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REVIEW

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LC-MS analysis in the aquatic environment and in water treatment – a critical review

Part I: Instrumentation and general aspects of analysis and detection

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Abstract LC-MS has become an invaluable technique for trace analysis of polar compounds in aqueous samples of the environment and in water treatment. LC-MS is of particular importance due to the impetus it has provided for research into the occurrence and fate of polar contaminants, and of their even more polar transformation products. Mass spectrometric detection and identification is most widely used in combination with sample preconcentration, chromatographic separation and atmospheric pressure ionization (API). The focus of the first part of this review is directed particularly toward instruments and method development with respect to their applications for detecting emerging contaminants, microorganisms and humic substances (HS). The current status and future perspectives of 1) mass analyzers, 2) ionization techniques to interface liquid chromatography (LC) with mass spectrometry (MS), 3) methods for preconcentration and separation with respect to their application for water analysis are discussed and examples of applications are given. Quadrupole and ion trap mass analyzers with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are already applied in routine analysis. Time-of-flight (TOF) mass spectrometers are of particular interest for accurate mass measurements for identification of unknowns. For non-polar compounds, different ionization approaches have been described, such as atmospheric pressure photoionization (APPI), electrochemistry with ESI, or electron capture ionization with APCI. In sample preconcentration and separation, solid phase extraction (SPE) with different non-selective sorbent materials and HPLC on reversed-phase materials (RP-HPLC) play the dominant role. In addition, various on-line and miniaturized approaches for sample extraction and sample introduction into the MS have been used. Ion chromatography (IC), size-exclusion chromatography (SEC), and capillary electrophoresis (CE) are alternative separation techniques. Furthermore, the issues of compound identification, matrix effects on quantitation, development of mass spectral libraries and the topic of connecting analysis and toxicity bioassays are addressed.

Keywords Mass spectrometry · Instrumentation · Contaminant · Water · Sample preparation

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Introduction

The improvement and preservation of water quality is one of the major tasks of water chemistry. A good ecological and chemical state of the waters of Europe is also required in the European Water Framework Directive [1]. Therefore, understanding of the fate and the cycles of natural and anthropogenic substances in the aquatic environment is an important prerequisite. The relevant processes include the distribution of the substances and their sinks, phase transfer and transformation. In addition, the effects of contaminants on the environment and on human health have to be investigated.

Because most of the so-called emerging contaminants, and even more of their metabolites, are highly polar and water-soluble, LC-MS is the method of choice for determination and quantification. Since the introduction of atmospheric pressure ionization techniques such as electrospray ionization (ESI) [2, 3] LC-MS has played an increasingly important role in environmental analysis. ESI and atmospheric pressure chemical ionization (APCI) are applicable for the analysis of a broad range of compounds and problems, including non-volatile, thermally labile and polar species. In addition, ESI and APCI provide high sensitivity, which is mandatory for environmental analysis where contaminants are often found at trace (ng/L or µg/L) levels.

Several recent reviews on water analysis [4], environmental analysis [5], environmental mass spectrometry [6, 7], and on the use of LC-MS in water analysis [8, 9] reveal that LC-MS methods have found their place in environmental analysis.

There are also several recent books on LC-MS [10, 11, 12, 13], including on its history and development [14]. Recent applications of LC-MS-MS methods for the analysis of emerging compounds are reported in Ferrer and Thurman [15]. Electrospray ionization is covered by Cole [16].

This review gives the current status and future perspectives of LC-MS in water analysis, with respect to its application for detecting emerging contaminants and related substances. This contribution is technique- and method-oriented. Therefore, the focus will be on mass analyzers, ionization techniques used for interfaces, and methods for compound preconcentration, separation, and identification. Furthermore, the issues of matrix effects on quantification, the development of mass spectral libraries of fragmentation data obtained by collision-induced dissociation, and the topic of connecting chemical analysis and toxicity bioassays will be addressed. In general, the most recent publications (covering the last three years approximately) are selected mainly with respect to the subject of water analysis. In addition, particular techniques and methods that have not yet been directly applied for water analysis will be considered, if they are thought to be of future interest in water analysis. This necessitates a critical, and not a comprehensive, review. Additional information can be found in recent reviews of specific subjects cited in this article.

Mass analyzers

The most widely used ionization techniques – ESI and APCI – are soft-ionization techniques, which in general produce protonated or deprotonated molecules. To cope with complex sample composition and not fully resolved chromatographic peaks, MS-MS methods are predominantly used in environmental analysis, applying triple quadrupole mass spectrometers or ion traps. LC-MS with single quadrupole mass spectrometers can also be used to produce fragmented spectra. This is done using in-source collision-induced dissociation, which can provide higher sensitivity in some cases but much less selectivity than MS-MS, because in this process co-eluting analytes and matrix components are also fragmented and can result in a mixed mass spectrum of the analyte and interfering compounds. Niessen has reviewed state-of-the-art mass analyzers and ionization techniques, with emphasis on high-throughput screening [17]. The major possibilities for the coupling together of liquid separation, ionization and mass spectrometric determination techniques for water analysis are shown in Fig. 1. The different methods are explained in the sections below.

