ANALYTICAL SCIENCES OCTOBER 2019, VOL. 35

Reviews

Paradigm Shift in the Arena of Sample Preparation and Bioanalytical Approaches Involving Liquid Chromatography Mass Spectroscopic Technique

Manish Kumar SHARMA, Pooja DHAKNE, Sidhartha NN, P Ajitha REDDY, and Pinaki SENGUPTA†

Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research—Ahmedabad, Palaj, Gandhinagar, Gujarat-382355, India

Sample preparation is a highly important and integral part of bioanalysis for cleaning up the complex biological matrices and thereby minimizing matrix effect. Matrix effect can jeopardize the precise quantification and adversely affect the reliability of liquid chromatography–mass spectrometry-based analytical results by alteration of analyte ionization. Matrix components result in suppression or enhancement of the intensity of analyte response. In spite of the high specificity and selectivity of tandem mass spectrometry, a relatively higher concentration of coeluted matrix elements present in biofluids may alter the efficiency of quantification of a bioanalytical method. Numerous literature reports different types of sample preparation techniques employed in bioanalysis. In this review, the strategies for selection of the appropriate sample clean-up technique in bioanalysis are discussed extensively. A paradigm shift in the arena of sample preparation and bioanalytical approaches involving the liquid chromatography–mass spectroscopic technique has been scrutinized. Current trends and possible future advancements in the field of biological sample extraction methods, including instrumental techniques are analyzed in detail.

Keywords Mass spectrometry, bioanalysis, sample preparation, matrix effect

(Received March 27, 2019; Accepted May 7, 2019; Advance Publication Released Online by J-STAGE May 17, 2019)

1 Introduction 1070
2 Mechanistic Investigation of Ionization Suppression or Enhancement in LC-MS 1070
3 Strategy for Selection of LC-MS/MS in Bioanalysis 1071
3·1 Triple quadrupole instrument
3·2 Q-TOF system
3·3 Quadrupole-ion mobility-TOF
3·4 Orbitrap-based analyzers
4 Challenges in Sample Preparation Technique 1072
4·1 Ion suppression or enhancement in LC-MS analysis
4·2 Physicochemical properties of drug and pH control
4·3 Stability issue of small molecules in biological matrices
5 Matrix Effects in LC-MS 1073
6 Strategy for Development and Optimization of Sample Extraction Method 1073
6·1 Protein precipitation
6·2 Liquid–liquid extraction
6·3 Solid-phase extraction
6·4 Solid phase microextraction
6·5 Monolithic spin column extraction
6·6 Microextraction by packed sorbent
7 Current Trend and Future Advancement 1079
8 Conclusion 1079
9 Conflict of Interest 1080
10 Acknowledgements 1080
11 References 1080

Manish Kumar SHARMA is a Ph.D research scholar in the Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research—Ahmedabad, India. He has done his master degree in Pharmaceutical Analysis from Institute of Pharmacy, Nirma University, Ahmedabad, India in 2011. He has 2.5 years of working experience in the pharmaceutical industry related to regulatory affairs as well as 1.5 years of experience in Anti-Doping analytical Science. His research area includes analytical method development of drugs and various growth hormone biomarkers, identification and characterization of drug degradation products, bioanalysis, metabolite profiling and pharmacokinetic studies of drugs.

Pooja DHAKNE is a Master of Science (Pharmacy) graduate in Pharmaceutical Analysis from National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad. She has completed her Bachelor of Pharmacy from Government College of Pharmacy, Aurangabad in 2017. Her research interest includes development and validation of bioanalytical methods for anticancer drugs, optimization of bioanalytical sample extraction techniques, and pharmacokinetics study of drugs. At present, she is associated with Drug Metabolism and Pharmacokinetics division of Sun Pharma Advanced Research Company Ltd., Vadodara, India.

All authors contributed equally to this work.

