, Kirk AT, Schlottmann F, Zimmermann S (2018) Coupling of a high-resolution ambient pressure drift tube ion mobility spectrometer to a commercial time-of-flight mass spectrometer. J Am Soc Mass Spectrom 29:2208–2217. https://doi.org/10.1007/s13361-018-2045-4
48. Regueiro J, Negreira N, Berntssen MHG (2016) Ion-mobility-derived collision cross section as an additional identification point for multiresidue screening of pesticides in fish feed. Anal Chem 88:11169–11177. https://doi.org/10.1021/acs.analchem.6b03381
49. Regueiro J, Negreira N, Hannisdal R, Berntssen MHG (2017) Targeted approach for qualitative screening of pesticides in salmon feed by liquid chromatography coupled to traveling-wave ion

mobility/quadrupole time-of-flight mass spectrometry. Food Control 78:116–125. https://doi.org/10.1016/j.foodcont.2017.02.053
50. Bauer A, Kuballa J, Rohn S, Jantzen E, Luetjohann J (2018) Evaluation and validation of an ion mobility quadrupole time-of-flight mass spectrometry pesticide screening approach. J Sep Sci 41:2178–2187. https://doi.org/10.1002/jssc.201701059
51. Bauer A, Luetjohann J, Hanschen FS, Schreiner M, Kuballa J, Jantzen E, Rohn S (2018) Identification and characterization of pesticide metabolites in Brassica species by liquid chromatography travelling wave ion mobility quadrupole time-of-flight mass spectrometry (UPLC-TWIMS-QTOF-MS). Food Chem 244:292–303. https://doi.org/10.1016/j.foodchem.2017.09.131
52. Goscinny S, McCullagh M, Far J, De Pauw E, Eppe G (2019) Towards the use of ion mobility mass spectrometry derived collision cross section as a screening approach for unambiguous identification of targeted pesticides in food. Rapid Commun Mass Spectrom 33:34–48. https://doi.org/10.1002/rcm.8395
53. Chen X-P, Zhang F, Guo Y-L (2019) Validating an ion mobility spectrometry-quadrupole time of flight mass spectrometry method for high-throughput pesticide screening. Analyst 144:4835–4840
54. Mlynek F, Himmelsbach M, Buchberger W, Klampfl CW (2020) A new analytical workflow using HPLC with drift-tube ion-mobility quadrupole time-of-flight/mass spectrometry for the detection of drug-related metabolites in plants. Anal Bioanal Chem 412:1817–1824. https://doi.org/10.1007/s00216-020-02429-7
55. Mlynek F, Himmelsbach M, Buchberger W, Klampfl CW (2021) A fast-screening approach for the tentative identification of drug-related metabolites from three non-steroidal anti-inflammatory drugs in hydroponically grown edible plants by HPLC-drift-tube-ion-mobility quadrupole time-of-flight mass spectrometry. Electrophoresis 42:482–489. https://doi.org/10.1002/elps.202000292
56. Seyer A, Mlynek F, Himmelsbach M, Buchberger W, Klampfl CW (2020) Investigations on the uptake and transformation of sunscreen ingredients in duckweed (Lemna gibba) and Cyperus alternifolius using high-performance liquid chromatography drift-tube ion-mobility quadrupole time-of-flight mass spectrometry. J Chromatogr A 1613:460673. https://doi.org/10.1016/j.chroma.2019.460673
57. Lang T, Himmelsbach M, Mlynek F, Buchberger W, Klampfl CW (2021) Uptake and bio-transformation of telmisartan by cress (Lepidium sativum) from sewage treatment plant effluents using high-performance liquid chromatography/drift-tube ion-mobility quadrupole time-of-flight mass spectrometry. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-021-14289-4
58. Emhofer L, Himmelsbach M, Buchberger W, Klampfl CW (2019) High-performance liquid chromatography drift-tube ion-mobility quadrupole time-of-flight/mass spectrometry for the identity confirmation and characterization of metabolites from three statins (lipid-lowering drugs) in the model plant cress (Lepidium sativum) after uptake from water. J Chromatogr A 1592:122–132. https://doi.org/10.1016/j.chroma.2019.01.049

Mass Spectrometry Imaging in Food and Environmental Chemistry

Katherine A. Maloof and Kevin R. Tucker

Contents

Abstract Mass spectrometry imaging (MSI) is a label-free, nonspecific tool that is effective in characterizing analytes from the surface of samples in situ by creating a two-dimensional image with high spatial resolution. Gaining molecular spatial information produces further insight into cellular processes, allowing for limitless possibilities when it comes to investigating food and environmental substances. MSI can be used exclusively to reveal the localization of material or in conjunction with other techniques to corroborate findings. In the field of food and environmental chemistry, MSI is slowly becoming a more popular technique owing to its countless applications and ease of use. MSI is governed by the ionization technique employed, and each ionization type has its own set of characteristics that control what classes of analytes can be studied. In this chapter, the applications of MSI within food and environmental chemical research are presented focusing on each of the ionization

K. A. Maloof and K. R. Tucker (✉)
Southern Illinois University Edwardsville, Edwardsville, IL, USA
e-mail: kmaloof@siue.edu; kevtuck@siue.edu

Yolanda Picó and Julian Campo (eds.), *Mass Spectrometry in Food and Environmental Chemistry*, Hdb Env Chem (2023) 119: 333–358, DOI 10.1007/698_2022_884,

Published online: 7 October 2022

techniques and their specific characteristics that make them appropriate for the individual examples highlighted.

Keywords Environmental analysis techniques, Environmental sampling, Mass spectrometry imaging, Matrix-assisted laser desorption/ionization, Multidimensional data, Sample preparation

1 Introduction

Over the last decade, there have been substantial increases in the amount of research dedicated to food and environmental science. This is due in part to the consumer's growing concern for safer foods and improving the environment. There has been a push for more research into eliminating harmful emerging contaminants from the environment [1], ridding marine environments of evasive microplastics [2], investigating the effects of pesticides on human health [3], and finding non-toxic substitutes for agrochemicals [4]. Safer food is also a concern with research efforts dedicated to detecting pesticides on the surface of food products and preventing adulterated food from entering the market [5]. Mass spectrometry imaging (MSI) has also been used to investigate endogenous molecules found in plant organ tissues to give a detailed look into how changes in the quantities of endogenous molecules affect the growth and development process of plants [6]. In this chapter, both dominant and novel ionization types used for MSI in food and environmental science and the advancements in each with the most recent, notable examples in the field will be explored.

MSI is preferred for specific applications where spatial information is relevant over the quantitative information provided by traditional analysis methods like gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LS-MS). As a sample is probed by laser beam, ion beam, or stream of charged droplets, a mass spectrum is collected at each individual pixel, ion intensity is plotted as a function of space for every *m/z* value, and a two-dimensional image can be generated from the individual mass spectra created for a specific *m/z* range. Each picture is generated based on the intensity of signal over a specific *m/z* window that the user defines. This picture shows a spatial analyte map where the abundances of analytes are depicted based on a defined color scale.

Choosing the best ionization method is critical for the successful analysis of target molecules. There are ongoing research efforts to achieve truly comprehensive analysis, but currently there is no ionization technique that allows for the detection of all compound types simultaneously [7]. In food and environmental science, there are thousands of possible compounds to analyze, each class with its own unique properties. One may want to analyze small molecule plant metabolites or larger molecules like proteins in food stuffs. Secondary ion mass spectrometry (SIMS) is better suited for atomics and very small molecules and matrix-assisted laser desorption/ionization (MALDI) was developed for handling larger molecular species,

while desorption electrospray ionization (DESI) works between those ranges. When ionizing molecules, hard and soft ionization techniques both exist within imaging techniques. Hard ionization techniques, like SIMS, transfer a large amount of energy to the target compound causing a high degree of fragmentation of precursor ions. This is evident within the spectrum as many peaks – most of them fragments – appear, making it difficult to detect the molecular ion, sometimes even in a pure sample. Since the first use of MALDI MSI in 1998 [8], soft ionization MSI has evolved into a highly selective and robust technique for qualitative and quantitative analysis of two- and three-dimensional media. Soft ionization does not impart as much energy, allowing the precursor ion to remain intact and providing information about the intact molecule [9, 10].

Throughput is a consideration for some fields of research. SIMS is slower than MALDI because achieving high resolution takes time. There are two modes to choose from in MSI – microscope and microprobe mode. SIMS microscope mode has been widely commercialized due to its ability to quickly generate whole images in sequence rather than the traditional microprobe mode which creates images via splicing together each individual pixel [11]. It is easier to move an ion beam rapidly, whereas the laser beam is harder to manipulate quickly. However, the scientists at Bruker have developed an advanced optics system that moves a laser beam within the lens without repositioning the stage. Bruker's rapifleX® is the highest throughput MALDI-time-of-flight (TOF) MS on the market and promises reliable data within minutes. This drastically improved the time factor, cutting down the time of analysis nearly 90%. This leads to a higher throughput and analysis that would normally take 3 h now can be accomplished in just 20 min. Cao et al. used the rapifleX to investigate the nutritional and physical state of used cooking oil and fresh edible oil to authenticate its quality. Vegetable oil is an edible oil with significant nutritional value that is commonly used commercially for frying foods. Repeated use of edible oil for frying food reduces the oil's quality and its prolonged use can be harmful to public health [12]. The researchers determined a method for checking the quality of fresh cooking oil to confirm that it is pure and not contaminated with used oil. Figure 1 shows samples of olive oil that have been contaminated with various quantities of used deep-fried oil and gutter, or recycled, oil. This figure illustrates vegetable oil that was adulterated with a ratio of 10% or higher of deep-fried oil or gutter oil. This method can be used in the future to help uphold the quality of vegetable oil used for commercial cooking purposes [13].

Dynamic range, in this context, is the range in concentration of compounds an instrument can analyze simultaneously. A single MSI analysis enables the detection of many different compounds simultaneously but can have difficulty detecting analytes that are low in abundance while much more abundant species are present leading to the need for specialized sample preparation to remove abundant classes of molecules (e.g., lipids). The development of TOF-MS mass analyzer drastically improves the limit of detection of the ionization technique and is used in MSI with MALDI and SIMS [14].

There have also been strides made in food science to develop new ionization types that do not include organic matrices and instead employ nanoparticles like

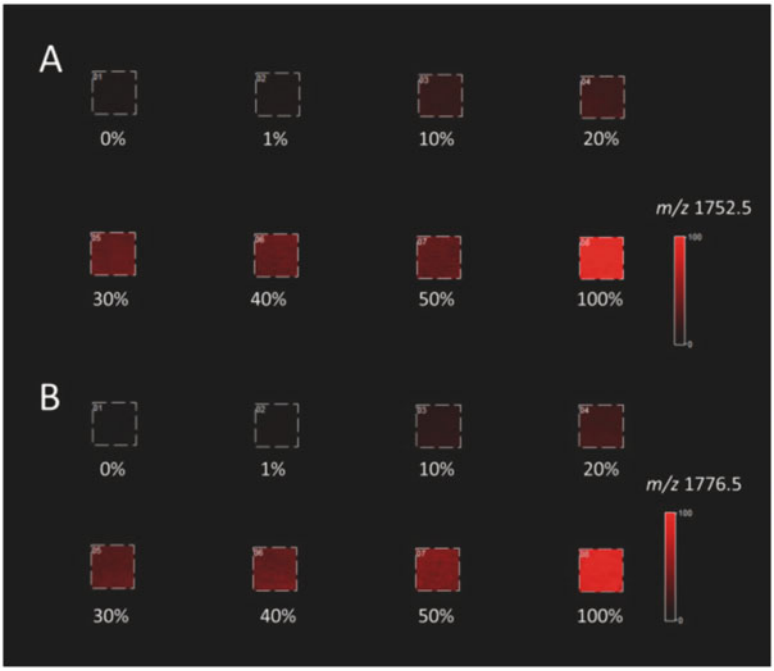


Fig. 1 Samples analyzed by MALDI-MSI show olive oil contaminated with varying volumes of (**a**) deep-fried oil and (**b**) gutter oil [13]

colloidal graphite (GALDI), silicon (DIOS)-MSI, and nanoparticle-enhanced target particles including gold (AuNPET) and silver (AgNPET). This technique called nanoparticle-based surface assisted laser desorption/ionization mass spectrometry imaging (NPs-ALDI-MSI) improves the selectivity and limit of detection but has difficulty ionizing all nanoparticles reasonably due to the complicated ternary interactions of the laser spot with the analytes and nanoparticles. Furthermore, these interactions are inconvenient to study under high vacuum conditions. Despite these complications, the future progress of new nanoparticle applications in MSI will result in advancements in food science [15].

2 Ionization Methods

2.1 Secondary Ion Mass Spectrometry (SIMS)

The first of the ionization types developed for MSI was secondary ion mass spectrometry (SIMS) in 1962 by Castaing and Slodzian [16]. SIMS is a hard ionization technique involving a high-energy primary ion beam that bombards the sample surface, smashing through the top surface layers and transferring energy

from those primary ions to the surface analytes in a process called secondary ionization. Static SIMS generates images with a spatial resolution between 1 and 5 μm, while TOF-SIMS achieves submicron spatial resolutions with high mass resolution ($m/\Delta m$ ~10,000) [17], which makes it the preferred ionization technique for small molecule and single cell analysis. The drawback of high-energy ion bombardment is that it causes fragmentation in the larger molecules and it damages the sample surface. The fragmentation limits the mass range of SIMS to less than 1,000 *m/z* [18]. However, because of the development of cluster ion beams, SIMS MSI has improved mass range up to 10,000 Da while maintaining its spatial resolution and improving the molecular ion yield [19, 20]. Ultimately, SIMS is widely utilized based on its unique capability for submicrometer spatial resolution.

2.2 MALDI

Developed in the late 1990s for imaging of larger molecules, MALDI is a soft ionization technique that involves the use of a sample coated in matrix to absorb energy from laser ablation converting analytes to the gas phase to be ionized [21]. When combined with a TOF analyzer, MALDI MSI has a mass range beyond 1,000,000 Da or 1 MDa [22]. In Boughton's review of mass spectrometry imaging for plant biology, MALDI was found to be the most common ionization type used in plant-based MSI research [23], which is likely due to its broad analyte coverage, high spatial resolution, and ease of use of commercial MALDI instruments. However, because MALDI is operated in microprobe mode, performing each scan is time-consuming. The spatial resolution of MALDI MSI is determined by the chosen matrix, matrix application technique, and the laser spot size [24]. Efforts have been made to improve the spatial resolution by reducing the laser spot size, using methods including a pinhole filter to achieve a 5 μm diameter [25] and implementing transition-mode geometry where the laser is projected through a target behind the sample rather than focused directly onto the matrix crystals which achieved a spot size of 1 μm [26]. In combination with transition-mode geometry, MALDI-2 – a post-ionization technique that uses a second laser pulse to generate ions from the original ion plume – can offer improved sensitivity up to three orders of magnitude, ultra-high spatial resolution, and improved ion yields [26, 27]. Heijs et al. used MALDI-2 to image N-glycans to study their role in cancer and auto-immune disease [28]. Parameters for negative-ion mode MALDI-2 MSI were optimized to generate high quality images of glycans in brain tissue as seen in Fig. 2. This same application of MSI could be extended to study foods high in N-glycan, which play an important role in promoting gut health in humans [29, 30]. MALDI-2 MSI was also used by Bien et al. to map sterols in marine flatworms to establish their role in a possible symbiotic relationship with algae [31]. This technique could be applied in the future to monitor environmental contamination by quickly detecting the presence of sterols in surface sediment [32].

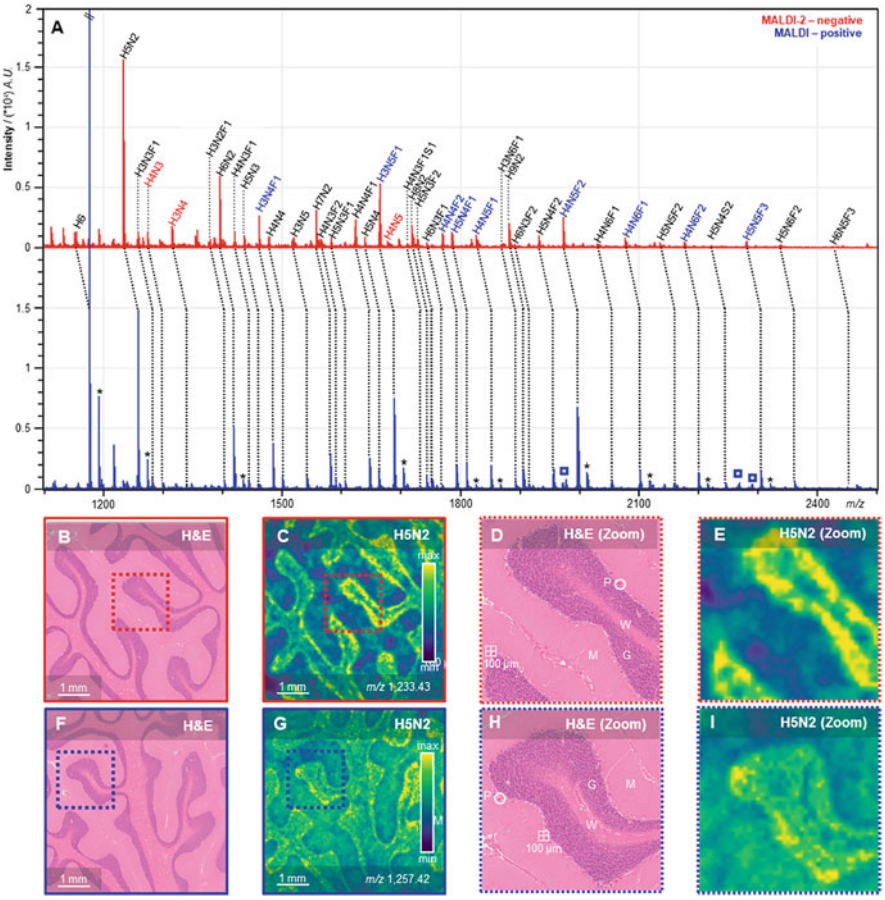


Fig. 2 (**a**) Mass spectra of MALDI-2-MSI operated in negative-ion mode (red) and MALDI-MSI in positive-ion mode (blue) to generate (**b–i**) images of *N*-glycan species in human brain tissue. The pink images are negative-ion mode MALDI-2-MSI and the blue-green images are positive-ion mode MALDI-MSI [28]

2.3 Desorption Electrospray Ionization (DESI)

Developed in 2004, DESI uses an electrospray ionization (ESI) source to spray the sample surface with charged microdroplets of solvent to desorb and ionize analyte ions simultaneously [33]. This soft ionization technique is performed at ambient conditions without the need for laser or ion beam which allows for in-situ analysis of molecules up to 2,000 Da without any sample preparation requirements [34]. The most distinctive parameter for DESI is in regard to the angle and focus of the solvent sprayer [35]. DESI-MSI analyzes molecules on the very surface of samples and the electrospray jet is only capable of achieving spatial resolution down to 200 µm with DESI or 30 µm using nano-DESI [36]. However, the ease of ambient analysis makes

DESI a popular technique for imaging [33]. Gallage et al. used DESI-MSI to look at the localization of the flavor compound vanillin – and its glucose-conjugated form vanillin glucoside – in *Vanilla planifolia* pods. Vanilla is a globally popular flavor, and these researchers set out to better understand the biosynthesis and accumulation of its flavor compound, vanillin. DESI-MSI was used to confirm the results of ^{14}C-radiolabeling used to find locations of vanillin and vanillin glucoside biosynthesis activity. The DESI images showed these compounds to be present in the same tissues where the ^{14}C-radiolabeled precursors suggested vanillin biosynthesis takes place [37].