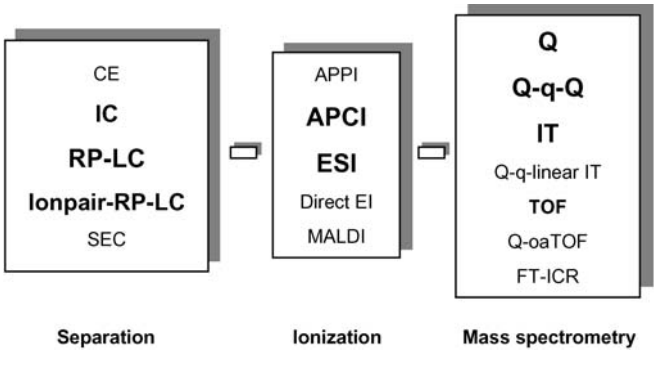


Fig. 1 Major possibilities for the linking together of liquid separation, ionization and mass spectrometric determination techniques used for water analysis. Size of acronyms correspond to the importance of the technique for water analysis

Quadrupole mass spectrometers

Triple quadrupole mass spectrometers are most widely used for sensitive and selective quantification of target compounds that show specific mass transitions in the multiple-reaction monitoring mode (MRM). In general, MRM operation is performed at fixed *m*/*z* values of the quadrupoles (Q) 1 and 3, whereas quadrupole 2 (Q2) serves as the collision cell. Further modes of operation are the constant neutral loss scan (NL), with both quadrupoles scanning at a constant *m*/*z* offset, the precursor-ion scan with Q1 scanning and Q3 set at a fixed *m*/*z* value, and finally the product-ion scan with selection of a precursor-ion in Q1 set at fixed *m*/*z*, and scanning of Q3 to record the collision-induced fragments produced in Q2.

Neutral loss scans have been successfully used for group specific detection: for instance for aromatic acids by NL of CO_2 (*m*/*z*=44; Fig. 2) [18], and for triazine herbicides by NL of a propylene group (*m*/*z*=42) [19, 20]. Precursor-ion scans have been used for the detection of 2,4-dinitrophenylhydrazine (DNPH) derivatives of carbonyls, by recording the product ions of the DNPH moiety, such as *m*/*z*=163 for aldehyde derivatives or *m*/*z*=182 for dicarbonyl and hydroxycarbonyl derivatives [21, 22]. These are unique features of triple quadrupole mass spectrometers, which have not been widely used in routine analysis. One reason may be found in the lower sensitivity

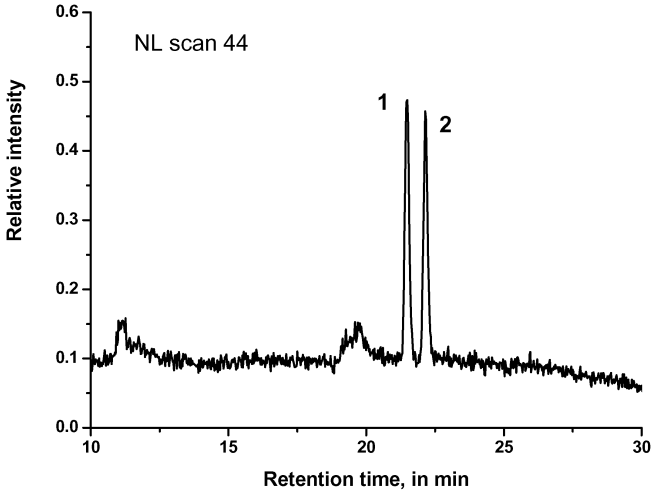


Fig. 2 Neutral loss scan of 44 of a ground water sample spike with a mixture of 1-naphthoic acid (1) and 2-naphthoic acid (2)

of triple quadrupole instruments in the scan mode, a major disadvantage of these instruments compared to ion trap and time-of-flight instruments.

A commercial system using quadrupoles with enhanced mass resolution without significant losses in ion transmission could be realized due to improved production capabilities [23, 24]. A mass resolution of 0.1 Da full width at half maximum (FWHM) instead of the usual 0.6 Da (typical for unit-mass resolution of quadrupole instruments) has been achieved.

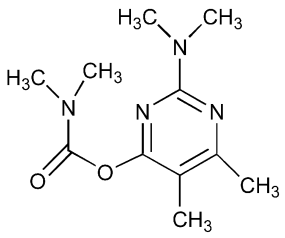
Quadrupole ion-traps

Three-dimensional quadrupole ion-trap mass spectrometers (ITMS) are now commonly used. They allow MS-MS in a time-sequenced series of ion isolation, fragmentation, and trapping of the product-ions formed. They also uniquely allow MS^n, which enables us to deduce fragmentation pathways easily, making them very useful for the identification of unknowns [25, 26, 27]. In general, quadrupole ion-traps also show high sensitivity in the scan mode, but neutral loss scans are not possible with this technique, and quantitation is less reliable than MRM with a triple quadrupole instrument.