† To whom correspondence should be addressed.
E-mail: psg725@gmail.com

1 Introduction

The eradication or minimization of matrix interference for quantitation of metabolites and biomarkers in biological matrices still remains a major challenge.[1] Erroneous results are often observed in bioanalysis due to the presence of complex biological interfering substances.[2] In context of the inevitable biological complex interactions, optimization of sample preparation method prior to bioanalysis demands special attention. Indeed, recent approaches have made efforts to fulfill the demand of regulatory standard to ascertain sensitive, selective and rapid quantification of new chemical entities (NCEs) in drug discovery.[3] Advancements in mass spectrometry (MS) techniques have opened a new window to face these challenges in rapid identification, characterization, and quantification of drug candidates in various biological samples like plasma, urine, serum, hair, oral fluids, *etc.*[4] The application of tandem mass spectrometric (MS/MS) technique has been extended to enhance the selectively of a compound and resolve the matrix effect-related issues in bioanalysis.[1] Matrix effect might have a deleterious effect on the overall performance of the chromatographic separation as well as the ionization efficiency of a target analyte in liquid chromatography–mass spectrometry (LC-MS) based analysis.[5] Matrix effects may compromise the selectivity and sensitivity, resulting in reduced precision, accuracy, and robustness of the bioanalytical method. Numerous reports point to the importance of matrix interference in bioanalysis involving LC-MS.[6-8] The aim of selecting an appropriate sample preparation technique in bioanalysis is to minimize the influence of the endogenous components as well as to eliminate variability among the samples. There is a need to implement versatile sample clean-up techniques and suitable MS ionization methods, which would help not only to enable good chromatographic separation but also to overcome the matrix effect by selectively isolating the target analyte from interferences.[9,10] According to the United State Food and Drug Administration (USFDA) bioanalytical guidelines, necessary steps need to be incorporated to eliminate variability of the matrix in LC-MS based bioanalysis. Physiological nature of different matrices is another criterion to be considered in developing a bioanalytical method. Recent guidance for industries on "Bioanalytical Method Validation, May 2018" addresses the importance of the fit-for-purpose (FFP) concepts in the assessment of matrix effects for LC-MS based bioanalytical assays. It must be ensured that there is no variability in matrix effect across the anticipated range of concentration when compared to a blank sample at the time of validating a method.[11,12]

In this review, we have extensively discussed different sample preparation strategies and modifications to the conventional sample extraction techniques in order to increase the sensitivity and recovery of a target analyte in complex biological fluids. The application of advanced yet simple and most preferable ionization techniques like electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) has been highlighted in this review. The potential issues encountered during sample extraction and the uses of different LC-MS/MS techniques have been scrutinized. Various strategies to reduce the time of analysis and cost-effective sample clean up in different biological matrices have also been discussed with case studies.

2 Mechanistic Investigation of Ionization Suppression or Enhancement in LC-MS

The development of integrated knowledge on the LC-MS/MS based technique has revolutionized the quantification strategy of drugs in complex biological matrices.[13–15] High resolution and sensitivity of LC-MS/MS instruments have contributed to enhance the specific function of accurate mass precursor data that helps in the confirmation or rejection of identity of a molecule.[16,17] The modern MS/MS approach has remarkably reduced the total time required for bioanalysis. However, sample preparation protocols still hold major challenges, like ineffective elimination of the interference of matrix with the analyte in different biofluids. High-resolution mass spectrometry (HRMS) detection is based on the type and concentration of the analyte ions.[2,18] Development of sensitive and robust bioanalytical assays is a prerequisite in early drug development stages.[19] An LC-MS/MS method offers several advantages over other conventional techniques in bioanalysis. ESI and APCI are the two widely used ionization methods, which can be applied with varying degrees of success to produce intact ions.[20-23] The level of matrix interference may not be similar in ESI and APCI ionization, as it depends on the method of ion production. Both of these techniques experience ion suppression or enhancement to a certain extent, which occurs due to co-elution of matrix components present in biological samples.[24] In addition to endogenous components, exogenous sources of interfering substances reported in the literature are plastic and polymer residues,[25] degradation products, reagents,[26] calibration standards, buffers,[27] or substances leached from the associated tubing or columns of the instrument.

ESI source encounters the molecule to be ionized with electrons, transforming small molecules into gas-phase ions.[16] A high electric voltage is applied at the capillary tip that assists

Sidhartha NN is a Master of Science (Pharmacy) graduate in Pharmaceutical Analysis from National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad. He has completed his Bachelor of Pharmacy from Avanthi Institute of Pharmaceutical Sciences, Hyderabad in 2016. His research area includes bioanalytical sample extraction techniques, development and validation of analytical and bioanalytical methods for anticancer drugs and their pharmacokinetic safety profiling in biological matrices. Currently, he is a research associate in the Analytical Chemistry Department at Aten Porus Life Sciences Pvt. Ltd., Bangalore, India.