2.4 Other Ambient Ionization Types

While ionization methods involving a vacuum chamber attain better resolutions than ambient ionization methods, there are many advantages with ambient ionization. Since the development of ambient mass spectrometry in the form of DESI, other ambient techniques have gained popularity in MSI [38]. Being able to analyze samples in their native environment reduces the risk of sample changes due to introduction into the vacuum system, preserves volatile compounds, and does not involve time-consuming sample preparation [38, 39]. The following ambient techniques have been applied to MSI and are related to food and environmental science.

2.4.1 Laser Ablation Electrospray Ionization (LAESI)

LAESI combines the use of a mid-IR laser beam with a nanoelectrospray source [40]. This ambient ionization technique is matrix free, high throughput and requires no sample preparation [41]. It is used in samples with a high-water content because the IR laser corresponds to the OH vibrations of the water molecule [41]. This makes LAESI perfect for the analysis of living tissue. LAESI can also be used on samples with rough surfaces without sample treatment [42]. Kooijman et al. created a probe that combines ESI with atmospheric pressure chemical ionization (APCI) and is compatible with commercial LAESI instruments. They improved the pixel-to-pixel repeatability for both ionization methods and successfully imaged the fungicide Isotianil and its metabolites from tomato plant leaves [43]. Spatial resolution in LAESI can be achieved down to 30 μm as demonstrated by Shrestha et al. for imaging metabolites in onions associated with their purple pigmentation [44].

2.4.2 AP-MALDI

Atmospheric pressure (AP) MALDI is traditional MALDI that still requires sample tissue to be sliced and coated with a matrix but has the advantage of handling samples at atmospheric pressure. While analysis at atmospheric pressure has its

advantages, ion loss is experienced with this technique as ions travel from the ablation plume to the mass analyzer [45]. Li et al. were the first to use AP-MALDI MSI to gain spatial information about the distribution of endogenous metabolites in the endosperm of the coffee plant ripe fruit. The researchers studied eight varieties of coffee beans from regions in Africa, Central and South America, and Asia to investigate the differences in endogenous metabolites of coffee beans from wet and dry processing methods. Images of caffeine, sucrose, and caffeoylquinic distribution were visualized in both positive-ion and negative-ion mode with a spatial resolution of 75 μm as seen in Fig. 3. Colombia and Tanzania were found to have the highest levels of caffeine (*m/z* 195.09, $[M + H]^+$) and Colombia, Tanzania, Nicaragua, and China had the highest levels of sucrose (*m/z* 381.08, $[M + K]^+$). The distribution of caffeoylquinic acid (*m/z* 393.06, $[M + K]^+$) can also be seen [39].

2.4.3 Liquid Extraction Surface Analysis (LESA)

In 2011, Eikel et al. developed a fully automated LESA that uses a nanoelectrospray source applied via pipette tip to ionize surface compounds from fruits and vegetables to be analyzed by tandem mass spectrometry (MS/MS) [46]. Ten years later, in a study conducted by Xiao et al. LESA-MSI was used to study the distribution of perfluoroalkyl substances (PFASs) in zebrafish [47]. PFASs are highly stable synthetic chemicals that cause harm to the environment due to their ability to resist degradation. PFASs are known to be detrimental to humans, causing children to have adverse immune responses [48]. Determining the spatial distribution of PFASs throughout fish tissue would help to better understanding the mechanism of action of toxic compounds in fish. Figure 4 shows the distributions of perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in different regions of the zebrafish with the highest accumulation in the organs, roe, pelvic fin, and gills. While the spatial resolution is well below average due to the limitation of the pipette tip diameter (600 μm), it clearly shows where most of the PFASs accumulated in the body of the zebrafish [47]. This technique is to be used for imaging analysis that does not require high resolution, making it a good use for discovery purposes like guaranteeing that food surfaces do not contain any pesticides or harmful chemicals [48, 49].

2.4.4 Thermal Desorption Electrospray Ionization Mass Spectrometry (TD-ESI-MS/MS)

TD-ESI/MS is an ionization technique that uses an oven to desorb analytes from a metal sample probe. The analytes after desorbing are carried via nitrogen gas to the ESI plume. This technique is easy to use and allows for rapid analysis with no sample preparation. Jeng et al. imaged pesticide residues on the surface of strawberries by TD-ESI-MS/MS. The sampling probe – a 2 mm stainless steel inoculating loop attached to a handle – is swept across the surface of the strawberry for 1 cm

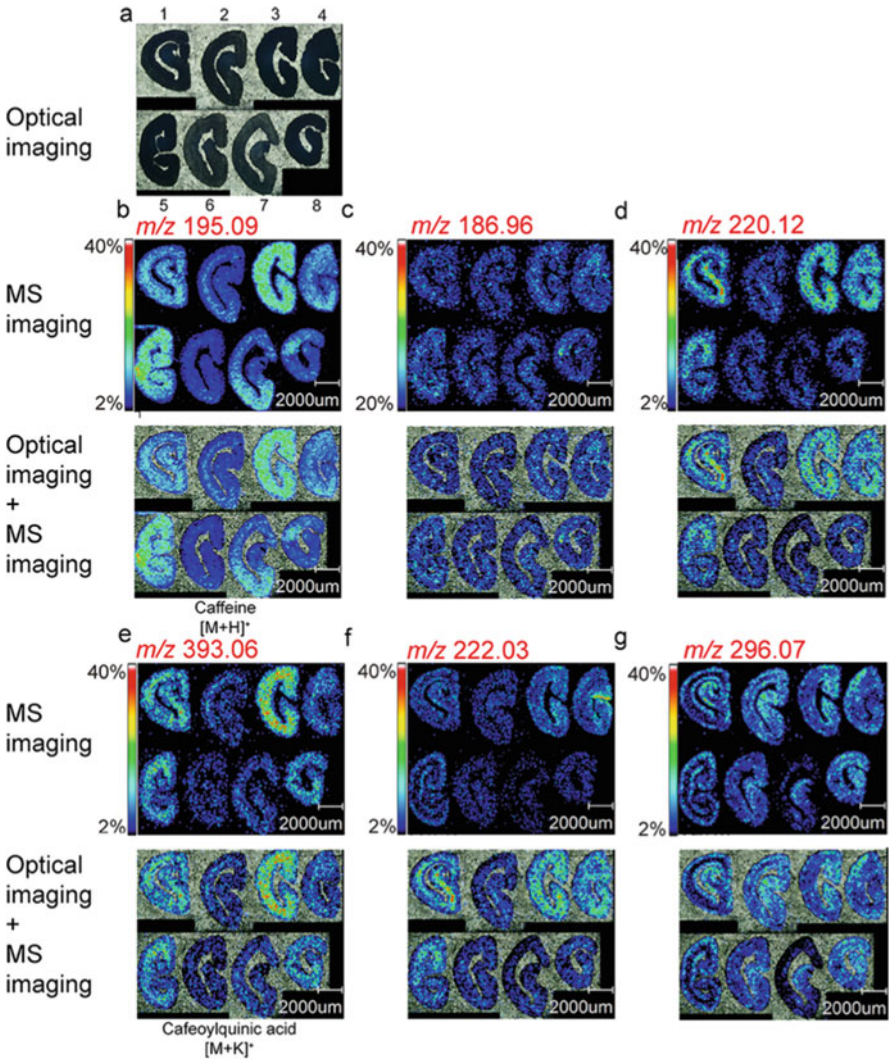


Fig. 3 Coffee bean sections analyzed via MALDI MSI show various distributions of caffeine, sucrose, and caffeoylquinic acid. (**a**) Eight coffee beans were imaged from 1, Ethiopia; 2, Brazil; 3, Colombia; 4, Nicaragua; 5, Tanzania; 6, Guatemala; 7, Uganda; 8, China. Distributions of (**b**) caffeine (*m/z* 195.09, $[M + H]^+$), (**c**) sucrose (*m/z* 381.08, $[M + K]^+$), and (**c**–**g**) caffeoylquinic acid (*m/z* 393.06, $[M + K]^+$) can be seen throughout the endosperm and outer edge of the beans [39]

once in each of the 40 predetermined sample locations. The probes were then placed into the TD-ESI unit where they are heated and sprayed with the electrospray source for desorption of analytes. The spatial resolution is not comparable to the other techniques currently being used in MSI, however, this technique could be used for

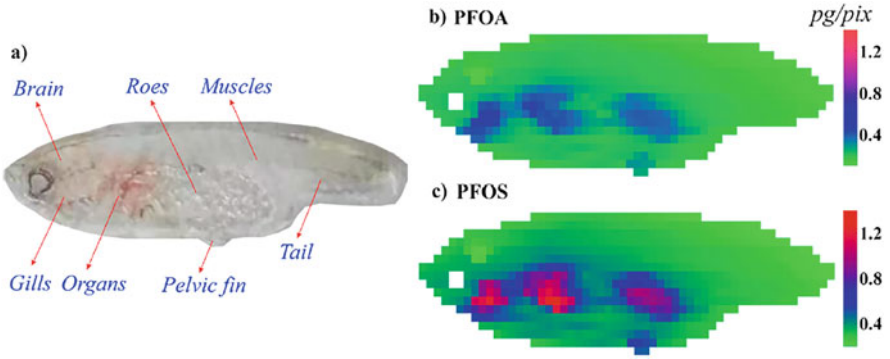


Fig. 4 (**a**) Optical image of a whole-body frozen section of zebrafish. Distributions of (**b**) perfluorooctanesulfonic acid (PFOS) and (**c**) perfluorooctanoic acid (PFOA) in different regions of the zebrafish with the highest accumulation seen in the gills, organs, roes, and pelvic fin with a spatial resolution of 600 μm [47]

many food quality and safety applications to confirm or deny the presence of adulterants [50].

3 Sample Preparation

Sample preparation is the act of treating a certain sample to prepare it for analysis. Although some methods require little to no sample preparation in MSI, it is one of the most important steps to guaranteeing accurate results. It is vital that samples are handled with extreme care and processed immediately after collection to ensure that the integrity of the sample is upheld, and no degradation occurs [6]. The goal of sample preparation is to retain all spatial details of the sample without degrading or changing its chemical structure so that the information gathered is a true representation of the analytes found on the surface. If samples are not prepared correctly, it will have negative impacts on analyte detection and can produce inaccurate results [51]. Any inconsistencies in sample preparation can skew analyte abundances making the reproducibility of sample handling techniques crucial components for successful MSI experiments.

Preparing samples for vacuum techniques in MSI involve freezing the sample, thinly sectioning it, thaw-mounted to a plate or slide, then drying for insertion into the vacuum [52]. When food and environmental science involves working with plant samples, preparation of those samples can be challenging. Plant samples are inherently more difficult to work with than mammalian samples due to the higher water content and lower lipid content of the tissue. While the walls of plant cells are more rigid, the complex cellular structure is inherently complicated and can be easily broken. This challenge has inspired various advances in the sample preparation techniques for plant tissue [53].

Histological sectioning is a popular method used for the preparation of root and stem tissue that can also be applied to fruit and vegetable tissue. Pereira et al. used this technique to detect fungicide 6 mm below the surface of apple tissue [54]. However, when dealing with tissues that are difficult to slice due to fragility or heavy water content, histological sectioning may not be ideal. Imprinting is the sample preparation technique used for more fragile sample types. Gold nanoparticle (AuNP)-immersed paper imprinting MSI can be used on delicate tissue samples to visualize pesticide metabolites on the surface and internally [5, 55].

One limitation in laser based MSI experiments is that samples need to be relatively flat to allow the laser to properly focus and to ensure mass accuracy across the image. Topography of sample surfaces is nonexistent for tissues that have been sectioned via a microtome or cryostat, however, for the analysis of samples with variation in the surface topography like whole leaves or fruits and vegetables, it is harder to achieve acceptable resolution [42]. If the surface topography is not within the range of lateral resolution, with an aspect ratio near 1, false imaging results and signal variation can occur [56]. To combat this, MALDI MSI implements an autofocusing mode to investigate 3D sample surfaces. Using a triangulation laser system integrated with atmospheric pressure scanning microprobe matrix-assisted laser desorption/ionization (AP-SMALDI) MSI, height variations of up to 1.5 μm can be resolved. While this method gains resolution, the measurement time increases by 25% [57].

To help prevent tissue breakage or analyte delocalization, a pre-embedding medium that preserves the sample can be used. Using gelatin as embedding medium prior to mounting cryosectioned leaf tissue results in less diffusion of analytes and better preservation of the sample structure compared to ice and carboxymethyl cellulose [58]. Imprinting and fractioning are two complementary techniques that can also be used when preparing thin plant tissues as first demonstrated by Klein et al. to look at internal metabolites of soybean leaves via MALDI-MSI [59].

With any type of tissue in MSI, one challenge that must be overcome is analyte delocalization on the sample surface. When analytes diffuse on the surface of sample tissue, the image created will not be a true representation and causes results to be skewed. A large portion of food and environmental science studies plant material. The Kawamoto method – originally used to prepare cryosections of hard, fragile animal bone tissue to study the movement of calcium [60] – uses an adhesive film or tape to help preserve sample structure during the adhering process and is an alternative to thaw mounting. The thaw-mounted method uses the heat from the fingertip to adhere the sample to the plate for analysis which causes delocalization of analytes. Kim et al. used adhesive tape on sections of corn seed tissue and compared the results to the thaw-mounted method [61]. They demonstrated that using adhesive tape does not cause surface analyte delocalization, but the thaw-mounted method does which can be seen in Fig. 5. Enomoto successfully used this adhesive tape to keep sections of delicate strawberry tissue intact to study metabolite functions within [55].

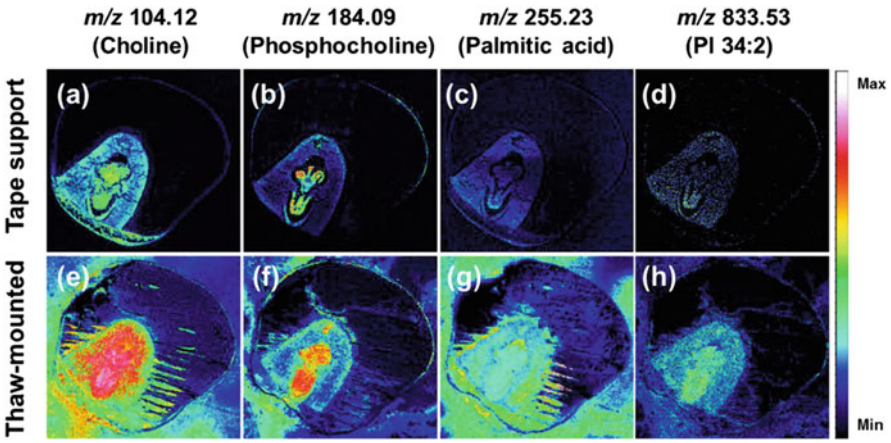


Fig. 5 Images of corn seed tissue by TOF-SIMS show excessive analyte diffusion in the thaw-mounted samples (**e-h**) as compared to the tape support method (**a-d**) for choline (**a, e**), phosphocholine (**b, f**), palmitic acid (**c, g**), and phosphatidylinositol (**d, h**). [61]

3.1 Secondary Ion Mass Spectrometry (SIMS)

Because SIMS ionization is performed in a vacuum, a big obstacle to overcome with SIMS MSI is the requirement that samples must be dried prior to placement in a high vacuum chamber. This can cause the sample to be damaged or altered in the process. The ultra-high vacuum pressure can also be problematic for samples that have a high-water content like plants, fruits, and vegetables. To date, there have been studies that use MeV-SIMS, a SIMS method that uses heavy, high-energy ions (e.g., $^{35}Cl^{6+}$ and $^{63}Cu^{4+}$) for ionization, in leaves [62, 63], but none that employ the use of Wet-SIMS. Wet-SIMS was developed to use a low vacuum and MeV-energy ion beams which allows for analysis of samples without drying steps. This technique could be useful to detect heavy metals, like lead, in delicate plant or produce tissue.

In 2021, SIMS was employed for the analysis of microplastics (MPs) in paramecia. MPs pose a large threat to the environment due to their harmful effects on living organisms. This study by Feng et al. offers a simple SIMS method for the in-situ analysis and imaging of six different types of MPs – with a size range between 1 and 50 μm – from paramecia that requires no sample preparation. In situ analysis of MPs via mass spectrometry is not typically performed due to the destructive sample pretreatment that is required to separate the MPs from the paramecia. The authors of this study were able to generate a spatial map of six types of MPs – polypropylene (PP), polyamide 6 (PA6), polyethylene terephthalate (PET), polymethyl methacrylate (PMMA), polyglycidyl methacrylate (GMA), and polyvinyl chloride (PVC) – ingested by paramecia with a high degree of chemical specificity, sensitivity, and spatial resolution of 1 μm (Fig. 6). The cellular structures of contaminated paramecia were also visualized to help investigate the interaction of plastics with living

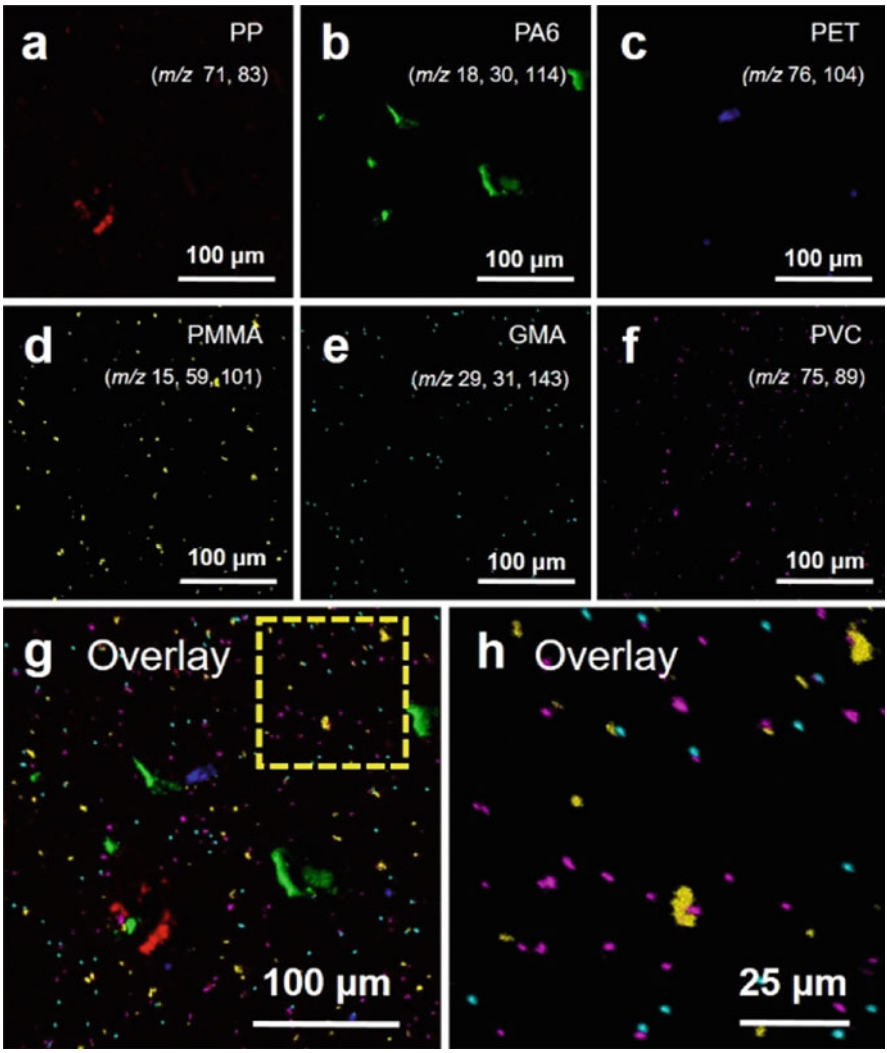


Fig. 6 Generated spatial map of six types of identified MPs. (**a**) PP (*m/z* 71, 83) (**b**) PA6 (*m/z* 18, 30, 114) (**c**) PET (*m/z* 76, 104) (**d**) PMMA (*m/z* 15, 59, 101) (**e**) GMA (*m/z* 29, 31, 143) (**f**) PVC (*m/z* 75, 89) (**g**) Merged image of MPs (**a**–**f**) (**h**) Zoomed in area from (**g**) [65]

organisms (Fig. 7). These findings will aid future studies to examine MPs influence in the environment [64].