A linear two-dimensional ion trap mass spectrometer is a new instrumental development. The instrumental apparatus is based on the ion path of a triple quadrupole mass spectrometer, using the collision cell or the final mass analyzer as the linear trap [28]. Ions can be trapped along the centerline of the linear trap and mass selectively ejected in the axial direction by an auxiliary quadrupole field. So many of the scan functions of conventional 3D traps can also be applied to a linear 2D trap. The larger volume of the linear 2D trap relative to the 3D device results in a higher storage capacity of ions due to space charge effects in the traps; in a 3D trap, ions are almost focused on a center point (one dimension) compared to a center line (two dimensions) in a 2D trap. Furthermore, the trapping efficiency of a pressurized linear ion trap (collision cell) can be up to 100%, compared to 1–10% for a 3D ion trap. With a triple quadrupole instrument, and the inclusion of a linear ion trap, significantly enhanced product ion scanning performance can be obtained while retaining all of the triple quadrupole capabilities like precursor ion and neutral loss scans.

Time-of-flight instruments

Time-of-flight (TOF) mass spectrometers have to a large extent replaced high-resolution double-focusing magnetic sector instruments for LC-MS applications. For unknown identification, the hybrid quadrupole orthogonal-acceleration time-of-flight tandem mass spectrometers (Q-oaTOF) instrument – in general simply called Q-TOF– has proven very useful due to its accurate mass measurement of fragments at high sensitivity. The TOF always gives full-scan data. Using a Q-TOF mass spectrometer, the elemental



	m/z (calc)	m/z (exp)	Δ (mDa)
$C_{11}H_{19}N_4O_2$	239.1508	239.1501	-0.7
↓ $-CO_2$			
$C_{10}H_{19}N_4$	195.1610	195.1627	1.7
↓ $-H_3C-N=C=O$			
$C_9H_{16}N_3O$	182.1293	182.1306	1.3

Fig. 3 Assignment of the collision-induced fragmentation of pirimicarb based on accurate mass measurements by Q-TOF (data based on [29])

composition for precursor and product-ions were calculated based on exact mass for unknowns in water samples and structures were proposed [29]. This is illustrated for the fragmentation of pirimicarb (Fig. 3). Based on accurate mass measurements, the losses of 44 and 57 Da from the protonated molecule can be attributed to the losses of CO_2 and H_3C-N=C=O, respectively. Therefore rearrangements of the dimethylamino and a methyl group have to be proposed to explain the losses. Furthermore, HPLC-TOF-MS has been applied to the analysis of pesticides and metabolites in ground and surface waters [30]. Exact mass measurements at a resolving power of 3500 and 5000 were used for the confirmation of 10 carbamate, urea, and thiourea pesticides [31] and the measurement of acetolachlor and alachlor degradates [32]. However, Q-TOF instruments are much more expensive and therefore have been applied much less frequently in environmental analysis compared to Q and IT instruments.

Fourier-transform ion cyclotron resonance instruments

Ultra-high resolution (up to 100,000) is achievable with Fourier-transform ion cyclotron resonance mass spectrometers (FT-ICR-MS). ESI with FT-ICR-MS has been shown to be a valuable tool for biomacromolecules, as well as for exact elemental composition and mass spectrometric characterization of highly complex mixtures like petroleum products, and humic and fulvic acids in aqueous samples [33, 34, 35]. Because resolution is defined by mass divided by the mass difference (δm) of the next resolved mass, only with ultra-high resolving power can exact mass data be obtained on high molecular weight material. However, a major disadvantage of this method is the high instrument cost, although, due to its high performance, it still may become an increasingly important technique in environmental analysis.

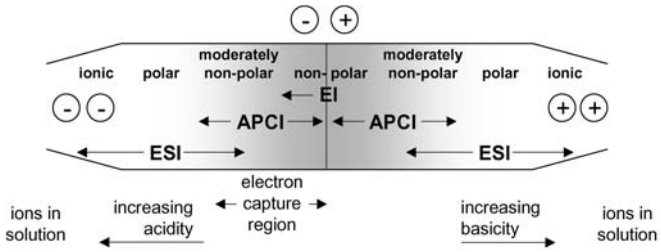


Fig. 4 Ionization continuum diagram showing the interrelationship between analyte properties and the appropriate API mode (reprinted with permission from Ref. [38], copyright (2001) American Chemical Society)

Ionization techniques

ESI and APCI

Some general principles of atmospheric pressure ionization are covered in recent reviews [17, 36, 37]. The ionization efficiency of 75 pesticides by ESI was compared with APCI, and this comparison revealed a more sensitive ionization of neutral and basic pesticides (phenylureas, triazines) by APCI, while the determination of cationic and anionic herbicides (bipyridylium ions, sulfonic acids) was more sensitive using ESI [38]. A diagram called the "ionization continuum" shows that proton affinity in the gas phase and polarity in solution (pK_a) are useful for selecting APCI or ESI (Fig. 4).