P Ajitha Reddy is a Master of Science (Graduate) in Pharmaceutical Analysis from National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad. She completed her Bachelor of Pharmacy in Teegala Ram Reddy college of Pharmacy, Hyderabad in 2016. Her research interest is development of Bioanalytical method, applying the method for the determination of the pharmacokinetic parameters and investigating the pharmacokinetic drug-drug interaction.

Pinaki Sengupta is a faculty member of the Department of Pharmaceutical Analysis, National Institute of Pharmaceutical Education and Research, Ahmedabad, India. He is a Ph.D in Pharmacy from Jadavpur University, India. Dr. Pinaki has more than ten years of experience in academic and industry-based research. He has published more than fifty articles in various high impact international journals. He is serving as the Editorial Board Member of four scientific journals. His work has been cited more than 750 times in scientific articles.

an analyte molecule to turn into a positive or negative ion.[28,29] Matrix components if present in high amounts may alter the viscosity and the surface tension of the droplets formed during the ESI ionization process, and decrease the proficiency of the analytes to convert to declustured ions.[30-32] The versatile ionization technique called atmospheric pressure chemical ionization (APCI) consists of a corona discharge needle and a probe, an inert gas flow for solvent evaporation and desolvation. Sampling cone and transfer optics transfer the ions into the mass analyzer.[29] The main advantage of APCI ionization source over ESI is the reduced ion suppression.[33,34]

3 Strategy for Selection of LC-MS/MS in Bioanalysis

Successful bioanalysis often requires a high degree of specificity due to the presence of many interfering matrix signals. Selected reaction monitoring (SRM) mode of detection in LC-MS has some limitations in bioanalysis as it analyzes based on parent ions only. New hybrid mass analyzers using full-scan acquisition mode can acquire quantitative information without the need for modification of specific sample preparation.[35] In other words, a full-scan acquisition would eliminate the need for sample preparation and enable the collection of quantitative (at ng/mL to pg/mL level) as well as qualitative information.[3] In recent years, a shift in drug discovery approach from SRM to non-targeted HRMS has been seen, which enables simultaneous parent drug quantitation and identification of metabolite and biomarker in biofluids.[35,36] The innovation in hybrid mass analyzers with higher resolving power and mass accuracy, advancement in ionization sources, development of novel LC columns and database for structural elucidation are being well explored for regulated bioanalysis.[37]

In this review, some insights focused on the applications of different hybrid mass analyzers employed in the bioanalysis of drugs, their metabolites and biomarkers in different biological matrices. The hybrid mass analyzers such as ion trap time of flight (TOF), quadrupole TOF, and quadrupole Orbitrap, facilitate high resolution, sensitivity, selectivity, dynamic range, full-scan acquisition speed with less mass error.[38] In the present scenario, HRMS-based analysis has become the preferred choice for drug discovery and development, biomarker discovery, biotransformation reaction in non-clinical studies, dope control studies, toxicological investigations, and forensic applications.[37]

3·1 Triple quadrupole instrument

Triple quadrupoles (QqQ), can be used in quantitative analysis based on full scan, precursor ion scan, product ion scan or neutral loss scan for sensitive, accurate, and precise quantification.[39,40] However, the limitation of this system includes the fact that it is less useful in qualitative analysis.

3·2 Q-TOF system

The main advantage of Q-TOF systems is its high scan speed, enhanced sensitivity, high resolution and capability to measure the accurate masses of protonated or deprotonated molecules and their fragment ions in full scan spectra.[20] The samples are analyzed by Q-TOF in two steps. The first step is quantification in QqQ followed by ion transfer into TOF mass analyzer for qualitative analysis. However, a main drawback of Q-TOF in bioanalysis includes weaker mass resolving power at a lower concentration level due to the interferences from endogenous compounds. Nowadays, improved technology has enabled stronger resolution power by increasing the length of the flight path and setting up new ion optics to ensure high ion transmissions. Modern TOF-based mass spectrometers have a resolution between 10000 and 20000, and scan rates of 200 Hz.[41] Introduction of the high-resolution power and reflector ion optics with high ion transmission also permit multiple MS/MS experiments.[13]