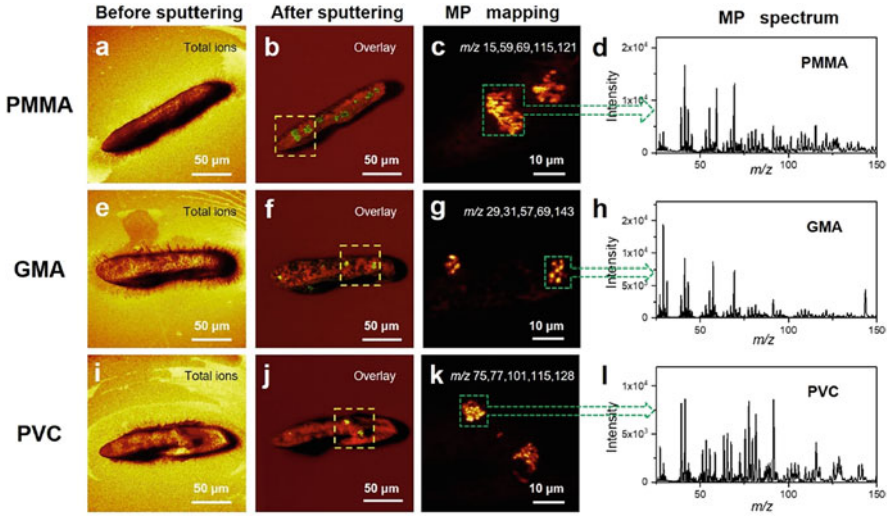


Fig. 7 The imaging of MPs in a paramecium in situ by TOF-SIMS. (**a**, **e**, **i**) Before SIMS sputtering, the total number of ions was seen. (**b**, **f**, **j**) After SIMS sputtering, the image of MPs inside the paramecium (**c**, **g**, **k**). The high-resolution images of MPs from the highlighted areas in (**b**, **f**, **j**). (**d**, **h**, **l**) PMMA, GMA, and PVC mass spectra collected from the paramecium [64]

3.2 MALDI

The focus of MALDI MSI sample preparation is the application of a matrix to aid in the ionization of surface analytes. The technique of application is important for creating quality images of high spatial resolution [45]. Homogenous application of the matrix is crucial which is commonly achieved using automated sprayers [65]. Sublimation is another common method that is a low-cost way to uniformly deposit dry matrix onto sample surfaces [66]. Traditional organic matrices cause spectral interferences for analysis of compounds with low molecular weight which makes application into small molecule investigations more challenging [67]. To avoid using a matrix, Enomoto et al. developed a new ionization method for MSI called desorption ionization using through-hole alumina membrane (DIUTHAME). They developed this method for analyzing large whole strawberry sections too large to fit onto glass slides for MALDI analysis [68].

3.3 Desorption Electrospray Ionization (DESI)

DESI-MSI is performed at ambient conditions and therefore does not require any sample preparation [69]. This is an advantage to DESI since sample preparation is time-consuming and expensive [22]. All that is required of DESI MSI is sectioning

and mounting of sample tissue which is nearly identical to the methods described above.

4 Specific Applications of MSI in Food and Environmental Science

Since the beginning of 2020, several related studies assess the quality of food products. Likely due to its popularity in commercial use and accounting for more than 50% of all publications referencing mass spectrometry imaging, MALDI MSI has been employed in these studies. Ambient techniques DESI and AP-SMALDI are also mentioned in this section due to their nondestructive nature even though they account for less than 20% of all similar publications.

Billions of people worldwide consume fish [70]. Since 30% of all fish products are frozen prior to being sold, the quality of frozen fish should be evaluated [71] because its nutritional and physical quality is deteriorated progressively. Morisasa et al. used MALDI MSI to detect formaldehyde (FA) in frozen fish filets to help determine their freshness. Trimethylamine N-oxide (TMAO), a natural compound found in the muscle tissue of sea fish, decomposes to equal parts dimethylamine (DMA) and formaldehyde (FA) in fish post-mortem [72]. FA denatures proteins and causes fish fillets to harden – a process that occurs after their frozen. This study used MSI to determine fish "hardness" by visualizing protein buildup in frozen fish that correlate with increasing concentrations of FA. As seen in Fig. 8, peaks at 624.2 *m/z* and 651.1 *m/z* were resolved showing peptides determining FA accumulation [73].

The proper storage of food is important to upholding its quality and to guarantee food safety. Glycoalkaloids (GAs) are toxic secondary metabolites that can form in solanaceous plants from improper storage due to temperature extremes, material damage, or exposure to light [74]. Deng et al. stored potato tubers for different lengths of time and used the iMScope to image the spatially resolved distribution of four GAs – dehydrochaconine, α-chaconine, dehydrosolanine, and α-solanine. In 2009, Harada et al. developed a "mass microscope" – which would later be developed and sold by Shimadzu as the iMScope [75] – to analyze samples of freshly cut ginger root. This instrument operates at atmospheric pressure to avoid damage to volatile metabolites or the water-rich structure of ginger rhizome. Coupled with atmospheric pressure quadrupole ion trap (AP-QIT) TOF MSI, the iMScope imaged GAs in the potato tubers with 5 μm resolution and saved the researchers time and money with limited sample preparation. As seen in Fig. 9, potato tubers were imaged at 0, 10, 15, 20, 30, 40, and 60 days showing increased accumulation of four toxic GAs. The images revealed that different parts of the potato tubers have different amount of GAs with the sprout showing the greatest increase over the 60 days. These findings can help procure future guidelines for the proper storage of potato tubers [76].

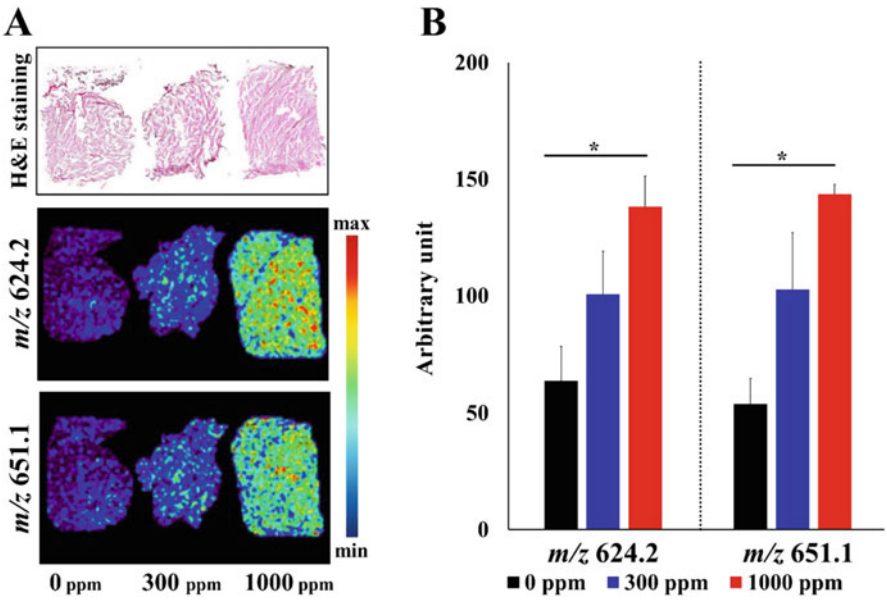


Fig. 8 (**a**) Three fish fillets treated with 0, 300, and 1,000 ppm FA were imaged by MALDI MSI and (**b**) their distinguished molecular ions shown ($n = 3$) [73]

Tea is the second most consumed beverage in the world – only coming in second to water [77]. This popular beverage is not only admired for its taste, but also for its numerous health benefits. Research shows that there are correlations with consumption of tea and higher likelihood for overcoming diseases like cancer [78], obesity [79], and cardiovascular disease [80] and can even lower the risk for type 2 diabetes [77]. There are many kinds of metabolites in tea leaves and studying their localization can offer insight into regulating and improving the quality of tea. Liao et al. used DESI MSI to show the catechin distribution found in tea leaves and roots. Catechins (flavan-3-ols) are specialized metabolites in tea leaves that have been found to have a positive impact on human health, and they also play a role in antistress regulation and pathogen defense in tea plants [81–83]. The nondestructive nature of DESI is what allows for the complete investigation of metabolites found on both sides of the tea leaves. Due to their high wax content, porous polytetrafluoroethylene (PTF) imprints were optimized and used as sample pretreatment. This study successfully imaged the localization of some fatty acids, polyphenols, and catechins within the tea tissue and will allow for future research into determining their function. In Fig. 10, different concentrations of catechins can be seen throughout various structures of the tea leaves showing that distributions of catechins are not uniform throughout the individual features of the tea leaf [84].

Honeybees play an important role in the environment because they are responsible for the pollination of many plants and crops around the world [85]. Bees are greatly affected by exposure to insecticides. When bees are exposed to them, it

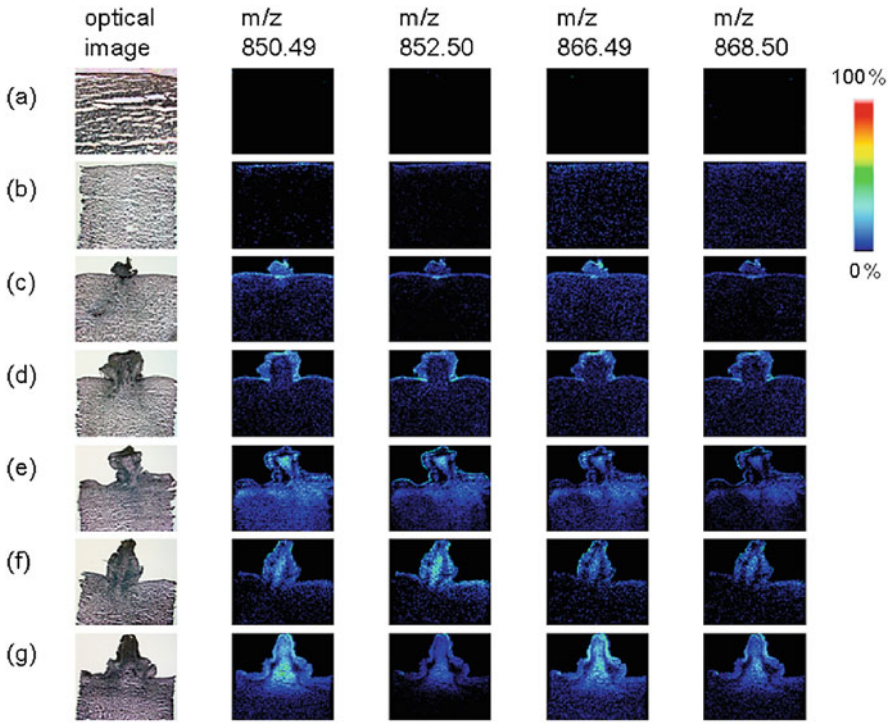


Fig. 9 Four toxic GAs, dehydrochaconine ($[M + H]^+$ at *m/z* 850.49), α-chaconine ($[M + H]^+$ at *m/z* 852.50), dehydrosolanine ($[M + H]^+$ at *m/z* 866.49), and α-solanine ($[M + H]^+$ at *m/z* 868.50), in potato tubers were imaged by the iMScope at varying storage times: (**a**) 0 days (**b**) 10 days (**c**) 15 days (**d**) 20 days (**e**) 30 days (**f**) 40 days and (**g**) 60 days [76]

affects the bee's behavior and can threaten their ability to function making them more vulnerable to disease [86] and causes entire colony to disappear [87]. Neonicotinoids are the most used class of insecticides globally due to their success in target pests [88]. In 2017, neonicotinoids were detected in 75% of honey samples collected from all continents and are believed to be the cause of the global decline of honeybees [89]. An increasing number of studies have been addressed to determine their exact mechanism of toxicity in bees, but the spatiotemporal distribution of neonicotinoids has yet to be understood. Zhang et al. employed MALDI MSI to visualize neonicotinoid distribution in honeybees. Healthy adult honeybees were captured and exposed orally and via direct contact to two neonicotinoids, dinotefuran and acetamiprid with co-application of the fungicide tebuconazole. After collection, the bees were frozen at −80°C, embedded with optimum cutting temperature (OCT) as per Phan et al. [90] and sliced at −20°C to a thickness of 25 μm using a freezing microtome (CM1950, Leica Microsystems Inc., Wetzlar, Germany) to obtain whole-body, parallel sections were cut from forehead to thorax. After homogenous matrix application, the deposited neonicotinoids were analyzed by MALDI MSI using a TOF-MS that was built and modified by the researchers.

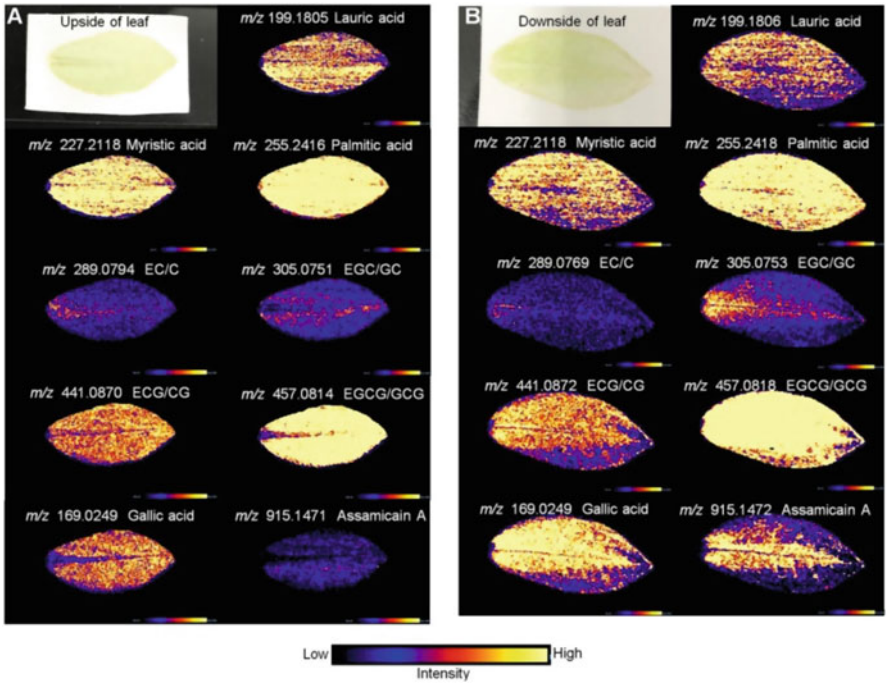


Fig. 10 Both sides of tea leaves were imaged by DESI-MSI to reveal the spatial distributions of specialized metabolites. (**a**) The front side of imprinted tea leaf and metabolites. (**b**) The back side of the imprinted tea leaf and metabolites [84]

The laser used was focused to 30 μm using an aspheric lens and pulsed three times for 5 ns per pixel to create each image. The images generated showed that both dinotefuran and acetamiprid rapidly penetrate the honeybee's tissues dispersing throughout the whole-body sections in just 2 h as shown in Fig. 11. This figure illustrates that when pretreated with piperonyl butoxide (PBO), an insecticide synergist, acetamiprid becomes 14 times more toxic than dinotefuran. Upon further examination, the degradation rates of dinotefuran and acetamiprid were found to be different. It was determined from the spatiotemporal distributions that these degradation rates cause the toxicity rather than their ability to penetrate through their various barriers. These findings can be used for further research into mechanistic determination of insecticides in honeybees [91].