Direct-electron ionization interface

A new direct-electron ionization (EI) interface was used for direct coupling of nano-HPLC to a mass spectrometer [39]. The device can be looked at as a kind of optimization of direct liquid introduction, with a heated chamber housing the nebulizer, and an electron impact ionization source. It is designed to work at very low flow rates, between 0.3 and 1.5 µL/min of mobile phase. The particle beam interface can be seen as the progenitor of direct EI. The system was applied for detecting organophosphorus pesticides in water at the low ng/L level. Ionization under typical EI conditions provided library-matchable EI spectra [40].

APPI

In addition to ESI and APCI, there have been approaches for expanding the range of applications to less polar compounds. The different approaches were recently discussed in a review [41]. Atmospheric pressure photoionization (APPI) uses photons in the vacuum UV region. Compounds which show lower first ionization potentials than the energy of the photons are ionized. A key application of APPI may be seen in the determination of PAHs. Due to their conjugated π-electron systems and therefore low ionization potentials, they work very well with APPI, but have bad response with APCI. Improved performance of APPI can be achieved by addition of a dopant – a mobile phase additive like acetone or toluene, which is first ionized itself and then aids ionization of the analytes in further reactions. Compounds like naphthalene, acridine, diphenyl sulfide, and 5-fluorouracil could be ionized by an APPI source. With a vacuum-ultraviolet lamp as a source of 10 eV photons, sensitivities eight times higher than those of a commercial corona discharge APCI source have been obtained [42]. Despite being a very new approach, APPI-MS it is expected to become an important complimentary technique to APCI for low and nonpolar analytes in the future.

Dissociative electron capture ionization

Another approach for less polar compounds with electronegative substituents is to use dissociative electron capture ionization. In APCI, the corona discharge can provide a source of electrons, which react with (for instance) pentafluorobenzyl (PFB) derivatives. PFB derivatives of estrogens yield the $[M\text{-}PFB]^-$ ion, but not a deprotonated $[M\text{-}H]^-$ ion [43]. Steroids and oxosteroids have been derivatized with various boronic acid and hydrazine derivatives with electron-capturing moieties, and were measured at higher sensitivities than by APCI-MS [44, 45]. For instance, the detection limit of 216 fmol of the intact molecule of 24,25-dihydroxyvitamin D_3 could be lowered to about 1 fmol if its derivative with [3-(4-nitrophenyl)aminophenyl]dihydroxyborane was measured. For nitroaromatic compounds which do not easily undergo deprotonation, both dissociative and non-dissociative electron capture was observed [46].

Electrochemistry

The use of an ESI source as electrochemical reactor for oxidation and reduction expands the utility of ESI to compounds, which are in general not ionized in ESI. The half-wave oxidation potential of the analytes is an important factor in the formation of radical ions. Recent reviews report on the history, applications and different coupling of an electrochemical cell with a variety of ionization interfaces [41, 47]. The applicability of the method was demonstrated for different compound classes like metal complexes, metal porphyrins, fullerenes, and other compounds that form stable organic radicals. Neutral mono- and disaccharides could be oxidized as their cyclic ferrocenyl boronic esters and analyzed by MS-MS and MS-MS-MS [48]. Ferrocenecarboxylic acids [49] were used for derivatization and even alkenes could be ionized with electrochemical oxidation by derivatization with an electroactive group like ferrocene boronic acid [50, 51]. Additional electrochemical cells were coupled to electrospray to provide a specific oxidation or reduction potential. For example, electrochemical polymerization [52] and biological redox reactions [53] were studied using this set-up.

MALDI

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is a useful technique for the analysis of large molecules (>1 kDa), and is therefore only of particular interest for water analysis. MALDI is a soft ionization technique and leaves molecules intact. It was used to investigate the biodegradation and fate of a water-soluble poly-(vinylpyrrolidone) (PVP) polymer with a mass distribution >2 kDa. On simulated sewage sludge PVP was found to be recalcitrant [54]. MALDI was further used for rapid detection and identification of microorganisms, using whole or treated cells [55, 56]. This technique provides the very exciting possibility of developing methods for very rapid determination of whether pathogens are present in a water sample or not.

Other sample introduction systems

MIMS

Membrane introduction mass spectrometry (MIMS) is an approach for on-line preconcentration and simultaneous introduction into the MS, which allows the analysis of organic compounds directly in water. The aqueous sample and the MS are separated by the membrane, which serves as material for preconcentration and transport to the MS. For MIMS the composition of the membrane is an important factor. Therefore, in addition to poly-(dimethylsiloxane), different tailored materials are used [57]. Phenols have been analyzed by flow injection analysis coupled with MIMS [58]. An in-membrane preconcentration/thermal desorption technique has been used for MIMS to measure the biodegradations of 4-fluorobenzoic acid and 4-fluorocinnamic acid [59], and a cryotrapping step prior to MIMS allowed sensitive detection of organohalogens in water [60].