Plants are incredibly adaptable, able to conform in many ways to enhance their survival. Because they are immobile, plants have developed ways to control the environment around them by communicating with other plant species through airborne signals [92] or by exchanging compounds via the soil–root interface [93]. Plants react to foreign substances by producing metabolites for defense or enzymes to degrade xenobiotics [94, 95]. Current research focuses mainly on the study of compounds that are harmful to plants, but few study the relationship between those compounds and plants in terms of uptake, distribution, and

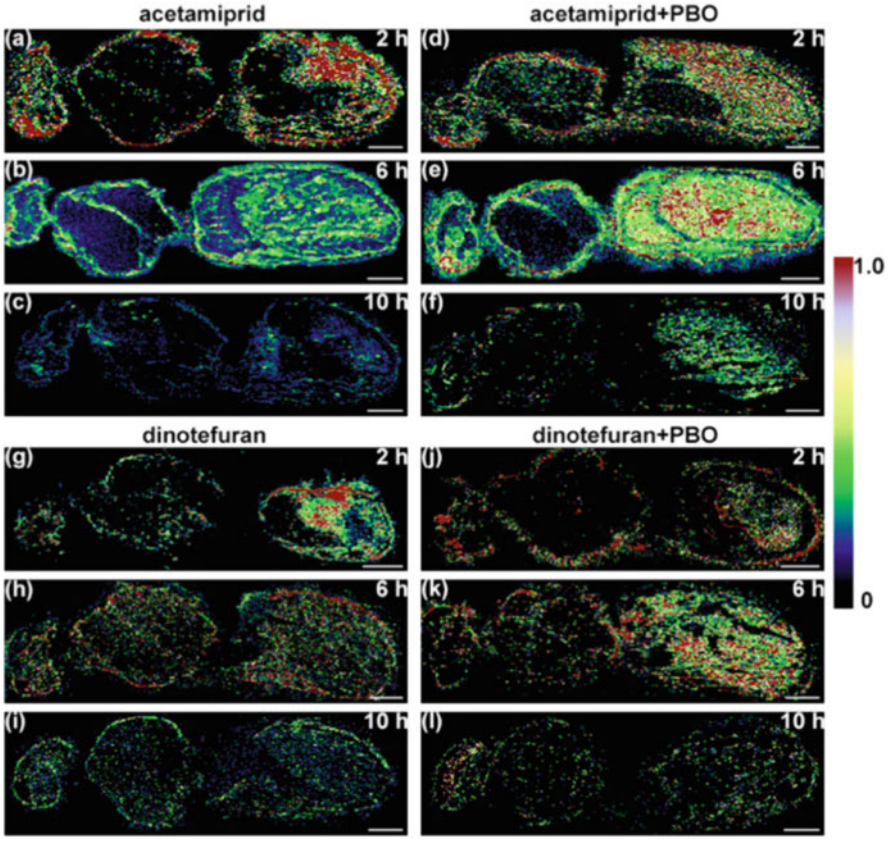


Fig. 11 Images of whole-body honeybee sections by MALDI MSI showing acetamiprid and dinotefuran after different exposure times. Acetamiprid (*m/z* 223.1) after a duration of (**a**) 2 h, (**b**) 6 h, and (**c**) 10 h. Acetamiprid + PBO after (**d**) 2 h, (**e**) 6 h, and (**f**) 10 h. Dinotefuran (*m/z* 158.2) after (**g**) 2 h, (**h**) 6 h, and (**i**) 10 h. Dinotefuran + PBO after (**j**) 2 h, (**k**) 6 h, (**l**) 10 h. Scale bar is 1 mm [91]

transformation. Righetti et al. used AP-SMALDI MSI to visualize the spatial distribution of aflatoxin B1 (AFB1) and its metabolite aflatoxin M2 (AFM2) to give insight into the defense metabolites produced by maize. Maize roots soaked in a medium containing AFB1 for 7 and 14 days were analyzed via AP-SMALDI. The generated images in Fig. 12 depict sections of AFB1 ($[M + K]^+$, *m/z* 351.0265) in treated maize root and the lacking distribution of cyanidin glucoside ($[M]^+$, *m/z* 449.1076), an anthocyanidin compound responsible for stress response in plants, and pheophytin ($[M + H]^+$, *m/z* 871.5729), a derivative of chlorophyll, in the treated tissue. These results show that AFB1 has significant inhibitory effects on the biosynthesis of these compounds [96].

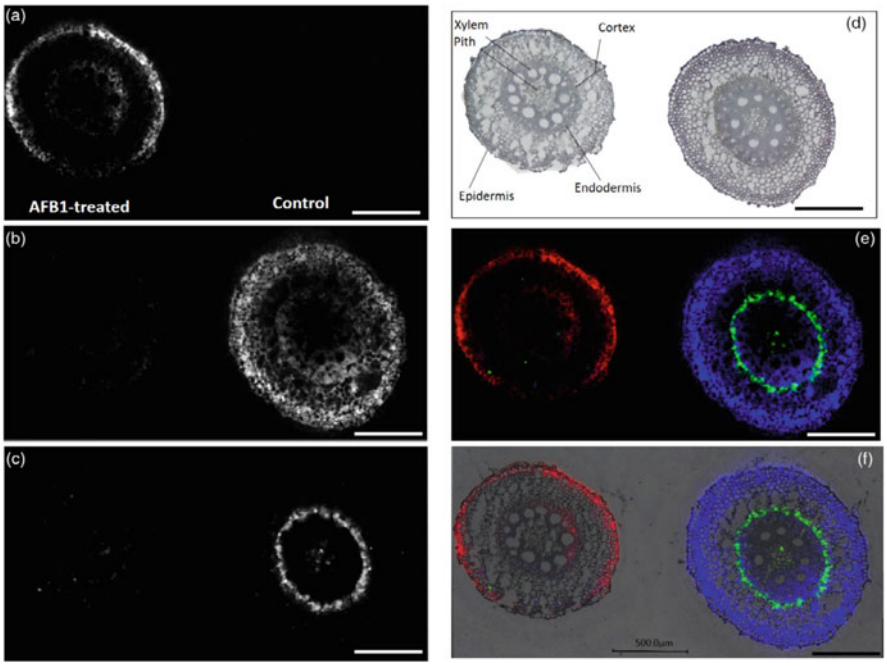


Fig. 12 AP-SMALDI images of control and AFB1 treated maize root after 14 days. (**a**) AFB1 accumulation shown in the treated root vs. the control root with no AFB1. (**b**) Cyanidin glucoside and (**c**) pheophytin distribution. (**d**) The optical image of (**a**) with key features labeled. (**e**) Overlay of (**a**) AFB1 (red), (**b**) Cyanidin glucoside (blue) and (**c**) pheophytin (green). (**f**) Red-green-blue image (**e**) merged with optical image (**d**) [96]

5 Conclusions and Future Trends in Mass Spectrometry Imaging

MSI is able to study almost anything owing to the wide variety of ionization choices and sample preparation strategies that can be paired depending upon your analyte (s) of interest. From pesticides to peptides and microplastics to metabolites, the future of MSI promises to extend the answerable questions in environmental and food science beyond those that were previously limited to temporal and quantitative data sets. Implementing MSI in environmental and food science research space means that spatial data will become part of routine analysis which will drive these fields forward in the same fashion that MSI has driven biochemical and analytical research since the late 1990s. Providing data that overlaps temporal and spatial information for non-targeted analysis will inform future experiments through identification of unknown adulterants that are needing to be tracked by quantitative analysis in the future. To even further the progress of MSI in this field, there will need to be collaboration between the scientists in traditional MSI fields (e.g., analytical chemistry, human medicine) and newer MSI fields like food and

environmental science. For example, Caprioli determined a method for imaging the whole body of a mouse which could easily be applied in food and environmental MSI to analyze larger sample types to locate toxins [97]. While quantitative MSI (qMSI), a way to absolutely quantify target molecules via MSI, has yet to be used for food or environmental science, it is worth noting here for the significance of its potential future use [98]. Its prevalence in the MSI community, as developed by Tobias and Hummon, has raised the standard that environmental and food science should be looking towards as they become more familiar with the technique of MSI.

References

1. Taheran M, Naghdi M, Brar SK, Verma M, Surampalli RY (2018) Emerging contaminants: here today, there tomorrow! Environ Nanotechnol Monit Manag 10:122–126. https://doi.org/10.1016/j.enmm.2018.05.010
2. Ivar do Sul JA, Costa MF (2014) The present and future of microplastic pollution in the marine environment. Environ Pollut 185:352–364. https://doi.org/10.1016/j.envpol.2013.10.036
3. Kalliora C, Mamoulakis C, Vasilopoulos E, Stamatiades GA, Kalafati L, Barouni R, Karakousi T, Abdollahi M, Tsatsakis A (2018) Association of pesticide exposure with human congenital abnormalities. Toxicol Appl Pharmacol 346:58–75. https://doi.org/10.1016/j.taap.2018.03.025
4. Carvalho FP (2017) Pesticides, environment, and food safety. Food Energy Secur 6(2):48–60. https://doi.org/10.1002/fes3.108
5. Qin R, Li P, Du M, Ma L, Huang Y, Yin Z, Zhang Y, Chen D, Xu H, Wu X (2021) Spatiotemporal visualization of insecticides and fungicides within fruits and vegetables using gold nanoparticle-immersed paper imprinting mass spectrometry imaging. Nanomaterials (Basel) 11(5):1327. https://doi.org/10.3390/nano11051327
6. Heyman HM, Dubery IA (2016) The potential of mass spectrometry imaging in plant metabolomics: a review. Phytochem Rev 15(2):297–316. https://doi.org/10.1007/s11101-015-9416-2
7. Wang Y, Sun J, Qiao J, Ouyang J, Na N (2018) A “Soft” and “Hard” ionization method for comprehensive studies of molecules. Anal Chem 90(24):14095–14099. https://doi.org/10.1021/acs.analchem.8b04437
8. Maloof KA, Reinders AN, Tucker KR (2020) Applications of mass spectrometry imaging in the environmental sciences. Curr Opin Environ Sci Health 18:54–62. https://doi.org/10.1016/j.coesh.2020.07.005
9. Kind T, Fiehn O (2010) Advances in structure elucidation of small molecules using mass spectrometry. Bioanal Rev 2(1–4):23–60. https://doi.org/10.1007/s12566-010-0015-9
10. Portolés T, Pitarch E, López FJ, Hernández F, Niessen WMA (2011) Use of soft and hard ionization techniques for elucidation of unknown compounds by gas chromatography/time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 25(11):1589–1599. https://doi.org/10.1002/rcm.5028
11. Burleigh RJ, Guo A, Smith N, Green A, Thompson S, Burt M, Brouard M (2020) Microscope imaging mass spectrometry with a reflectron. Rev Sci Instrum 91(2):023306. https://doi.org/10.1063/1.5142271
12. Goswami G, Bora R, Rathore MS (2015) Oxidation of cooking oils due to repeated frying and human health. Int J Sci Technol Manag 4(1):7
13. Cao G, Hong Y, Wu H, Chen Z, Lu M, Cai Z (2021) Visual authentication of edible vegetable oil and used cooking oil using MALDI imaging mass spectrometry. Food Control 125:107966. https://doi.org/10.1016/j.foodcont.2021.107966

14. Zavalin A, Todd EM, Rawhouser PD, Yang J, Norris JL, Caprioli RM (2012) Direct imaging of single cells and tissue at sub-cellular spatial resolution using transmission geometry MALDI MS. J Mass Spectrom 47(11):i–i. https://doi.org/10.1002/jms.3132
15. Yoshimura Y, Zaima N (2020) Application of mass spectrometry imaging for visualizing food components. Foods 9(5):575. https://doi.org/10.3390/foods9050575
16. Castaing R, Slodzian G (1981) Analytical microscopy by secondary ion imaging techniques. J Phys E 14(10):1119–1127. https://doi.org/10.1088/0022-3735/14/10/002
17. Vanbellingen QP, Castellanos A, Rodriguez-Silva M, Paudel I, Chambers JW, Fernandez-Lima FA (2016) Analysis of chemotherapeutic drug delivery at the single cell level using 3D-MSI-TOF-SIMS. J Am Soc Mass Spectrom 27(12):2033–2040. https://doi.org/10.1007/s13361-016-1485-y
18. Benninghoven A (1994) Surface analysis by secondary ion mass spectrometry (SIMS). Surf Sci 299–300:246–260. https://doi.org/10.1016/0039-6028(94)90658-0
19. Winograd N (2005) The magic of cluster SIMS. Anal Chem 77(7):142A–149A. https://doi.org/10.1021/ac053355f
20. Bich C, Touboul D, Brunelle A (2014) Cluster TOF-SIMS imaging as a tool for micrometric histology of lipids in tissue. Mass Spectrom Rev 33(6):442–451. https://doi.org/10.1002/mas.21399
21. Amstalden van Hove ER, Smith DF, Heeren RMA (2010) A concise review of mass spectrometry imaging. J Chromatogr A 1217(25):3946–3954. https://doi.org/10.1016/j.chroma.2010.01.033
22. Bodzon-Kulakowska A, Suder P (2016) Imaging mass spectrometry: instrumentation, applications, and combination with other visualization techniques: IMAGING MASS SPECTROMETRY. Mass Spectrom Rev 35(1):147–169. https://doi.org/10.1002/mas.21468
23. Boughton BA, Thinagaran D, Sarabia D, Bacic A, Roessner U (2016) Mass spectrometry imaging for plant biology: a review. Phytochem Rev 15(3):445–488. https://doi.org/10.1007/s11101-015-9440-2
24. Hansen RL, Lee YJ (2018) High-spatial resolution mass spectrometry imaging: toward single cell metabolomics in plant tissues. Chem Rec 18(1):65–77. https://doi.org/10.1002/tcr.201700027
25. Zavalin A, Yang J, Caprioli R (2013) Laser beam filtration for high spatial resolution MALDI imaging mass spectrometry. J Am Soc Mass Spectrom 24(7):1153–1156. https://doi.org/10.1007/s13361-013-0638-5
26. Spivey EC, McMillen JC, Ryan DJ, Spraggins JM, Caprioli RM (2019) Combining MALDI-2 and transmission geometry laser optics to achieve high sensitivity for ultra-high spatial resolution surface analysis. J Mass Spectrom 54(4):366–370. https://doi.org/10.1002/jms.4335
27. timsTOF fleX MALDI-2 | Bruker https://www.bruker.com/en/products-and-solutions/mass-spectrometry/timstof/timstof-flex-maldi-2.html. Accessed 26 Nov 2021
28. Heijs B, Potthoff A, Soltwisch J, Dreisewerd K (2020) MALDI-2 for the enhanced analysis of *N* -linked glycans by mass spectrometry imaging. Anal Chem 92(20):13904–13911. https://doi.org/10.1021/acs.analchem.0c02732
29. Menapace M (2018) Recent advances in nutritional sciences: an overview of glycans and MiRNAs. J Nutr Food Sci 8(6). https://doi.org/10.4172/2155-9600.1000734
30. Koropatkin NM, Cameron EA, Martens EC (2012) How glycan metabolism shapes the human gut microbiota. Nat Rev Microbiol 10(5):323–335. https://doi.org/10.1038/nrmicro2746
31. Bien T, Hambleton EA, Dreisewerd K, Soltwisch J (2021) Molecular insights into symbiosis—mapping sterols in a marine flatworm-algae-system using high spatial resolution MALDI-2-MS imaging with ion mobility separation. Anal Bioanal Chem 413(10):2767–2777. https://doi.org/10.1007/s00216-020-03070-0
32. Yao X, Lu J, Liu Z, Ran D, Huang Y (2013) Distribution of sterols and the sources of pollution in surface sediments of Ulungur Lake, Xinjiang. Water Sci Technol J Int Assoc Water Pollut Res 67(10):2342–2349. https://doi.org/10.2166/wst.2013.107

33. Cooks RG (2006) Ambient mass spectrometry. Science 311(5767):1566–1570. https://doi.org/10.1126/science.1119426
34. Takats Z (2004) Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. Science 306(5695):471–473. https://doi.org/10.1126/science.1104404
35. Claude E, Jones EA, Pringle SD (2017) DESI mass spectrometry imaging (MSI). In: Cole LM (ed) Imaging mass spectrometry. Methods in molecular biology, vol 1618. Springer, New York, pp 65–75. https://doi.org/10.1007/978-1-4939-7051-3_7
36. Campbell DI, Ferreira CR, Eberlin LS, Cooks RG (2012) Improved spatial resolution in the imaging of biological tissue using desorption electrospray ionization. Anal Bioanal Chem 404(2):389–398. https://doi.org/10.1007/s00216-012-6173-6
37. Gallage NJ, Jørgensen K, Janfelt C, Nielsen AJZ, Naake T, Duński E, Dalsten L, Grisoni M, Møller BL (2018) The intracellular localization of the vanillin biosynthetic machinery in pods of Vanilla planifolia. Plant Cell Physiol 59(2):304–318. https://doi.org/10.1093/pcp/pcx185
38. Beneito-Cambra M, Gilbert-López B, Moreno-González D, Bouza M, Franzke J, García-Reyes JF, Molina-Díaz A (2020) Ambient (desorption/ionization) mass spectrometry methods for pesticide testing in food: a review. Anal Methods 12(40):4831–4852. https://doi.org/10.1039/D0AY01474E
39. Li N, Dong J, Dong C, Han Y, Liu H, Du F, Nie H (2020) Spatial distribution of endogenous molecules in coffee beans by atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry imaging. J Am Soc Mass Spectrom 31(12):2503–2510. https://doi.org/10.1021/jasms.0c00202
40. Etalo DW, Díez-Simón C, de Vos RCH, Hall RD (2018) Laser ablation electrospray ionization-mass spectrometry imaging (LAESI-MS) for spatially resolved plant metabolomics. In: António C (ed) Plant metabolomics: methods and protocols. Methods in molecular biology. Springer, New York, pp 253–267. https://doi.org/10.1007/978-1-4939-7819-9_18
41. Kulkarni P, Wilschut RA, Verhoeven KJF, van der Putten WH, Garbeva P (2018) LAESI mass spectrometry imaging as a tool to differentiate the root metabolome of native and range-expanding plant species. Planta 248(6):1515–1523. https://doi.org/10.1007/s00425-018-2989-4
42. Bartels B, Kulkarni P, Danz N, Böcker S, Saluz HP, Svatoš A (2017) Mapping metabolites from rough terrain: laser ablation electrospray ionization on non-flat samples. RSC Adv 7(15):9045–9050. https://doi.org/10.1039/C6RA26854D
43. Kooijman PC, Lamprecht S, Lamshoeft M, Beine B, Verhoeven BJHT, Ellis SR, Heeren RMA (2021) A novel dual ionization modality source for infrared laser ablation post-ionization mass spectrometry imaging to study fungicide metabolism and transport. Int J Mass Spectrom 465:116602. https://doi.org/10.1016/j.ijms.2021.116602
44. Shrestha B, Patt JM, Vertes A (2011) In situ cell-by-cell imaging and analysis of small cell populations by mass spectrometry. Anal Chem 83(8):2947–2955. https://doi.org/10.1021/ac102958x
45. Li Y, Shrestha B, Vertes A (2007) Atmospheric pressure molecular imaging by infrared MALDI mass spectrometry. Anal Chem 79(2):523–532. https://doi.org/10.1021/ac061577n
46. Eikel D, Henion J (2011) Liquid extraction surface analysis (LESA) of food surfaces employing chip-based Nano-electrospray mass spectrometry: LESA of food surfaces by NanoESI-MS. Rapid Commun Mass Spectrom 25(16):2345–2354. https://doi.org/10.1002/rcm.5107
47. Xiao Y, Deng J, Fang L, Tu L, Luan T (2021) Mapping the distribution of perfluoroalkyl substances in zebrafishes by liquid extraction surface analysis mass spectrometry. Talanta 231:122377. https://doi.org/10.1016/j.talanta.2021.122377
48. Sunderland EM, Hu XC, Dassuncao C, Tokranov AK, Wagner CC, Allen JG (2019) A review of the pathways of human exposure to poly- and Perfluoroalkyl substances (PFASs) and present understanding of health effects. J Expo Sci Environ Epidemiol 29(2):131–147. https://doi.org/10.1038/s41370-018-0094-1
49. Spraker JE, Luu GT, Sanchez LM (2020) Imaging mass spectrometry for natural products discovery: a review of ionization methods. Nat Prod Rep 37(2):150–162. https://doi.org/10.1039/C9NP00038K