FAIMS

ESI-high field asymmetric waveform ion mobility spectrometry (FAIMS)-MS is a technique for efficient separation of ionized analytes in the gas phase in the drift region of an IMS at atmospheric pressure. FAIMS acts as an ion filter, tuneable by control of electrical voltages, which permits continuous transmittance of selected ions from a complex sample mixture. Improved signal-to-noise ratio is obtained due to the removal of low-mass solvent cluster ions and reduction of background noise in electrospray [61]. ESI-FAIMS-MS was used for the determination of nine halogenated acetic acids at detection limits in the submicrogram per liter range [62, 63]. Other FAIMS applications are the trace determination of perchlorate in water and human urine [64], and the analysis of microcystins in water by ESI-FAIMS-MS-MS [65].

Sample preparation

Sample preparation is quite a large field, and therefore only a selection of the most widely used preconcentration methods are described here, with a focus on sorptive extraction. More innovative methods which may play a role in water analysis in future applications are also emphasized.

Adsorptive extraction

Solid-phase extraction (SPE) with a multitude of broad spectrum and chemical class-specific materials is the most important method for sample preparation. A recent review on environmental analysis includes advances in sample preparation for LC-MS [5]. Sample preparation is also included in a review on the application of LC-MS to detection of selected emerging contaminants [66] and pesticides [67]. Solid phase extraction is also discussed in an excellent book [157].

For a variety of water contaminants, off-line SPE disks, or most frequently cartridges have been used. Octadecyl (C_{18})-bonded silica has been the most widely used adsorbent, although polymeric sorbents (like styrene divinylbenzene copolymers) and graphitized carbon black (GCB) have also been employed. Applications range from estrogens (C_{18}-material [68]; GCB [69, 70]), drugs [71, 72], over surfactants (C_{18}-material [73]; GCB [74]), and pesticides [75], to algal and cyanobacterial toxins [76, 77].

Immunosorbent extractions are an interesting field, with the capability of highly-selective, effect-related preconcentration and sample fractionation. However, the application of immunosorbent extraction to water analysis has been hampered by difficulties in obtaining antibodies and in the high selectivity which may narrow analysis to almost single target analytes or analytes with very similar structures. As an example, immunosorbent extraction was applied to sample purification to analyze estrogens in wastewater effluents [78].

Another approach to improving the selectivity in SPE can be achieved by molecularly-imprinted polymers (MIP) [79]. Analyte-selective MIP materials have been produced by polymerization under inclusion of the analytes into the polymer. After removing the analyte molecules from the polymer, cavities are obtained which may serve as analyte selective sorption sites. The development of MIP materials for SPE is described in detail in [80].

Restricted access materials (RAM) have been applied in LC-LC coupling and they helped to reduce problems with the hump of humic acids in the chromatogram during the analysis of acidic pesticides in water samples [81, 82].

The general move to lower sample volumes and less sample handling fosters the development of on-line methods. Solid-phase microextraction (SPME) and in-tube SPME are tools with these features, used for miniaturized and on-line extraction of aqueous samples [83, 84, 85, 86].

LC-MS with membrane introduction mass spectrometry (MIMS) is another approach for integrated sample preparation and determination [60]. Furthermore, using on-line SPE-LC-ESI-MS-MS, sample volumes of 1.3 mL were found to be sufficient to analyze about 30 pesticides down to 0.1 pg/L [87]. Additional literature on this topic may be found in a review article [88].

Pressurized liquid extraction

Extraction techniques for solids are of particular interest for the investigation of the fate of chemicals in the aquatic environment and in sewage water treatment. For that purpose, pressurized liquid extraction (PLE) and subcritical water extraction (SWE) have become routine technologies for sediments and sewage sludge, for example to extract 4-nonylphenol and bisphenol A [89], triclosan [90], steroid hormones [91], fluoroquinolone antibacterial agents [92] or benzalkonium chlorides [27]. Therefore PLE and SWE are already frequently used and rapidly replacing conventional solvent extraction, like Soxhlet or ultrasonic extraction [93].

Separation methods

Liquid chromatographic separation (LC) in its various modes (reversed-phase, ion exchange, size exclusion) and capillary electrophoresis are the methods of choice for less volatile, polar compounds. Since the physico-chemical properties of environmental contaminants like molecular mass, acidity/basicity and hydrophobicity cover a wide range of values, different kinds of separation materials and techniques are required to address the analytical challenges. The choice of the appropriate chromatographic separation method based on analyte properties was visualized in a diagram by Reemtsma (Fig. 5).

Reversed-phase HPLC

The most important and most widely used separation technique for environmental compounds in aqueous samples is HPLC on reversed-phase material. In general octadecyl (C_{18})-bonded silica and octyl (C_8)-bonded silica can be considered as the standard separation materials. However, other bonded phase materials on polymeric, monolithic, non-porous and superficially porous supports have also become available. Special aqueous phase materials are designed for application to aqueous mobile phases, which are necessary for the more polar analytes.