50. Jeng J-Y, Jiang Z-H, Cho Y-T, Su H, Lee C-W, Shiea J (2021) Obtaining molecular imagings of pesticide residues on strawberry surfaces with probe sampling followed by ambient ionization mass spectrometric analysis. J Mass Spectrom 56(4):e4644. https://doi.org/10.1002/jms.4644
51. Goodwin RJA (2012) Sample preparation for mass spectrometry imaging: small mistakes can lead to big consequences. J Proteomics 75(16):4893–4911. https://doi.org/10.1016/j.jprot.2012.04.012
52. Rae Buchberger A, DeLaney K, Johnson J, Li L (2018) Mass spectrometry imaging: a review of emerging advancements and future insights. Anal Chem 90(1):240–265. https://doi.org/10.1021/acs.analchem.7b04733
53. Qin L, Zhang Y, Liu Y, He H, Han M, Li Y, Zeng M, Wang X (2018) Recent advances in matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI) for *in situ* analysis of endogenous molecules in plants. Phytochem Anal 29(4):351–364. https://doi.org/10.1002/pca.2759
54. Pereira I, Banstola B, Wang K, Donnarumma F, Vaz BG, Murray KK (2019) Matrix-assisted laser desorption ionization imaging and laser ablation sampling for analysis of fungicide distribution in apples. Anal Chem 91(9):6051–6056. https://doi.org/10.1021/acs.analchem.9b00566
55. Enomoto H (2021) Adhesive film applications help to prepare strawberry fruit sections for desorption electrospray ionization-mass spectrometry imaging. Biosci Biotechnol Biochem 85(6):1341–1347. https://doi.org/10.1093/bbb/zbab033
56. Wang T, Cheng X, Xu H, Meng Y, Yin Z, Li X, Hang W (2020) Perspective on advances in laser-based high-resolution mass spectrometry imaging. Anal Chem 92(1):543–553. https://doi.org/10.1021/acs.analchem.9b04067
57. Kompauer M, Heiles S, Spengler B (2017) Autofocusing MALDI mass spectrometry imaging of tissue sections and 3D chemical topography of nonflat surfaces. Nat Methods 14(12):1156–1158. https://doi.org/10.1038/nmeth.4433
58. Li B, Zhang Y, Ge J, Liu K, Li P (2018) Sample preparation for mass spectrometry imaging of leaf tissues: a case study on analyte delocalization. Anal Bioanal Chem 410(28):7449–7456. https://doi.org/10.1007/s00216-018-1355-5
59. Klein AT, Yagnik GB, Hohenstein JD, Ji Z, Zi J, Reichert MD, MacIntosh GC, Yang B, Peters RJ, Vela J, Lee YJ (2015) Investigation of the chemical interface in the soybean-aphid and Rice-bacteria interactions using MALDI-mass spectrometry imaging. Anal Chem 87(10):5294–5301. https://doi.org/10.1021/acs.analchem.5b00459
60. Kawamoto T, Kawamoto K (2021) Preparation of thin frozen sections from nonfixed and undecalcified hard tissues using Kawamoto's film method (2020). Methods Mol Biol 2230:259–281. https://doi.org/10.1007/978-1-0716-1028-2_15
61. Kim SH, Kim J, Lee YJ, Lee TG, Yoon S (2017) Sample preparation of corn seed tissue to prevent analyte relocations for mass spectrometry imaging. J Am Soc Mass Spectrom 28(8):1729–1732. https://doi.org/10.1007/s13361-017-1682-3
62. Jenčič B, Jeromel L, Ogrinc Potočnik N, Vogel-Mikuš K, Vavpetič P, Rupnik Z, Bučar K, Vencelj M, Kelemen M, Matsuo J, Kusakari M, Siketić Z, Al-Jalali MA, Shaltout A, Pelicon P (2017) Molecular imaging of alkaloids in Khat (Catha Edulis) leaves with MeV-SIMS. Nucl Instrum Methods Phys Res Sect B Beam Interact Mater At 404:140–145. https://doi.org/10.1016/j.nimb.2017.01.063
63. Jenčič B, Jeromel L, Ogrinc Potočnik N, Vogel-Mikuš K, Kovačec E, Regvar M, Siketić Z, Vavpetič P, Rupnik Z, Bučar K, Kelemen M, Kovač J, Pelicon P (2016) Molecular imaging of cannabis leaf tissue with MeV-SIMS method. Nucl Instrum Methods Phys Res Sect B Beam Interact Mater At 371:205–210. https://doi.org/10.1016/j.nimb.2015.10.047
64. Feng J, Zhao H, Gong X, Xia M-C, Cai L, Yao H, Zhao X, Yan Z, Li Z, Nie H, Ma X, Zhang S (2021) In situ identification and spatial mapping of microplastic standards in paramecia by secondary-ion mass spectrometry imaging. Anal Chem 93(13):5521–5528. https://doi.org/10.1021/acs.analchem.0c05383

65. Veličković D, Zhang G, Bezbradica D, Bhattacharjee A, Paša-Tolić L, Sharma K, Alexandrov T, Anderton CR, KPMP Consortium (2020) Response surface methodology as a new approach for finding optimal MALDI matrix spraying parameters for mass spectrometry imaging. J Am Soc Mass Spectrom 31(3):508–516. https://doi.org/10.1021/jasms.9b00074
66. Dong Y, Li B, Malitsky S, Rogachev I, Aharoni A, Kaftan F, Svatoš A, Franceschi P (2016) Sample preparation for mass spectrometry imaging of plant tissues: a review. Front Plant Sci 7: 60. https://doi.org/10.3389/fpls.2016.00060
67. Calvano CD, Monopoli A, Cataldi TRI, Palmisano F (2018) MALDI matrices for low molecular weight compounds: an endless story? Anal Bioanal Chem 410(17):4015–4038. https://doi.org/10.1007/s00216-018-1014-x
68. Enomoto H, Kotani M, Ohmura T (2020) Novel blotting method for mass spectrometry imaging of metabolites in strawberry fruit by desorption/ionization using through hole alumina membrane. Foods 9(4):408. https://doi.org/10.3390/foods9040408
69. Parrot D, Papazian S, Foil D, Tasdemir D (2018) Imaging the unimaginable: desorption electrospray ionization – imaging mass spectrometry (DESI-IMS) in natural product research. Planta Med 84(9–10):584–593. https://doi.org/10.1055/s-0044-100188
70. Belton B, Bush SR, Little DC (2018) Not just for the wealthy: rethinking farmed fish consumption in the global south. Glob Food Secur 16:85–92. https://doi.org/10.1016/j.gfs.2017.10.005
71. Nielsen J, Jessen F (2007) Quality of frozen fish. In: Nollet LML (ed) Handbook of meat, poultry and seafood quality. Blackwell, Ames, pp 577–586. https://doi.org/10.1002/9780470277829.ch44
72. Sikorski Z (1982) Trimethylamine N-oxide demethylase: its occurrence, properties, and Rôle in technological changes in frozen fish. Food Chem 9(3):213–222. https://doi.org/10.1016/0308-8146(82)90099-1
73. Morisasa M, Kimura K, Sumida M, Fukumoto S, Tamura T, Takeuchi R, Mori T, Goto-Inoue N (2020) Application of matrix-assisted laser desorption/ionization mass spectrometry imaging for evaluating the quality of fish fillets. Foods 9(4):402. https://doi.org/10.3390/foods9040402
74. Şengül M, Keleş F, Keleş MS (2004) The effect of storage conditions (temperature, light, time) and variety on the glycoalkaloid content of potato tubers and sprouts. Food Control 15(4):281–286. https://doi.org/10.1016/S0956-7135(03)00077-X
75. Shimadzu Corporation (2014) Shimadzu J 2:2
76. Deng Y, He M, Feng F, Feng X, Zhang Y, Zhang F (2021) The distribution and changes of glycoalkaloids in potato tubers under different storage time based on MALDI-TOF mass spectrometry imaging. Talanta 221:121453. https://doi.org/10.1016/j.talanta.2020.121453
77. Sanlier N, Gokcen BB, Altuğ M (2018) Tea consumption and disease correlations. Trends Food Sci Technol 78:95–106. https://doi.org/10.1016/j.tifs.2018.05.026
78. Yang CS, Maliakal P, Meng X (2002) Inhibition of carcinogenesis by tea. Annu Rev Pharmacol Toxicol 42(1):25–54. https://doi.org/10.1146/annurev.pharmtox.42.082101.154309
79. Hsu C-L, Yen G-C (2008) Phenolic compounds: evidence for inhibitory effects against obesity and their underlying molecular signaling mechanisms. Mol Nutr Food Res 52(1):53–61. https://doi.org/10.1002/mnfr.200700393
80. Grassi D, Aggio A, Onori L, Croce G, Tiberti S, Ferri C, Ferri L, Desideri G (2008) Tea, flavonoids, and nitric oxide-mediated vascular reactivity. J Nutr 138(8):1554S–1560S. https://doi.org/10.1093/jn/138.8.1554S
81. Wan XC (2003) Tea biochemistry.3rd edn. China Agriculture Press, Beijing
82. Wan XC, Xia T (2015) Secondary metabolism of tea plant, 1st edn, Science Press, Beijing
83. Wu Z-J, Li X-H, Liu Z-W, Xu Z-S, De Zhuang J (2014) Novo assembly and transcriptome characterization: novel insights into catechins biosynthesis in Camellia sinensis. BMC Plant Biol 14(1):277. https://doi.org/10.1186/s12870-014-0277-4
84. Liao Y, Fu X, Zhou H, Rao W, Zeng L, Yang Z (2019) Visualized analysis of within-tissue spatial distribution of specialized metabolites in tea (Camellia *sinensis*) using desorption electrospray ionization imaging mass spectrometry. Food Chem 292:204–210. https://doi.org/10.1016/j.foodchem.2019.04.055

85. Klein A-M, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. Proc R Soc B Biol Sci 274(1608):303–313. https://doi.org/10.1098/rspb.2006.3721
86. Sánchez-Bayo F, Goulson D, Pennacchio F, Nazzi F, Goka K, Desneux N (2016) Are bee diseases linked to pesticides? — a brief review. Environ Int 89–90:7–11. https://doi.org/10.1016/j.envint.2016.01.009
87. Gill RJ, Ramos-Rodriguez O, Raine NE (2012) Combined pesticide exposure severely affects individual- and colony-level traits in bees. Nature 491(7422):105–108. https://doi.org/10.1038/nature11585
88. Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke CH, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Stark JD, Tapparo A, Van Dyck H, Van Praagh J, Van der Sluijs JP, Whitehorn PR, Wiemers M (2015) Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environ Sci Pollut Res Int 22(1):5–34. https://doi.org/10.1007/s11356-014-3470-y
89. Mitchell EAD, Mulhauser B, Mulot M, Mutabazi A, Glauser G, Aebi A (2017) A worldwide survey of neonicotinoids in honey. Science 358(6359):109–111. https://doi.org/10.1126/science.aan3684
90. Phan NTN, Mohammadi AS, Dowlatshahi Pour M, Ewing AG (2016) Laser desorption ionization mass spectrometry imaging of *drosophila* brain using matrix sublimation versus modification with nanoparticles. Anal Chem 88(3):1734–1741. https://doi.org/10.1021/acs.analchem.5b03942
91. Zhang Y, Chen D, Du M, Ma L, Li P, Qin R, Yang J, Yin Z, Wu X, Xu H (2021) Insights into the degradation and toxicity difference mechanism of neonicotinoid pesticides in honeybees by mass spectrometry imaging. Sci Total Environ 774:145170. https://doi.org/10.1016/j.scitotenv.2021.145170
92. Heil M, Karban R (2010) Explaining evolution of plant communication by airborne signals. Trends Ecol Evol 25(3):137–144. https://doi.org/10.1016/j.tree.2009.09.010
93. van Dam NM, Bouwmeester HJ (2016) Metabolomics in the rhizosphere: tapping into below-ground chemical communication. Trends Plant Sci 21(3):256–265. https://doi.org/10.1016/j.tplants.2016.01.008
94. Siminszky B (2006) Plant cytochrome P450-mediated herbicide metabolism. Phytochem Rev 5(2):445–458. https://doi.org/10.1007/s11101-006-9011-7
95. D'Abrosca B, Scognamiglio M, Fiumano V, Esposito A, Choi YH, Verpoorte R, Fiorentino A (2013) Plant bioassay to assess the effects of allelochemicals on the metabolome of the target species aegilops geniculata by an NMR-based approach. Phytochemistry 93:27–40. https://doi.org/10.1016/j.phytochem.2013.03.016
96. Righetti L, Bhandari DR, Rolli E, Tortorella S, Bruni R, Dall'Asta C, Spengler B (2021) Unveiling the spatial distribution of aflatoxin B1 and plant defense metabolites in maize using AP-SMALDI mass spectrometry imaging. Plant J 106(1):185–199. https://doi.org/10.1111/tpj.15158
97. Reyzer ML, Chaurand P, Angel PM, Caprioli RM (2010) Direct molecular analysis of whole-body animal tissue sections by MALDI imaging mass spectrometry. In: Rubakhin SS, Sweedler JV (eds) Mass spectrometry imaging. Methods in molecular biology, vol 656. Humana Press, Totowa, pp 285–301. https://doi.org/10.1007/978-1-60761-746-4_17
98. Tobias F, Hummon AB (2020) Considerations for MALDI-based quantitative mass spectrometry imaging studies. J Proteome Res 19(9):3620–3630. https://doi.org/10.1021/acs.jproteome.0c00443

Chip-Based Separation Devices Coupled to Mass Spectrometry in Food and Environmental Chemistry

Ángel Ríos and Mohammed Zougagh

Contents

Abstract Nowadays, chip-based separation devices coupled to mass spectrometry are a leading analytical technique in many science areas of major interest. This interest is based on their economic, environmental, and analytical advantages, such as low volumes consumption of samples and reagent, gain on separation performance, and enhanced sensitivity with MS detectors. The application in food and environmental fields will keep in constant spreading, and new and miniaturized instrumental advances in chip technology will continue to be necessary for the

Á. Ríos (✉)
Analytical Chemistry and Food Technology Department, University of Castilla-La Mancha, Ciudad Real, Spain

Regional Institute for Applied Scientific Research, IRICA, Ciudad Real, Spain
e-mail: angel.rios@uclm.es

M. Zougagh
Regional Institute for Applied Scientific Research, IRICA, Ciudad Real, Spain

Analytical Chemistry and Food Technology Department, Faculty of Pharmacy, University of Castilla-La Mancha, Albacete, Spain
e-mail: mohammed.zougagh@uclm.es

Yolanda Picó and Julian Campo (eds.), *Mass Spectrometry in Food and Environmental Chemistry*, Hdb Env Chem (2023) 119: 359–380, DOI 10.1007/698_2022_888,

Published online: 18 May 2022

coming years. This chapter summarizes the main features of chipLC–MS, chipGC–MS, and chipCE–MS, outlining their main parts. Finally, a description about some diverse applications of chip-based separations mass spectrometry devices is summarized. In this case, showing remarkable examples using chipLC–MS, chipGC–MS, and chipCE–MS to the analysis of food and environmental samples.

Keywords Chip-based separation devices, Chip-CE, Chip-GC, Chip-LC, Environmental, Food, Mass spectrometry

1 Introduction

The current trend in analytical chemistry is miniaturization using chip technology to improve sensitivity and speed of analysis. Chip-based analyses reduce the sample volume and the consumption of chemical reagents. As a result, chip-based separation devices have been applied to various food and environmental studies. In addition to miniaturized separation devices sensitivity is another key issue. Further confirmation on molecular structures of analytes is generally required for various food and environmental applications. Mass spectrometry (MS) offers additional high-quality structural information for qualitative identification and good sensitivity for quantitative determination of target molecules [1]. Therefore, MS has been widely utilized for sensitive detection in many chip-based separation devices. Coupling of MS to chip-based separation systems has been intensively studied. These developments have been focused nearly exclusively on chip technology. Although chip-based separation devices were first reported in 1976 in gas chromatography (GC) [2], the scientific breakthrough of the idea took until the 1990s when liquid separation techniques such as liquid chromatography (LC) and capillary electrophoresis (CE) [3–7] and their coupling with MS [8, 9] were introduced. Nowadays, areas of the technology have reached a rather mature status which is reflected by the availability of several commercial products like chipCE–MS and chipLC–MS. The crucial point for online coupling of chip-based separation devices with MS instruments is the interface that transfers the dissolved molecules in the chip to gas phase ions which is necessary for subsequent MS analysis. The introduction of miniaturized electrospray ionization, in the middle of the 1990s, immediately revelated outstanding detection becoming the preferential choice to hyphenate with chipLC and chipCE [10]. Wilm and Mann developed the first microelectrospray emitter [10], which by the reduction of the tip internal diameter (from 100 μm to 1–20 μm) provided much higher sensitivity. Likewise, Emmett et al. [11], Davis et al. [12], and Gatlin et al. [13] also demonstrated similar detectability improvements through miniaturization of the electrospray ionization (ESI) source, even at the nanoscale. Nevertheless, micro-/nano-ESI-MS emitters are not suitable for coupling chipGC instruments. Microchips atmospheric pressure chemical ionization MS (APCI-MS) and atmospheric pressure photoionization MS (APPI-MS) systems [14] which

provided flow rates from tens of nanolitres to several microlitres per minute, efficient ionization, excellent sensitivity, and good reproducibility offer a potential ionization technique for chipGC–MS. In this chapter, the advances of chip-based separation devices, including chipGC, chipLC, and chipCE, all coupled to MS will be discussed. In addition, the usage of these chips to determine molecules in various sample matrices for environmental and food fields will also be presented.

2 Chip Chromatography–Mass Spectrometry

The hyphenation of chip-based separation devices like GC or LC to MS is a highly active research area in modern separation techniques. Such methods are particularly attractive for comprehensive food and environmental chemistry. They can handle extremely low sample amounts, with low solvent consumption. Furthermore, they provide unsurpassed analysis speed together with the prospect of integrating several functional elements on a single multifunctional platform. In this part, we describe the latest developments in this emerging field.