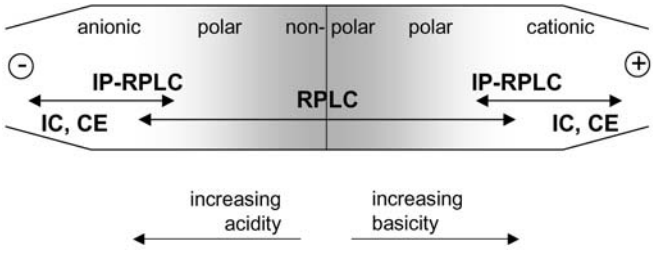


Fig. 5 Diagram showing the interrelationship between analyte properties and the appropriate chromatographic separation method (reprinted with permission from Ref. [37], copyright (2003) Elsevier)

The chemistry of the bonded phase, the properties of the support material and various shielding of the residual silanol groups determine the selectivity and applicability of a column. Excellent overviews on column selectivity in RP-LC can be found in the literature [94, 95, 96, 97]. RP-LC retention can be described as a function of the column and other conditions by a so-called solvation relationship. Different terms in the equation account for intermolecular interactions of the solute with the mobile and the stationary phase, for example as a result of dispersion, cavity formation, dipole moment, polarizability, and various hydrogen bonding conditions [94]. A further approach to characterizing stationary phases based only on the lipophilic capacity (Pl) has also been pursued [98]. Pl can be measured by the retention of a set of standards with regular differences in lipophilicity, such as homologous alkylbenzenes. However, for particular separation problems, the most used approach in practice may be still the trial-and-error method.

New stationary phases of high stability and robustness, even at extreme pH values and high temperatures, have been developed based on zirconia (ZrO_2) [99]. Porous graphitic carbon as a stationary phase has its specific applications, such as in the separation of organometallic compounds, but is not yet widely used [100]. Another alternative for the separation of polar compounds is the application of hydrophilic interaction liquid chromatography (HILIC) with amide, cyclodextrin, cyano, and amino-based stationary phases [101]. Low molecular weight compounds such as amino acids, glycoconjugates, and organic acids have been separated on an amide gel phase with an organic gradient of increasing water content. Problems with such stationary phases occur concerning the long-term stability due to deterioration and reaction with sample matrix constituents.

For conventional RP materials, the dissociation of weakly acidic or basic analytes have to be suppressed by adding volatile acid or base (such as ammonium acetate) to the eluent [18, 37, 102, 103, 104, 105, 106]. Ion-pairing with corresponding counter-ions has also been done for acidic and basic analytes. In particular, the ammonium cation is the weakest ion-pairing agent, while tri- and dialkylamines are the stronger ones [107, 108, 109, 110, 111, 112]. Perfluorinated organic acids serve as anionic counterions [113, 114, 115]. The type and quantity of organic modifier added to the eluent, however, has to be a compromise between improvement of separation and minimization of suppression of the API ionization. A diagram may support the choice of an appropriate separation method based on analyte properties (Fig. 5).

LC on microbore columns (1 mm i.d.) is a promising technique that can achieve greater separation efficiencies,

sensitivities, and reduced solvent use, as well as lower operating costs [116]. Open-tubular or monolithic silica capillary columns are expected to assume the same importance as capillary columns in GC. Up to now there are only a few applications due to the lack of instrumental adaptation of LC, detectors, and availability of stable columns. In recent applications, microbore LC coupled to positive electrospray MS was particularly used to determine carbamate, urea and thiourea pesticides and herbicides in water [117]. A nano-HPLC was coupled to a new direct electron ionization interface [40].

Ion chromatography

For ionic compounds and where ion-pair chromatography is not useful, ion chromatography with weak cation exchangers has been used. Applications have been reported for the analysis of organic acids [118], complexing agents [119], inorganic or organometallic compounds like species of arsenic, selenium, and chromium (IV) [120, 121, 122, 123], or oxoanions [124] in water.

Again the use of ionic eluents compromises ionization efficiency in API. To remove non-volatile ionic constituents of an eluent, a post-column suppressor has been used in a few applications [125].

Capillary electrophoresis

Progress has been made with capillary electrophoresis (CE), another technique applicable for ionic compounds, where improved separation efficiency is of importance [126]. Late fabrication of microchips from plastics or polymeric materials are of great interest due to the flexibility of the production process [127]. But there are still concerns whether techniques that rely on electroosmotic flow can generally meet the reproducibility requirements for certain applications in environmental water analysis.

A few recent applications have used CE-MS for the analysis of carboxylic acids [128, 129], aromatic sulfonates [130], and arsenic species [131].

Size-exclusion chromatography

Size-exclusion chromatography (SEC) is a well established technique for analysis of polymers and humic substances (HS). With SEC, compounds are separated on separation gels with defined pore diameters due to their different effective sizes. On the SEC column the analytes are partitioned between the pores and the mobile phase, and will appear at retention volumes between the exclusion volume (larger size than pores) and the pore volume (smaller and intermediate size). Another typical application of SEC is for sample clean-up in the analysis of complex matrices, like the determination of water soluble arsenic species in biological matrices. Multi-dimensional LC (SEC-anion-exchange-cation-exchange) was applied with ESI-TOF-MS [122]. Recent applications used SEC coupled to ESI-MS and APCI-MS for the characterization of HS. This is a promising approach, but due to the complex nature of HS, including the unavailability of authentic matter for calibration, it is still of limited value as an applied technique [132, 133, 134].