2.1 Chip gas Chromatography–Mass Spectrometry (ChipGC–MS)

The main components of the ChipGC–MS system are gas injector and preconcentrator, column, MS detector, and electronics for data pre-processing that play an important role in a system integration perspective. Recently, numerous research efforts have benefited from the advances in micro-electromechanical systems (MEMS) and nanotechnology-based solutions to miniaturize the key components of GC instrument. Reducing the dimensions of GC systems enhances the analytical performances and analysis of small-volume samples and increases analysis speed. A chipGC–MS system requires several components to function properly: preconcentrator, micro-valves for injecting the sample into the carrier gas, microfabricated columns well-functionalized for the specific use, heaters, temperature sensors for controlling column temperature, and MS interfaced detector for detecting different types of molecules. Temperature stability is also critical for GC operation, as the adsorption/desorption processes responsible for molecular separation in the column are very sensitive to temperature. The issues of microfluidic integration are therefore critical in chipGC–MS systems. Despite the fact that the first work on chipGC system was a miniaturized GC in 1979 [2] using micro-electromechanical systems (MEMS), this development was hardly pursued afterward, probably because the resolving power was poor, when compared to the conventional ones and the analytical community was not yet ready to embrace this new technology. Unsatisfactory results, due to difficulties in producing

homogeneous stationary phases, may have contributed to this feeling. However, miniaturized GC are of great interest in several application fields. These microsystems could potentially be used for breath analysis, indoor air-quality monitoring, and warfare agent detection, allowing the onsite and portable monitoring of gases in a fast and reliable way. A typical ChipGC–MS system consists of microinjector, micropreconcentrator, column or microcolumn, MS detector, and electronics for data processing in miniaturized form, thus promising an enhanced performance in terms of size, power consumption, resolution, and speed.

2.1.1 Microinjector and Preconcentrator

The microinjector is a device used for introducing during milliseconds very small plug of sample vapour (nanolitres) into the carrier gas stream at the head of the capillary column. Terry et al. were the first to report a gas sample microinjector much smaller than that used in GC commercially [2]. It consists of an etched silicon seat and orifice, a nickel diaphragm, and a solenoid actuator and plunger assembly. Both the solenoid assembly and the diaphragm are removable to facilitate cleaning of the internal valve areas. Since this first development, several authors designed new and improved microinjectors [15–18]. Bessoth et al. [19] reported the first injector applied for gas injection having the geometry commonly used in microchip capillary electrophoresis (Fig. 1a). In the sample load step, valve 1 connected flow restrictor and pressure regulator while valve 4 fed the outlet of detector. Valves 2 and 3 were opened and gas was transported from the sample inlet to the waste outlet by the

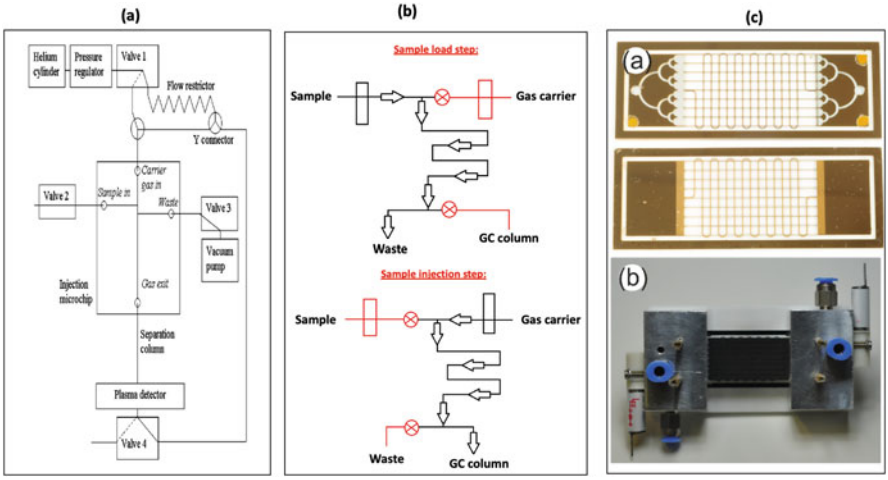


Fig. 1 Different methods of introducing sample mixture in separation channel: (**a**) gas injector having the geometry commonly used in microchip capillary electrophoresis (adapted from [19]), (**b**) six low-leak valves with 250 nL sample volume, (**c**) large-capacity preconcentrator device (adapted from [20]: (**a**) top view with Carbosieve SII as filling material and housing (**b**))

suction of the vacuum pump. The sample is introduced and flushed out into the waste channel. In the sample injection step, valves 1 and 4 are switched to their alternative positions and valves 2 and 3 are closed. As a result, the carrier gas pushes a plug of sample towards the separation column and detector. More recently, Nachef et al. [21] developed a six low-leak microvalve (Fig. 1b) made from polyetheretherketone (PEEK) membranes on silicon substrates, when the inlet and outlet holes are closer to each other, with their centres being distant by 300 μm. The valves are pneumatically actuated, and work in two steps: (1) sample load, which consists of filling the largest microchannel with a known sample volume (250 nL) and (2) sample injection, during which the sample is injected into the separation column.

2.1.2 Micropreconcentrators

Micropreconcentrators allow the collection and concentration of analyte(s) followed by their release and injection into the ChipGC system. Since the first micro-machined preconcentrator designed and fabricated by the ChemLab at Sandia National Laboratories in 1999 [21–23], many work has been done with various preconcentrating microstructures of different designs combined with a wide range of adsorbents [20, 23–27]. Alfeeli et al. reported a microthermal preconcentrator (TPC) device with high-aspect-ratio 3D structures which comprises more than 3,500 micro-pillars and allows large adsorption surface area [23]. This device was used as a GC injector to demonstrate its ability to concentrate a dilute mixture of multicompound hydrocarbons into a sharp pulse suitable for GC analysis. Tian et al. developed a preconcentrator-focuser (PCF), consisting of a thick micro-machined Si heater packed with graphitized carbon granules (200 μm diameter) with high surface area [24]. The PCF was connected directly to GC detector through a six-way valve using passivated fused-silica capillary tubing. A small aliquot (250 μL) of sample was extracted by gas-tight syringe and injected into the sample stream which was drawn through the PCF by a downstream diaphragm pump. Subsequent heating of the PCF led to desorption of the captured vapours, which were passed on a positive pressure of carrier gas to GC detector. Jasen et al. designed and fabricated a large-capacity on-chip preconcentrator device with a micro-machined silicon cartridge and an integrated heater [20]. The absorption material was Carbosieve S-II immobilized in PC device channels housed in stainless-steel unit, as represented in Fig. 1c. Zampolli et al. reported a preconcentration column consisting of a micro-machined silicon cartridge (25 × 12 × 1.3 mm), containing quinoxaline-bridged cavitand (QxCav) selective for retention of aromatic compounds. This preconcentrator component has been integrated into a miniaturized gas chromatograph and allowed real-time monitoring of sub-ppb concentrations of aromatic volatiles [25].

2.1.3 Column or Microcolumn

Column is the most critical component in the ChipGC system. Two types of columns have been explored for its use, capillary columns and chip-based columns. Conventional capillary columns are circular in cross section, while chip-based columns, known as microcolumns, are typically rectangular in cross section. Figure 2 shows the cross-sectional view of different separation columns of both types used in ChipGC system. Microcolumns offer several advantages. The small size of microcolumns enables high speed and low power heating; large-scale batch production of these columns may result in low manufacturing cost; monolithic integration of the columns with other components potentially minimizes the dead volumes and cold spots; and fabrication of regularly arranged support structures inside the channels enhances the separation performance [28]. Major limitation, however, of these columns is the accumulation of the stationary phase in sharp corners (known as pooling effect) resulting in non-uniform film thickness inside the column leading to band broadening. Nonetheless, in the case of high-aspect-ratio rectangular columns, the efficiency can be increased by decreasing the width of the channel, and the sample capacity (maximum amount of sample that can be injected without overloading). Flow rate can be increased by increasing the height, thereby offering a possibility of getting better efficiencies compared to conventional capillary columns. Considerable efforts are being made to increase the separation efficiency of

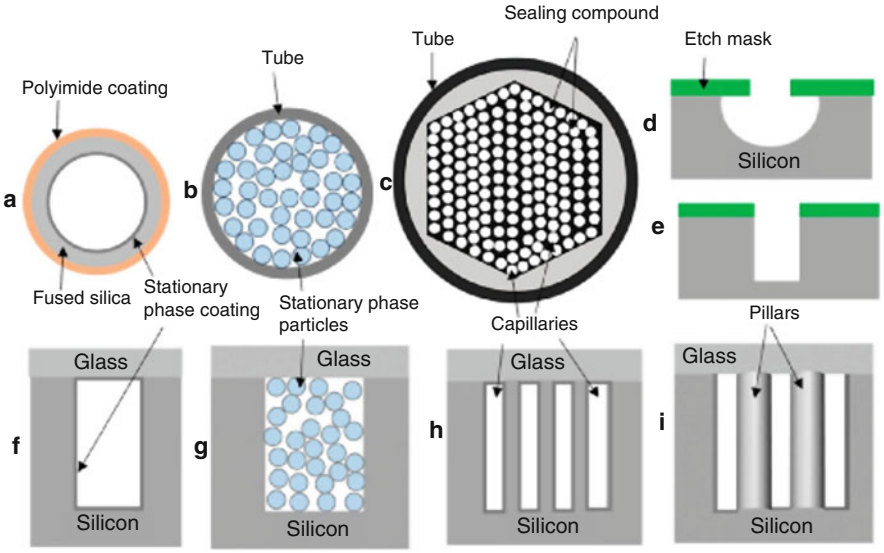


Fig. 2 Cross-sectional view of different GC columns and etched profiles: (**a**) wall-coated capillary column, (**b**) packed capillary column, (**c**) multicapillary column, (**d**) isotropic etch profile, (**e**) anisotropic etch profile, (**f**) wall-coated microcolumn, (**g**) packed microcolumn, (**h**) multicapillary microcolumn, and (**i**) pillar-array column. Adapted from [28]

the columns by exploring different column architectures and stationary phase coating techniques.

Several materials have been used as a substrate to fabricate microcolumns such as silicon wafer [29], metal (nickel, steel, and titanium) [30], polymeric materials and glass [31]. Gaddes et al. [29] microfabricated a 2 m of silicon wafer column using silicon microfabrication techniques and dynamic coating process for PDMS (OV-1) stationary phase. This GC microcolumn along with the high temperature fixture offers a better solution towards the achievement of a ChipGC system for the detection of semi-volatile environmental pollutants and explosives without the thermal limitations reported to date with GC microcolumns using epoxy-based interconnect technology. Lewis and Wheeler developed a nonplanar microcolumn using a planar nickel substrate containing circular through-holes that further apply to separate polar as well as nonpolar analytes [30]. Rankin et al. [31] developed the first moulded GC microcolumn in polymeric material. This microcolumn consists of a single microtextured thermoset polymer composite which acts as both substrate and stationary phase.

Depending on type of microcolumns, several materials have been used as stationary phases. Graphitized carbon black [32], polyethylene glycol (PEG)-coated graphitized carbon black [25, 33], porous polymer beads [25, 34], and carbon molecular sieves have been used for packed columns, particularly for separation of permanent gases and light hydrocarbons. In open and semi-packed columns, PDMS and its derivatives have been predominantly used to coat microcolumns [35, 36]. For separation of polar compounds, polymers of PEGs have been frequently used [37]. There are two types of methods for coating the columns, named static and dynamic methods. Static methods are the more appropriate, because they obtain more homogenous stationary phase films and are easier to implement. First, a known concentration stationary phase solution is prepared in a solvent mixture and the entire column is filled with this dilute solution by means of a pressurized gas. One of the ends of the column is then sealed, and the solvent is evaporated under vacuum through the other end. Static coating produces a more uniform coating, and the coating thickness can be easily calculated by employing the surface area of the column, concentration of the stationary phase in the solution, and the density of the coating solution. One of the disadvantages of this coating method is the formation of bubbles during the evaporation of the solvent. For this reason, extreme precautions are required during this coating process. A 5.9 m long cylindrical fused-silica microcolumn, fabricated in a silicon wafer using deeper reactive ion etching (DRIE) and high temperature fusion bonding, was coated with a 1% vinyl, 5% phenyl, 94% methyl polysiloxane stationary phase using a static method [38]. The authors found that the coating was not uniform, due to the bubbles originated from inefficient degassing of the coating solution in the micro-cavities of the channel walls or at the capillary lead/microchip interfaces. As an alternative, the dynamic method forcing the introduction of the stationary phase through the column by an inert gas and then, evaporating the solvent by continuous gas flow can be used to fabricate columns. This method is fast, but advanced prediction of film thickness is difficult, due to its alteration as the concentration of stationary phase solution and the

velocity change during the coating process. In both methods (static and dynamic), the limitation is accumulation of the stationary phase at the sharp corners (known as the pooling effect) resulting in the band broadening. To minimize the pooling effect, the walls of microcolumns are rounded using isotropic etching after the DRIE process. In recent years, laser etching technology (LET) has become an important etching method. As it can easily etch deep channels on glass wafers as well as on silicon wafers, the column depth can be substantially increased by bonding the channels on the silicon wafer together with the channels on the glass wafer. Consequently, the column can achieve a channel with a depth of 1.2 mm to 1.5 mm, which greatly enhances the aspect ratio and the sample capacity, improving the separation efficiency of micropacked GC columns. Sun et al. fabricated a micropacked gas chromatograph column integrated with a microheater by using LET for analysing environmental gases [34]. LET is a powerful tool to etch deep well-shaped channels on the glass wafer, and it is the most effective way to increase depth of channel. A number of stationary phases have been developed, with different features depending on the field and analytes to which they are applied. However, research efforts in capillary ChipGC have been focused on achieving high-resolution separations by means of porous selective layers that create new microcolumns. In this sense, the use of nanomaterials (NMs) such as AuNPs, SiO_2NPs, graphene, carbon nanotubes (CNTs), and metal-organic frameworks (MOFs), constitutes a very interesting alternative to conventional stationary phases because of the extraordinary properties that these kind of materials have, including high surface area, thermal stability, and chemical inertness [39]. Moreover, their ability to be functionalized increases their versatility making the separation of target analytes even possible.

MOFs are the most attractive nanomaterials class for stationary phases because of their chemical functionality and pore size. MOFs are porous materials that consist of organic ligands trust that bridge inorganic canters (typically metals or metal clusters). The MOF structure is templated by the specific ligand used in the synthesis. MOFs are highly porous, thermally stable, and possess tuneable chemical sorption affinity/selectivity through modification of their chemical functionality and pore structure. Certain MOFs can also be deposited using layer-by-layer techniques directly on selected substrates as conformal thin films [40]. These so-called surface-mounted MOFs (SURMOFs) allow for the direct deposition of a MOF thin film with a known thickness, both attributes being ideal from a chromatography perspective. SURMOFs can be tuned in terms of their chemical functionality and pore size and as such make an attractive class for stationary phases over porous layer open tubular (PLOT) with proprietary formulation, and thus inaccessible for in-house of non-commercial stationary phases. Read and Sillerud from National Laboratories fabricated GC microcolumn using MOF thin films as stationary phases [41]. The authors demonstrated the first reported MOF-stationary-phase, high-speed chromatographic separation of natural gas using GC columns – outperforming commercial Porous Layer Open Tubular (PLOT) stationary phases in terms of both polarity and absolute partition coefficients. Xie et al. developed chiral MOF coated open tubular microcolumns that were used in the GC separation of chiral compounds. The

microcolumns have excellent selectivity and also possess good recognition ability towards a wide range of organic compounds such as alkanes, alcohols, and isomers [42].

2.1.4 Mass Spectrometry Detectors

MS detectors provide the determination of ChipGC separated compounds with high sensitivity. However, because of their sizes, weight, and power consumption, MS have rarely accompanied GC in portable instrument. That is why a large part of efforts for the development of MS micro-detectors have been made around the conception of micro-mass sensors [43, 44]. Kostiainen research group [44, 45] microfabricated nebulizer chip for combining atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization mass spectrometry (APPI-MS) with GC (Fig. 3). The chip consists of a silicon plate and one or two glass plates. The chip includes a sample inlet channel, auxiliary gas and dopant inlet, vaporizer channel, nozzle, and platinum heater. The sample from GC is directed via heated transfer line inserted from the rear edge into a stopper inside the vaporizer channel of the APCI chip. In the ChipGC-APPI-MS, the sample eluted from the GC is mixed with auxiliary gas and dopant (toluene) in the heated vaporizer. The chip forms a confined jet of the sample vapour, which is photoionized as it exits the chip. This ChipGC-APPI-MS provides an efficient method for qualitative and quantitative analysis with detection limits down to the nanomole per litre range and providing the same type of information as classical chemical ionization for structure elucidation.

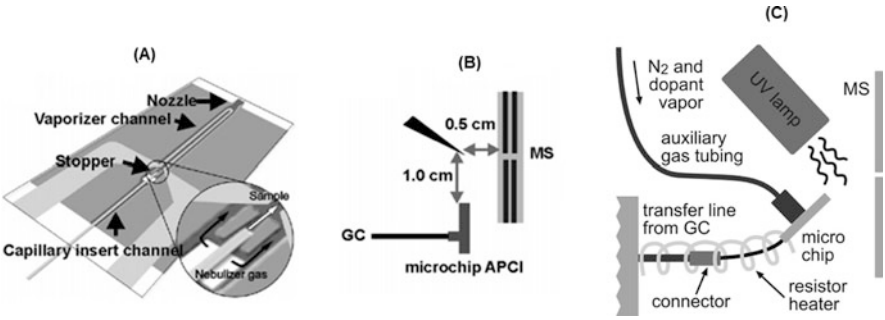


Fig. 3 (**a**) Schematic presentation of the microchipGC. Sample inlet capillary is inserted from the rear edge of the chip to the narrowing. The nebulizer gas flows from behind and along the capillary to the flow channel. (**b**) Schematic presentation of the positioning of the microchip APCI and the corona discharge needle in front of the mass spectrum. (**c**) Schematic view of the ChipGC-APPI-MS. Adapted from [44, 45]

2.2 *Chip-Based Liquid Chromatography–Mass Spectrometry (ChipLC–MS)*

Much of the articles reporting the use of chipLC use commercially available systems. ChipLC–MS can be presented as micro-LC–MS or nano-LC–MS systems, which provide extremely sensitive analyses, although some robustness problems can be produced due to dead volumes and leaks in their designs. ChipLC offers a solution for these challenges. In fact, the first chipLC was fabricated by Manz et al. in 1990 [3]. It consists of an open tubular spiral column made on a silicon wafer, interfaced with a conventional valve injector, and a conductometric detector (cell volume of 1.2 pL) fabricated on a Pyrex glass chip. Although actual experiments have not been performed using this chipLC system, this work opened the possibility for LC miniaturization in a chip format, and presented its potential advantages, allowing the commercialization of various formats of chipLC columns and interfaces with various detectors, especially with MS. Based on this model, Agilent chipCL is described [46] (Fig. 4a). It consists of an inert polyimide device integrating a nano-electrospray interface connected to a mass spectrometer, an analytical chromatographic column of appropriate size to the nano-electrospray flow rate, and an enrichment column for online sample concentration. In this device, there are no fittings, adapters, connectors, or any other dispersive flow element negatively affecting the performance in μ-LC and nano-LC. Transfer volume between the enrichment column and the analytical column is minimized by installing the chipLC within an LC rotary valve. In this way, the chipLC is interposed between the rotor and the stator with a precise registration. In one position the flow from the

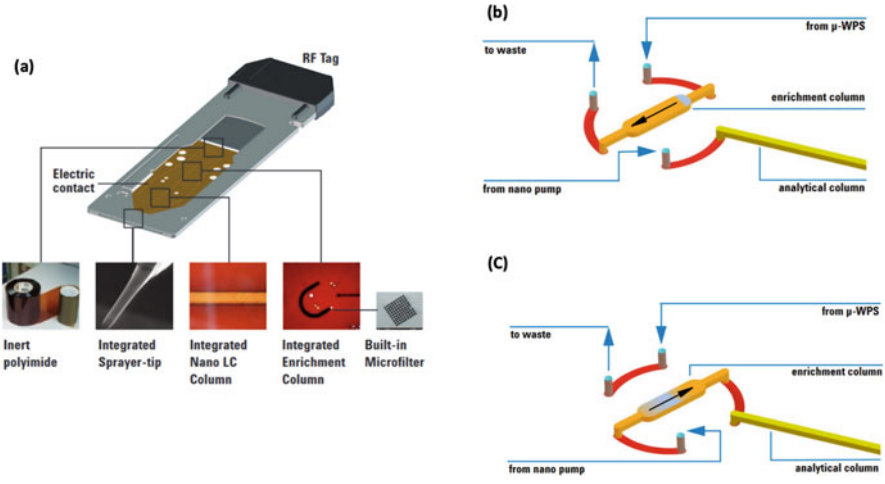


Fig. 4 (**a**). View of the commercial Agilent HPLC Chip and scheme of the chip–rotor interface in the LC run mode (**b**) and in the loading process: the sample-loading configuration (**c**) and the LC-running configuration. Reproduced from [46]

liquid autosampler is directed to an enrichment area (a short, packed column of RP material for adsorbing the sample components), and then to waste (Fig. 4b). When the rotor travels 60°, the flow from the nanopump enters the enrichment column, sweeping the sample(s) into the analytical column (Fig. 4c).