Chiral separations

Chiral separation is a rather niche technique, but it is of particular interest in pesticide and pharmaceutical research. Chiral isomers can behave very differently regarding their biological effects and microbiological degradation. A review of chiral separation techniques was published in 2000 [135]. The most popular chiral selectors are still cyclodextrins (CD). Other selectors are proteins, polysaccharides, crown ethers, polyacrylamides, polymeric chiral surfactants, macrocyclic antibiotics, and ergot alkaloids. Polymeric molecular micelles have been used as pseudo stationary phases for CE [136]. They show several advantages compared to CD and can be used very well in combination with MS. Reversed-phase HPLC methods have been developed with the aim of ESI-MS coupling [137]. As an example, chiral separation has been used to analyze the 1′S and 1′R diastereomers of metolachlor in surface and groundwater [138]. However, applications of chiral separations in water analysis are still quite rare.

Compound identification

Criteria for the identification and quantification of organic residues in food are given by a new EU guideline [139]. The approach is based on the use of identification points, which are earned according to the quality criteria for spectrometric identification and confirmation (Table 1). For example, at low mass resolution, for each precursor ion can earn one identification point (IP) and each transition ion can earn 1.5 IPs, if the allowable tolerances of the ion intensity ratios can be met. For the identification of legal compounds three IPs are necessary, for an illegal compound four IPs. Therefore, for a triple quadrupole MS, at least two transition ions and the molecular ion are necessary to meet the identification criteria for an illegal compound. Using high resolution mass spectrometry, two IPs can be earned for each precursor ion and 2.5 IPs for each transition ion. The main advantage of this method is the

Table 1 Quality criteria for compound confirmation by MS based on identification points (IPs; data from [139])

Measured ion	IP for low-mass resolution	IP for high-mass resolution
Quasimolecular ion (MS)	1	2
Precursor-ion (MS-MS)	1	2
Product-ion (MS-MS)	1.5	2.5

fact that identification based on these criteria can be done in a well-established and internationally accepted way [140].

Mass spectral libraries

Mass spectral libraries are a useful tool in unknown identification. Unfortunately, most libraries with appropriate sets of data were created for electron impact ionization data under standardized conditions. For the soft-ionization techniques such as ESI and APCI, fragment patterns are only obtained by collision-induced fragmentation in the electrospray-transport region, in quadrupole collision cells, or in an ion trap, and are strongly dependent on operational and instrumental parameters. There are no standard conditions, and user libraries can be generated at best for an individual instrument.

One approach to improving the lack of library data is to use standardized tuning and performance-based standard conditions to get library data which can be used at least for different instruments of the same type [141]. Tune compounds have been tested to adjust the collision energies of mass spectrometers from two different manufacturers. At least two tune compounds such as haloperidol, paracetamol, metronidazole or metamizole were necessary to adjust CID energies to get similar fragmentation [142]. Another more promising approach predominantly uses only the *m*/*z* values of the fragments rather than abundances. In this way, a universal library can be created, which can be used in compound identification to cut down the number of possible matches in a library search on the basis of *m*/*z* values [143]. More data have yet to be compiled in such a library format to show its usefulness and versatility.

Quantitation and matrix effects

Direct aqueous sample injection is an attractive analysis approach which is supported by the high sensitivity and selectivity of LC-tandem mass spectrometry with atmospheric pressure ionization. However, co-eluting ionic and ionizable constituents of the sample and the sample matrix can show severe effects on the ionization yield of the analyte [144, 145, 146, 147]. Also, mobile phase additives, such as buffers or ion-pairing reagents, can severely influence signal intensity [148]. Perfluoroheptanoic acid and heptafluorobutanoic acid worked well as ion-pairing reagents to determine eight drugs with LC-ESI-MS, but signal intensities were significantly lowered by the fluorinated carboxylic acids [149].

Dissolved organic carbon from humic acids and calcium ions showed significant interferences with the ionization of acidic aromatic compounds like naphthoic acids measured by direct infusion ESI-MS-MS [18] (Fig. 6). The results of a standard addition method revealed that the acidic aromatic compounds could be determined without any matrix effects by LC-ESI-MS-MS due to sufficient chromatographic separation of the sample matrix from the analytes. LC-LC combined with ESI-MS-MS was found to be the most favorable approach for the on-line analysis of various acidic pesticides like bromoxynil, bentazone, 2-methyl-4-chlorophenoxypropionic acid (MCPP), 2-methyl-4-chlorophenoxyacetic acid (MCPA) and sulfometuron-methyl in DOC containing tap water [103]. The classical way to deal with matrix effects is to use adequate internal standards, ideally with co-eluting isotope standards. However, the signal intensity of the internal standard can also be suppressed by increasing concentrations of the co-eluting analyte [150]. The echo-peak internal standard calibration allows injection of an internal standard of the same identity as the target analyte, so that its retention time is very close to that of the analyte. With the echo-peak technique, accurate results have been obtained similar to those from matrix-matched calibration [151].

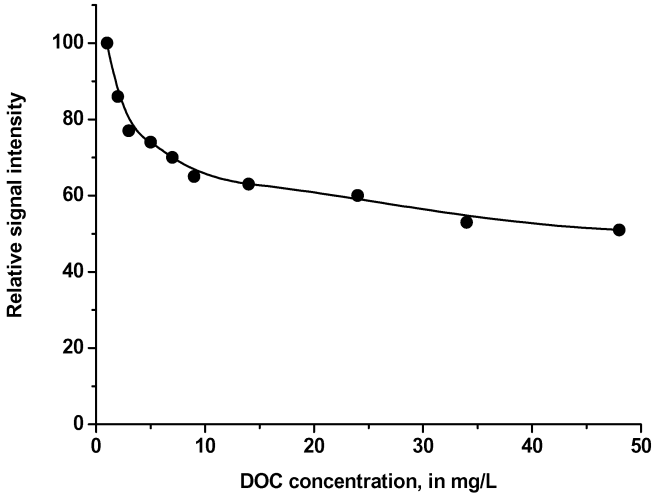


Fig. 6 Matrix effect of increasing concentration of humic acid on the signal intensity of 2-naphthoic acid (ESI-MS-MS, *m*/*z*=171→127; redrawn from [18])

Chemical analysis and toxicity bioassays

Increasing interest in water analysis requires an approach which links the information on identity and occurrence of chemical contaminants to their biological effect. In this way, detection and identification of a substance can be linked with the assessment of any biological effect. Initial development of this approach took place in the 1970 s, and since then has led to several strategies for toxicity tests for sample selection or fractionation (toxicity-directed fractionation, toxicity-directed analysis) [152], and for bioeffect detection after HPLC separation [153] or in parallel with instrumental analysis [105, 154, 155]. In this context, LC-MS methods play an important role in compound identification. The concept of the so-called bioresponse-linked instrumental analysis includes a real combination of biomolecular recognition elements, and high-resolution instrumental analysis, which greatly relies on mass spec-

trometric techniques [156]. This concept could be realized in particular by immunoaffinity extraction of steroid estrogens coupled with LC-ESI-MS [77]. A further logical step would be the direct determination of the receptor-bound analyte by high-resolution mass spectrometry.

Conclusions

LC-MS is a prerequisite for the analysis of a large number of polar contaminants in water samples in the investigation of the occurrence and fate of such compounds in the environment and in water treatment. In general, high sensitivity accompanied with high selectivity is obtained using atmospheric pressure ionization (API) and triple quadrupole (TQ) instruments. API-TQ-MS demonstrates its power if applied for the sensitive and selective quantitation analysis of target compounds. Structural information for screening and identification of unknowns can also be obtained with TQ and ion trap (IT) instruments, although this information is somewhat limited due to their low mass resolution. Fragmentation pathways can be successfully studied by IT-MS due its ability to perform MS^n. The recent introduction of time-of-flight (TOF) and Q-TOF instruments improves the possibility of unknown identification due to the possibility of accurate mass measurements at sensitivities comparable to the above-mentioned instruments.

Despite the selectivity of mass spectral detection, false positive findings can still occur, and therefore identification criteria in terms of molecular and fragmentation ions, relative abundances, retention times, and so on, are essential. Large mass spectral libraries for API and collision-induced fragmentation mass spectra similar to those available for GC-MS would be desirable for unknown identification. In this direction, the first steps have been taken by using predominantly the *m/z* values rather than abundance ratios to develop a library of instrument-independent data.

Still important for LC-MS are sample preconcentration and chromatographic separation. Both must be streamlined for the requirements of the specific properties of the analytes. Furthermore, limitations of specific operating parameters, like the choice of solvents and type and concentration of (ionic) additives, have to be observed with respect to the compatibility with API-MS. The most commonly-used separation method is therefore HPLC on reversed-phase stationary phases, since the used eluent mixtures (water-acetonitrile, methanol) work very well with electrospray (ESI) or atmospheric pressure chemical ionization (APCI). The separation of very polar or even ionic analytes on RP-phases, however, require the addition of volatile acids/bases or ion-pairing reagents. There is in all cases a compromise between the improvement of separation and deterioration of the ionization process. Ion chromatography (IC) may be an alternative but faces the same restrictions.

Solid-phase extraction (SPE) with non-selective adsorbents is clearly the method of choice for sample preconcentration in order to improve the sensitivity. SPE is also used for fractionation of analytes and other sample constituents (clean-up). A clear trend points to the use of on-line extraction and detection systems which need much less sample volume and less manual sample handling. A significant increase in selectivity can be achieved by the combination of selective sorbents, especially immunosorbents, with mass spectrometric detection.

The combination of chemical analysis and toxicity bioassays has found growing interest, since there is an increasing demand to focus analysis activities on compounds and samples showing a biological effect, and to answer questions about the direct meaning of the analytical results. A major challenge in this context is to overlap the different analytical windows of instrumental analysis and bioassays, since they inherently have different sensitivities and selectivities.

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