At the end of the column, the flow passes electrical contacts, which allow the biasing of the effluent to the electrospray. The highly stable spray exits through a 2 mm long 40 μm inner diameter tip and enters to the MS analyser. For coupling chipLC, ESI is the more efficient ionization mode, as described in [47]. The main analytical advantage of chipLC-ESI-MS systems is the enhanced detection capabilities derived from the synergistic compatibility shown by these techniques at a miniaturized scale [48]. Miniaturized flow rates favour the ESI process leading to enhanced MS detectability [49]. The miniaturization of the ESI sources leads to the more efficient transference of the targeted ions to the vapour phase, reducing matrix effects and susceptibility of the ionization process to the effluent composition [10]. On the other hand, nano-ESI sensitivity also directly depends on parameters such as the emitter internal diameter, geometry, material, and position. Hence, nano-emitters architecture has been under continuous evolution, and several works dedicated to improving emitters have been performed in the last decades [50]. Nowadays, two main strategies for coupling chipLC with nanoESI-MS can be distinguished: (a) spraying via microfabrication of integrated emitters and (b) spraying by coupling with external emitters. Matrix-assisted laser desorption ionization (MALDI) has been another versatile ionization source for interfacing chip-based separations with MS. In this case, the coupling has been achieved microfabricating MALDI reservoirs at the end of the separation channel, for on-chip ionization [51], or by dropping off the chip effluent in a MALDI probe, for offline analysis.

3 Chip Electrophoresis Mass Spectrometry

Despite the difference in size between a chipCE and a MS, and hence, the a priori difficulties in making them compatible, they are actually well matched due to the similarities in the flow rate generated by the chipCE and those required for the ESI. Various chipCEs have been coupled to MS [52–62] and the most reported chipCE–MS interfaces are ESI [63–66], and MALDI.

A typical chipCE generally included on-chip sample loading and separation without any connectors. The coupling of a chipCE to MS may be achieved using a sheath flow or a liquid junction to provide stable electrospray signals [55–62, 67, 68].

An illustrating possible layout of a chipCE device is shown in Fig. 5. Different approaches for combining ESI with chipCE are schematically shown in Fig. 5a. These are, spraying first, from the blunt end of a chip, second, from the corner of a thin chip, third, from an attached external capillary, fourth, from an attached confined nanospray emitter, and finally from a monolithically integrated confined

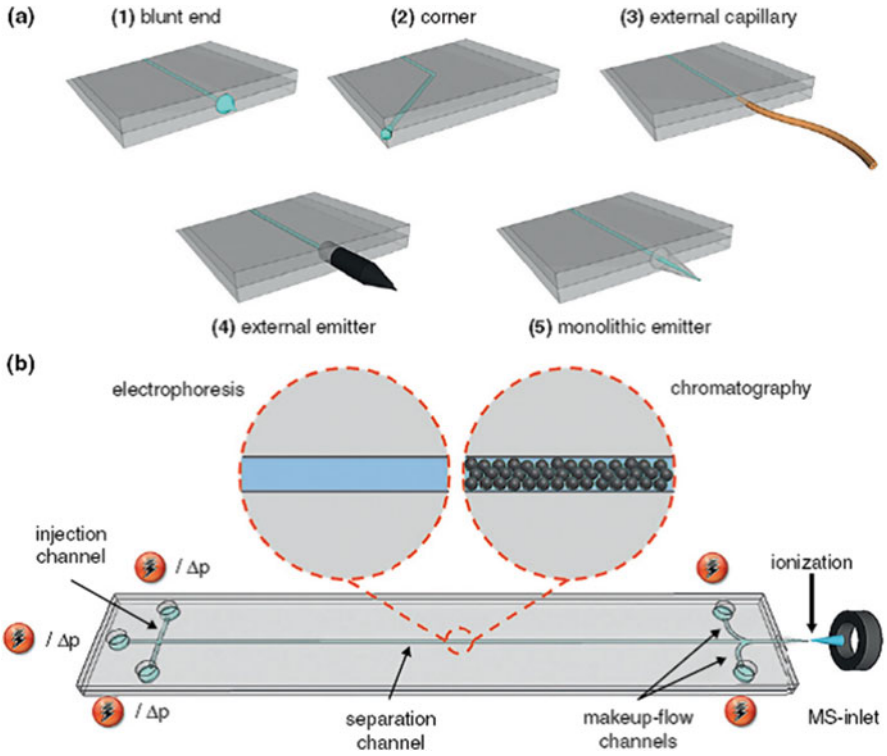


Fig. 5 MS coupling of chipCE. (**a**) Overview of different approaches for hyphenation of microfluidics with electrospray MS. (**b**) Schematic of a microfluidic device for chip electrophoresis or chip chromatography. (Reproduced from [69])

nanospray emitter. Such chips can have the footprint of a microscope slide containing crossed microchannels with widths of the size of a human hair or less (Fig. 5b). The short channel is often used for sample injection (especially in electro-driven separations), whereas the actual separation occurs in the long channel, which is filled with a stationary phase in the case of chip chromatography. The pros and cons of these interface types were discussed extensively in literature [70, 71]. To facilitate the electrospray process at the emitter, for example, for the application of an electrical potential, optional makeup-flow channels can be implemented.

Considering spraying, from the blunt end of a chipCE, three basic approaches can be distinguished [66]. The first possibility is to locate the electrospray directly at the end of the microfluidic platform (Fig. 6a and Fig. 6b). Although its simplicity, the position of the electrospray with respect to the chipCE is often difficult to control. To avoid this problem, an approach with the electrospray inserted in the proper chipCE was proposed (Fig. 6c). In another approach, the electrical field is applied between one of the inlet reservoirs on the chipCE and the inlet of the mass spectrometer, as shown in Fig. 6d [72]. The problem here is that a large potential difference between the end of the microfluidic channel and one of the inlets of the microfluidic device

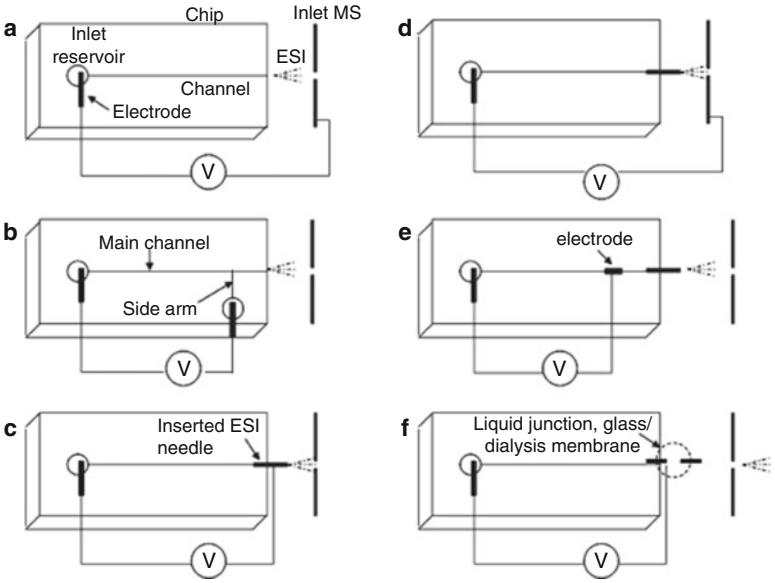


Fig. 6 Basic coupling configurations for chipCE–MS arrangements (a) Spray directly from the edge of a chip, with the spray potential applied between a reservoir and the inlet of the mass spectrometer; (b) spray from the edge of a chip, with potential applied between inlet reservoir and coated side-arm channel at the end of the main channel which can serve as a separation channel; (c) spray from an inserted fused-silica (FS) needle, with the potential applied between the reservoir and the FS needle; (d) as (c) but with the potential applied between the reservoir and the inlet of the MS; (e) electrode integrated in microfluidic channel for applying the electrical field for ESI; (f) spraying with an integrated liquid junction or dialysis/ porous membrane (Reproduced from [66] with permission of the Royal Society of Chemistry)

would be needed to perform a separation, resulting in the generation of heat. In other approaches, the electrical field is applied to a stainless-steel tube connected to the exit of a chipCE through which the eluent is guided [73], or to electrodes integrated in the microfluidic channel, which may be made of gold [74, 75], conductive carbon or conductive epoxy [76] (Fig. 6e). All these approaches have the advantage that compounds eluting from the column are directly electrosprayed without dilution. A different configuration consists of a liquid junction making use frequently of a sheath flow of make-up buffer. This approach has as advantage to be able to modify the composition of the separation buffer to make it suitable for ESI. Most liquid junctions are made by inserting a spray capillary into the device and coupling it to an existing ion source [77, 78]. Couplings where the potential is applied to a side channel in contact with the separation channel through a porous (glass) membrane [79] or a dialysis membrane made of polysulphone [80, 81] have also been reported (Fig. 6f).

One of the main problems associated to the manual insertion of spray needles into the microchannel is that the approach does not lend itself to mass fabrication [76]. As

a solution, integrated microchip emitters can be fabricated, mainly by using a photolithographic procedure, which allows to simultaneously fabricate many structures in a single step.

The direct integration of chipCE and MALDI targets within a single chip appears to be a particularly natural combination since both technologies generally employ planar substrates. The growing interest in this integration is reflected on the range of approaches developed for delivering analyte to on-chip MALDI target surfaces. From the point of view of microfluidic systems, the offline nature of MALDI–MS analysis represents a key advantage over ESI-MS. For example, the time scales for molecular separations and online MS data acquisition are often incompatible. However, offline MALDI–MS enables on-chip sample processing steps to be fully decoupled from the back-end MS analysis. In addition, offline MALDI–MS analysis provides a powerful method for coupling multiplexed parallel on-chip analyses with MS detection.

This type of detection is particularly suitable for analytical applications. MS is a powerful analytical tool. Its integration to CE in general (CE-MS) provides a valuable contribution, as it combines separation speed, high resolution, minimum sample consumption, selectivity, and structural information, including a highly accurate determination of molecular weights. Thus, this hyphenated technique opens very interesting possibilities for analytical purposes. In consequence, the chipCE–MS integration introduces the additional advantages of the separation in microchips, specially providing a high-throughput for the analysis of food and environmental samples, by using very small sample volumes.

4 Some Applications of Chip-Based Separation Devices and MS in Food and Environmental Chemistry

Generally, food and environmental samples are considered complex matrices containing numerous macro and micro interferents, which commonly demand highly effective analytical methods to being properly analysed. Within such a context, conventional LC and CE coupled to MS are highly effective techniques to achieve such goals; however, they are also expensive, involving complex operation steps, and time-consuming, in some cases. For these reasons, chipGC–MS, chipLC–MS, and chipCE–MS approaches are emerging to creating new procedures showing similar or even better performance compared to conventional GC–MS, LC–MS, and CE–MS, reducing steps to as few as possible. These platforms (chipGC–MS, chipLC–MS, and chipCE–MS) are excellent for the separation, profiling, and quantification of target analytes in various types of matrixes. In this section, most representative applications of these devices used as separation techniques in food and environmental fields will be addressed. In food field, several applications of chipGC–MS, chipLC–MS, and chipCE–MS have emerged. A great percentage of published papers use these chip systems in food quality and food safety evaluation,

by determining, phenolic compounds [80–88], peptides and amino acids, protein, vitamins [84], antibiotics, pesticides [87], and toxins. These compounds have been determined in different matrices like fruits [84], wine, [85], vegetable oils [84], edible nuts [86], milk and honey [87], and edible nuts [86, 88].

Chip–LC coupled to time-of-flight mass spectrometry (TOF-MS) was applied for accurate and routine characterization and quantification of phenolic compounds such as chlorogenic acid, p-coumaric acid, (+)-catechin, (−)-epicatechin, procyanidin A2, quercetin 3-O-glucoside, quercetin 3-O-rhamnoside, quercetin, and myricetin in cranberry or cranberry based-products [82]. The chromatographic parameters were optimized, and the method was validated using lock-mass calibration obtaining adequate linearity, with R^2 above 0.99, and acceptable values of intra- and inter-day repeatability of the retention time and peak area. The detection limits and quantification were between1.0–15.6 ng mL^{-1} and 2.0–62.5 ng mL^{-1}, respectively.

Another challenging topic of interest for using chipLC and chipCE is the separation of peptides and amino acids. Giusti et al. developed a selenopeptide mapping in a selenium–yeast protein digest by parallel chipLC–inductively coupled plasma, ICP–MS, and chipLC–MS after online preconcentration on a C18 capillary precolumn [83]. This method allowed the identification of whole series of Se/S substitutions in individual peptides and, in some cases, sequencing of isomers differing in the position of selenomethionine residues in the amino acid sequence. Other interesting application of chipCE–MS in food samples involves the determination of amino acids by microfluidic chip electrophoresis mass spectrometry method [89]. The described platform enabled the separation of 16 amino acids in <2 min and without the requirement for extensive sample preparation.

Recently, Aydoğan et al. [87] used integrated nano-LC–high-resolution orbitrap mass spectrometry (HR Orbitrap MS), for the analysis of antibiotics and pesticides in milk and honey. The stationary phase was prepared by polymerization procedure incorporating multiwall carbon nanotubes (MWCNTs). This monolithic stationary phase achieved the separation of eight antibiotics and six pesticides with high theoretical plate numbers up to 46,000 plates per min. The use of monolith-based nano-LC in combination with HR Orbitrap MS exhibited low limits of detection at the ng mL^{-1} level. The improvement was due to the use of a nanoscale monolith (75 μm i.d.), which increased ionization efficiency with respect to that originated by nanospray ionization.

Proteins are very important in wine quality, because they affect taste, clarity, and stability. In this sense, Kwon has determined the profile of soluble proteins in wine by nano-LC–MS [85]. Twenty proteins were identified from a white wine including five proteins derived from the grape, 12 from yeast, two from bacteria, and one from fungi. The proteins profiling in white wine was reported for the first time in this study using one-dimensional sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) coupled with nano-LC–MS.

Cerretani et al. [84] reported a very interesting work dealing with the analysis of vitamin E in vegetable oils with different botanical origins by using nano-LC with UV detection. The separation of tocopherols and tocotrienols was carried out in a C18 silica monolithic column (150 mm × 0.1 mm) using isocratic elution with

acetonitrile/methanol/water (acidified with 0.2% acetic acid) at a flow rate of 0.5 μL min^{-1}, giving a total analysis time below 18 min. Under the optimum conditions, limits of detection were lower than 0.16 μg mL^{-1}.

Apart from studies involving peptides and other endogenous compounds identification, chipLC–MS was also used for food quality/safe analysis of aflatoxin trace levels in peanut samples and peanut feedstuffs [88]. A remarkable feature is the sub-ng g^{-1} limits of detection reported for the five compounds, which underline the great sensitivity related to chipLC–MS approaches. Alcantara et al. [86] determined mycotoxins residues in edible nuts, using a commercial chip-LC system coupled with MS. Chromatographic parameters were optimized and good recoveries (ranging from 75 to 98%) and lowest concentration levels between 0.05 and 5 μg kg^{-1} (lower than the maximum levels established by the current legislation) were obtained. ChipLC coupled to TOF–MS was applied for accurate and routine characterization and quantification of 17 mycotoxins compounds in commercial peanut, almond, and pistachio samples.

When looking at the methods for environmental field in the conventional scale, the significant reduction in analysis time and solvent consumption is a great perspective to support the chipGC–MS, chipLC–MS, and chipCE–MS approaches in this field. In this same way, Haapala et al. [45] developed a chipGC-APPI-MS for determination of polycyclic aromatic hydrocarbons (PAHs) in environmental samples. This chip platform provides high sensitivity down to 0.8 fmol, repeatability (RSD = 7.5–14%), linearity ($r = 0.9952$–0.9987), and short analysis time (14 min).

Another interesting application in environmental field of chip-based approaches also involves analysis of pesticides on a single C_{30} bead via a chipLC combined with ESI-Q-TOF-MS [90]. Microfluidic device was introduced to manipulate the single C_{30} bead for its miniaturization. The trace level chemicals from the single C_{30} bead were detected and identified by mass spectrum, which showed accurate and clear characteristic peaks for aimed compounds. Significant potential on analysis of environmental samples on this combination system was demonstrated. It consists in reduction of analysis time to 5 min and the solvent consumption to 2.5 μL, obtention of high sensitivity detection avoiding the complex sample pretreatment.

5 Conclusions

Chip-based separation devices coupled to mass spectrometry are a promising platform for food and environmental analysis, which will particularly replace traditional separation systems (LC–MS, GC–MS, and CE–MS). Compared to these traditional systems, they offer several advantages: (1) they can provide quantitative and qualitative information of analytes at molecular level, (2) they have low cost and consume small volumes of samples and reagent, and (3) permit multi-step operations, since sample preparation, separation, and signal analysis can be simultaneously integrated into a single chip-based separation device. Although important technology innovations aiming the fully-integration of chip-based separation devices

and mass analysers have been accomplished, their use for routine analyses is still under evaluation. Continuing efforts of instrumentation development by manufacturers and researchers will provide more comprehensive chip-based MS in food and environmental fields. Glass chip-based separation devices offer lower background MS signals than polymer-based or polymer-modified chips, due to their good chemical stability. However, the fabrication processes of these chips are relatively more complicated compared with the processes of fabricating polymer-based chips. On the other hand, most chips have noncylindrical (such as quadratic or semicylindrical) cross sections of separation channels. In addition to the influence on the packing densities in packed channels, the corner regions are prone to form stagnant flow in channels to reduce separation efficiency. Improvements in fabricating chips, such as exploring new materials and constructing cylindrical cross section, require more attention to make the chip-based devices more rugged and cost-effective. The development of miniaturized MS detectors should also receive more attention from the scientific community. Simultaneously with the chip-based separation devices, the miniaturization and on-chip integration of the mass spectrometer could provide new application opportunities in food and environmental chemistry.

References

1. Lin SL, Lin TY, Fuh MR (2013) Recent developments in microfluidic chip-based separation devices coupled to MS for bioanalysis. Bioanalysis 5(20):2567–2580
2. Terry SC, Jerman JH, Angell JB (1979) A gas chromatographic air analyzer fabricated on a silicon wafer. IEEE Trans Electron Dev 26(12):1880–1886
3. Manz A, Miyahara Y, Miura J, Watanabe Y, Miyagi H, Sato K (1990) Design of an open-tubular column liquid chromatography using silicon chip technology. Sensors Actuators B 1: 249–255
4. Harrison DJ, Manz A, Fan Z, Ludi H, Widmer HM (1992) Capillary electrophoresis and sample injection systems integrated on a planar glass chip. Anal Chem 64:1926–1932
5. Manz A, Graber N, Widmer HM (1990) Miniaturized total chemical analysis systems: a novel concept for chemical sensing. Sensors Actuators B Chem 1:244–248
6. Harrison DJ, Fluri K, Seiler K, Fan Z, Effenhauser CS, Manz A (1993) Micromachining a miniaturized capillary electrophoresis based chemical analysis system on a chip. Science 261: 895–897
7. Jacobson SC, Hergenroder R, Koutny LB, Ramsey JM (1994) Open channel electrochromatography on a microchip. Anal Chem 66:2369–2373
8. Ramsey RS, Ramsey JM (1997) Generating electrospray from microchip devices using electroosmotic pumping. Anal Chem 69:1174–1178
9. Xue Q, Foret F, Dunayevskiy YM, Zavracky PM, McGruer NE, Karger BL (1997) Multichannel microchip electrospray mass spectrometry. Anal Chem 69:426–430
10. Wilm MS, Mann M (1994) Electrospray and Taylor-cone theory, Dole's beam of macromolecules at last? Int J Mass Spectrom Ion Process 136:167–180
11. Emmett MR, Caprioli RM (1994) Micro-electrospray mass spectrometry: ultra-high-sensitivity analysis of peptides and proteins. J Am Soc Mass Spectrom 5:605–613
12. Davis MT, Stahl DC, Hefta SA, Lee TD (1995) A microscale electrospray interface for online, capillary liquid chromatography/tandem mass spectrometry of complex peptide mixtures. Anal Chem 67:4549–4556

13. Gatlin CL, Kleemann GR, Hays LG, Link AJ, Yates JR (1998) Protein identification at the low femtomole level from silver-stained gels using a new frit less electrospray interface for liquid chromatography microspray and nano-spray mass spectrometry. Anal Biochem 263:93–101
14. Ostman P, Marttila SJ, Kotiaho T, Franssila S, Kostiainen R (2004) Microchip atmospheric pressure chemical ionization source for mass spectrometry. Anal Chem 76:6659–6664
15. Lorenzelli L, Benvenuto A, Adami A, Guarnieri V, Margesin B, Mulloni V, Vincenz D (2005) Development of a gas chromatography silicon-based microsystem in clinical diagnostics. Biosens Bioelectron 20:1968–1976
16. Bruns MW (1994) High speed portable gas chromatography. Erdol Kohle Erdgas Petrochemie 47:80–84
17. Lehman U, Krusemark O, Muller J, Vogel A, Binz D (2000) Micromachined gas chromatograph based on plasma polymerised stationary phase. Proc MicroTas 5:167–170
18. Dziuban JA, Mróz J, Szczygielska M, Matachowski M, Górecka-Drzazga A, Walczalk R et al (2004) Portable gas chromatograph with integrated components. Sensors Actuators B Chem 115:318–330
19. Bessoth FG, Naji OP, Eijkel JC, Manz A (2002) Towards an on-chip gas chromatograph: the development of a gas injector and a dc plasma emission detector. J Anal At Spectrom 17:794–779
20. Janssen S, Tessmann T, Lang W (2014) High sensitive and selective ethylene measurement by using a large-capacity-on-chip preconcentrator device. Sensors Actuators B 197:405–413
21. Nachef K, Marty F, Donzier E, Bourlon B, Dannaie K, Bourouina T (2012) Micro gas chromatography sample injector for the analysis of natural gas. J Microelectromech Syst 21: 730–738
22. Maginell RP, Frye-Mason GC (1999) U.S. Patent 6 171 378 and 6 527 835
23. Alfeeli B, Cho D, Ashraf-Khorassanib M, Taylor LT, Agah M (2008) MEMS-based multi-inlet/outlet preconcentrator coated by inkjet printing of polymer adsorbents. Sensors Actuators B 133:24–32
24. Tian WC, Pang SW, Lu CJ, Zellers ET (2003) Microfabricated preconcentrator–focuser for a microscale gas chromatograph. J Microelectromech Syst 12:264–272
25. Zampolli S, Elmi I, Mancarella F, Betti P, Dalcanale E, Cardinali GC, Severi M (2009) Real-time monitoring of sub-ppb concentrations of aromatic volatiles with a MEMS-enabled miniaturized gas-chromatograph. Sensors Actuators B 141:322–328
26. Lahlou H, Sanchez JB, Vilanova X, Berger F, Correig X, Fierro V, Celzard A (2011) Towards a GC-based microsystem for benzene and 1,3 butadiene detection: pre-concentrator characterization. Sensors Actuators B 156:680–688
27. Kim SK, Chang H, Zellers ET (2011) Microfabricated gas chromatograph for the selective determination of trichloroethylene vapor at sub-parts-per-billion concentrations in complex mixtures. Anal Chem 83:7198–7206
28. Regmi BP, Agah M (2018) Micro gas chromatography: an overview of critical components and their integration. Anal Chem 90:13133–13150
29. Gaddes D, Westland J, Dorman FL, Tadigadapa S (2014) Improved micromachined column design and fluidic interconnects for programmed high-temperature gas chromatography separations. J Chromatogr A 1349:96–104
30. Lewis PR, Wheeler DR (2007) Non-planar microfabricated gas chromatography column. U.-S. Patent 7,273,517, September 25
31. Rankin JM, Suslick KS (2015) The development of a disposable gas chromatography microcolumn. Chem Commun 51:8920–8923
32. Kaanta BC, Chen H, Zhang X (2010) A monolithically fabricated gas chromatography separation column with an integrated high sensitivity thermal conductivity detector. J Micromech Microeng 20:055016–055022
33. Zampolli S, Elmi L, Sturmann JS, Nicoletti S, Dori L, Cardinali GC (2005) Selectivity enhancement of metal oxide gas sensors using a micromachined gas chromatographic column. Sensors Actuators B 105:400–406

34. Sun J, Guan F, Zhu X, Ning Z, Ma T, Liu J, Deng T (2016) Micro-fabricated packed gas chromatography column based on laser etching technology. J Chromatogr A 1429:311–316
35. Reidy S, Lambertus G, Reece J, Sacks R (2006) High-performance, static-coated silicon microfabricated columns for gas chromatography. Anal Chem 78:2623–2630
36. Lin Z, Nuñovero N, Wang J, Nidetz R, Buggaveeti S, Kurabayashi K, Zellers ET (2018) A zone-heated gas chromatographic microcolumn: energy efficiency. Sensors Actuators B 254: 561–572
37. Cagliero C, Galli S, Galli M, Elmi I, Belluce M, Zampolli S, Sgorbini B, Rubiolo P, Bicchi CJ (2016) Conventional and enantioselective gas chromatography with microfabricated planar columns for analysis of real-world samples of plant volatile fraction. J Chromatogr A 1429: 329–339
38. Ghosh A, Johnson JE, Nuss JG, Stark BA, Hawkins AR, Tolley LT, Iverson BD, Tolley HD, Lee ML (2017) Extending the upper temperature range of gas chromatography with all silicon microchip columns using a heater/clamp assembly. J Chromatogr A 1517:134–141
39. Hussain CM (2018) Nanomaterials in chromatography: current trends in chromatographic research technology and techniques. Elsevier
40. Bétard A, Fischer RA (2012) Metal-organic framework thin films: from fundamentals to applications. Chem Rev 112:1055–1083
41. Read DH, Sillerud CH (2016) Metal-organic framework thin films as stationary phases in microfabricated gas-chromatography columns. Sandia Report SAND2016-0706. Unlimited release
42. Xie SM, Zhang ZJ, Wang ZY, Yuan LM (2011) Chiral metal organic frameworks for high-resolution gas chromatographic separations. J Am Chem Soc 133:11892–11895
43. Cruz D, Chang J, Showalter S, Gelbard F, Manginell R, Blain M (2007) Microfabricated thermal conductivity detector for the micro-ChemLabTM. Sensors Actuators B 121:414–422
44. Ostman P, Luosuja L, Haapala M (2006) Gas chromatography-microchip atmospheric pressure chemical ionization-mass spectrometry. Anal Chem 78:3027–3031
45. Haapala M, Luosujarvi L, Saarela V, Kotiaho T, Ketola RA, Franssila S, Kostiainen R (2007) Microchip for combining gas chromatography or capillary liquid chromatography with atmospheric pressure photoionization-mass spectrometry. Anal Chem 79:4994–4999
46. Yin H, Killeen K (2006) The fundamental aspects and applications of Agilent HPLC-Chip. J Sep Sci 30:1427–1434
47. Vargas DA, Soares EV, Mauro F (2020) Miniaturization of liquid chromatography coupled to mass spectrometry. 3. Achievements on chip-based LC-MS devices. Trends Anal Chem 131: 116003
48. Vargas DA, Maciel EVS, Toffoli ALFM (2020) Miniaturization of liquid chromatography coupled to mass spectrometry. Trends Anal Chem 128:115910
49. Abian J, Oosterkamp AJ, Gelpí E (1999) Comparison of conventional, narrow-bore and capillary liquid chromatography/mass spectrometry for electrospray ionization mass spectrometry: practical considerations. J Mass Spectrom 34:244–254
50. Gibson GTT, Mugo SM, Oleschuk RD (2009) Nanoelectrospray emitters: trends and perspective. Mass Spectrom Rev 28:918–936
51. Lazar LM, Kabulski JL (2013) Microfluidic LC device with orthogonal sample extraction for on-chip MALDI-MS detection. Lab Chip 13:2055–2065
52. Kitagawa F, Otsuka K (2011) Recent progress in microchip electrophoresis–mass spectrometry. J Pharm Biomed Anal 55(4):668–678
53. Zhang B, Foret F, Karger BL (2000) A microdevice with integrated liquid junction for facile peptide and protein ana lysis by capillary electrophoresis/electrospray mass spectrometry. Anal Chem 72(5):1015–1022
54. Deng Y, Zhang H, Henion J (2001) Chip-based quantitative capillary electrophoresis/mass spectrometry determination of drugs in human plasma. Anal Chem 73(7):1432–1439
55. Zheng Y, Li H, Guo Z et al (2007) Chip-based CE coupled to quadrupole TOF mass spectrometer for the analysis of glycopeptide. Electrophoresis 28(9):1305–1311

56. Li HF, Liu J, Cai Z et al (2008) Coupling a microchip with electrospray ionization quadrupole time-of-flight mass spectrometer for peptide separation and identification. Electrophoresis 29(9):1889–1894
57. Akashi S, Suzuki K, Arai A et al (2006) Top-down analysis of basic proteins by microchip capillary electrophoresis mass spectrometry. Rapid Commun Mass Spectrom 20(12): 1932–1938
58. Sikanen T, Tuomikoski S, Ketola RA et al (2007) Fully microfabricated and integrated SU-8-based capillary electrophoresis electrospray ionization microchips for mass spectrometry. Anal Chem 79(23):9135–9144
59. Li FA, Huang JL, Her GR (2008) Chip-CE/MS using a flat low-sheath-flow interface. Electrophoresis 29(24):4938–4943
60. Mellors JS, Gorbounov V, Ramsey RS et al (2008) Fully integrated glass microfluidic device for performing high-efficiency capillary electrophoresis and electrospray ionization mass spectrometry. Anal Chem 80(18):6881–6887
61. Sikanen T, Aura A, Franssila S et al (2012) Microchip capillary electrophoresis– electrospray ionization–mass spectrometry of intact proteins using uncoated OrmoComp microchips. Anal Chim Acta 711:69–76
62. Sainiemi L, Sikanen T, Kostiainen R (2012) Integration of fully microfabricated, three-dimensionally sharp electrospray ionization tips with microfluidic glass chips. Anal Chem 84(21):8973–8979
63. Limbach PA, Meng Z (2002) Integrating micromachined devices with modern mass spectrometry. Analyst 127:693–700
64. Lion N, Rohner TC, Dayon L, Arnaud IL, Damoc E, Youhnovski N, Wu ZY, Roussel C, Josserand J, Jensen H, Rossier JS, Przybylski M, Girault HH (2003) Microfluidic systems in proteomics. Electrophoresis 24:3533–3562
65. Freire SLS, Wheeler AR (2006) Proteome-on-a-chip: mirage, or on the horizon? Lab Chip 6: 1415–1423
66. Koster S, Verpoorte E (2007) A decade of microfluidic analysis coupled with electrospray mass spectrometry: an overview. Lab Chip 7:1394–1412
67. Brivio M, Tas NR, Goedbloed M, Gardeniers H, Verboom W, Van den Berg A, Reinhoudt DN (2005) A MALDI-chip integrated system with a monitoring window. Lab Chip 5:378–381
68. DeVoe DL, Lee CS (2006) Microfluidic technologies for MALDI-MS in proteomics. Electrophoresis 27:3559–3568
69. Ohla S, Belder D (2012) Chip-based separation devices coupled to mass spectrometry. Curr Opin Chem Biol 16:453–459
70. Sikanen T, Franssila S, Kauppila TJ, Kostiainen R, Kotiaho T, Ketola RA (2010) Microchip technology in mass spectrometry. Mass Spectrom Rev 29:351–391
71. Lee J, Soper AS, Murray KK (2009) Microfluidic chips for mass spectrometry-based proteomics. J Mass Spectrom 44:579–593
72. Lazar IM, Ramsey RS, Ramsey JM (2001) On-chip proteolytic digestion and analysis using "wrong-way-round" electrospray time-of-flight mass spectrometry. Anal Chem 73(8): 1733–1739
73. Shui W, Yu Y, Xu X, Huang Z, Xu G, Yang P (2003) Micro-electrospray with stainless steel emitters. Rapid Commun Mass Spectrom 17:1541–1547
74. Lion N, Gellon JO, Girault HH (2004) Flow-rate characterization of microfabricated polymer microspray emitters. Rapid Commun Mass Spectrom 18:1614–1620
75. Bindila L, Froesch M, Lion N, Vukelic Z, Rossier JS, Girault HH, Peter-Katalinic J, Zamfir AD (2004) A thin chip microsprayer system coupled to Fourier transform ion cyclotron resonance mass spectrometry for glycopeptide screening. Rapid Commun Mass Spectrom 18:2913–2920
76. Liu H, Felten C, Xue Q, Zhang B, Jedrezejewski P, Karger BL, Foret F (2000) Development of multichannel devices with an array of electrospray tips for high-throughput mass spectrometry. Anal Chem 72:3303–3310

77. Tachibana Y, Otsuka K, Terabe S, Arai A, Suzuki K, Nakamura S (2003) Robust and simple interface for microchip electrophoresis–mass spectrometry. J Chromatogr A 1011:181–192
78. Tachibana Y, Otsuka K, Terabe S, Arai A, Suzuki K, Nakamura S (2004) Effects of the length and modification of the separation channel on microchip electrophoresis–mass spectrometry for analysis of bioactive compounds. J Chromatogr A 1025:287–296
79. Yue GE, Roper MG, Jeffery ED, Easley CJ, Balchunas C, Landers JP, Ferrance JP (2005) Glass microfluidic devices with thin membrane voltage junctions for electrospray mass spectrometry. Lab Chip 5:619–627
80. Gao J, Xu J, Locascio LE, Lee CS (2001) Integrated microfluidic system enabling protein digestion, peptide separation, and protein identification. Anal Chem 73:2648–2655
81. Jiang Y, Wang PC, Locascio LE, Lee CS (2001) Integrated plastic microfluidic devices with ESI-MS for drug screening and residue analysis. Anal Chem 73:2048–2053
82. Contreras M, Arráez-Román D, Fernández-Gutiérrez A, Segura-Carretero A (2015) Nano-liquid chromatography coupled to time-of-flight mass-spectrometry for phenolic profiling: a case study in cranberry syrups. Talanta 132:929–938
83. Giusti P, Schaumloffel D, Preud'homme H, Szpunara J, Lobinski R (2006) Selenopeptide mapping in a selenium–yeast protein digest by parallel nanoHPLC-ICP-MS and nanoHPLC-electrospray-MS/MS after on-line preconcentration. J Anal At Spectrom 21:26–32
84. Cerretani L, Lerma-García MJ, Herrero-Martínez JM, Gallina-Toschi T, Simó-Alfonso EF (2010) Determination of tocopherols and tocotrienols in vegetable oils by nanoliquid chromatography with ultraviolet−visible detection using a silica monolithic column. J Agric Food Chem 58:757–761
85. Kwon SW (2004) Profiling of soluble proteins in wine by nano-high-performance liquid chromatography/tandem mass spectrometry. J Agric Food Chem 52:7258–7263
86. Alcántara J, Moreno D, García JF, Molina A (2019) Use of a modified QuEChERS method for the determination of mycotoxin residues in edible nuts by nano flow liquid chromatography high resolution mass spectrometry. Food Chem 279:144–149
87. Aydogan C, El Rassi Z (2019) MWCNT based monolith for the analysis of antibiotics and pesticides in milk and honey by integrated nano-liquid chromatography-high resolution orbitrap mass spectrometry. Anal Methods 11:21–28
88. Aldredge DL, Geronimo MR, Hua S, Nwosu CC, Lebrilla CB, Barile D (2013) Annotation and structural elucidation of bovine milk oligosaccharides and determination of novel fucosylated structures. Glycobiology 23:664–676
89. Da Silva MR, Zaborowska I, Carillo S, Bones J (2021) A rapid, simple and sensitive microfluidic chip electrophoresis mass spectrometry method for monitoring amino acids in cell culture media. J Chromatogr A 1651:462336
90. Wei H, Li H, Lin JM (2009) Analysis of herbicides on a single C_{30} bead via a microfluidic device combined with electrospray ionization quadrupole time-of-flight mass spectrometer. J Chromatogr A 1216:9134–9142