3·3 Quadrupole-ion mobility-TOF

A quadrupole-ion mobility-TOF setup has eight ion trajectories along with an extra-long flight path, which can be referred to as the traveling wave ion mobility technique. It enables high-resolution power of 100000 and fast acquisition rates of 200 spectra per second with the only limitation being scan speed. The TOF analyzer as an interface has great importance in measuring the mono-isotopic precursor at *m/z* 2500 in a TOF/TOF design.[42] These potential capabilities of the instrument with such specifications not only allow for a resolution up to 60000 but also provide extra drift times to acquire information of precursor ion and daughter ion accurately.[43] This innovative approach has promising applications and has fast scan rates, fast polarity switching, and simultaneous targeted and untargeted analysis with high mass accuracy for recording product ion spectra.[44]

3·4 Orbitrap-based analyzers

There are several distinguishing features of Orbitrap-based mass analyzers, which helps in the reduction of interference of endogenous matrix. A hybrid Orbitrap mass analyzer by means of a linear ion trap has good sensitivity, good duty cycle, wide dynamic range and high mass resolution.[43] The new generation Orbitrap-based mass spectrometers are equipped with a multipole collision cell, which overcomes the limitations of ion-trapping devices in tandem mass experiments regarding low mass cut-off. Orbitrap generates selective fragmentation information. Recent advancements in Orbitrap technology ensure dissociation of all ions entering the Orbitrap mass analyzer simultaneously through a dedicated collision cell.[44] However, the primary limitation of this type of analyzer is their slow scan speed during the analysis of multiple analytes in

Abbreviation: APCI, atmospheric pressure chemical ionization; APPI, atomic pressure photoionization; BIN, barrel insert and needle; CID, collision ion dissociation; CSF, cerebrospinal fluid; CV, coefficient of variation; DMPK, drug metabolism and pharmacokinetic study; EE, extraction efficiency; ESI, electron spray ionization; FDA, food and drug administration; FFP, fit-for purpose; FFT, fast Fourier transform; FT-ICR, Fourier transform cyclotron resonance; HILIC-MS/MS, hydrophilic interaction liquid chromatography–tandem mass spectroscopy; HN, heating nebulizer; HRMS, high-resolution mass spectroscopy; Ig, immunoglobulin; IMS, ion mobility spectroscopy; ISP, ion spray; LC-MS/MS, liquid chromatography–mass spectrometry/mass spectrometry; LPME, liquid-phase microextraction; LIT, linear ion trap; LLE, liquid–liquid extraction; LLOQ, lower limit of quantification; ME, matrix effect; MEPS, microextraction by packed sorbent; MRM, multiple reaction monitoring; MTBE, methyl tertiary butyl ether; MARC, multiple affinity removal columns; NCE, new chemical entity; PEth, phosphatidyl ethanol; PK, pharmacokinetic; PPT, protein precipitation; QqQ, triple quadrupole; QTOF, quadrupoles time-of-flight; QUAL, qualitative; QUAN, quantitative; RE, recovery; RF, radiofrequency; SALLE, salting-out assisted liquid–liquid extraction; SD, standard deviation; SLE, supported liquid extraction; SPME, solid phase microextraction; SPE, solid phase extraction; SRM, selected reaction monitoring; TBME, tertiary butyl methyl ether; UHPLC, ultra high-performance liquid chromatography; ULOQ, upper limit of quantification; USFDA, United States Food and Drug Administration.

different scan modes. This limitation of the Orbitrap mass analyzer has been resolved by introducing the quadrupole mass filter.[45]

4 Challenges in Sample Preparation Technique

4·1 Ion suppression or enhancement in LC-MS analysis

Ion suppression or enhancement of endogenous matrix components is a major concern in bioanalysis using LC-MS.[5,46] A breakthrough introduction of ultra performance liquid chromatography (UPLC) with HRMS has been perceived to have a wider application of ionization source like ESI, API/APCI as interfaces with hybrid mass analyzers for effectively eliminating matrix interferences.[1,5,18] Ion suppression is a major limitation regardless of the high sensitivity or selectivity of the mass analyzer in analysis of biofluids.[2,47] Matuszewski *et al.* reported a random error in signal response which reduces the precision and accuracy of a method. The alteration in ionization efficiency finally resulted in compromised sensitivity and selectivity of the method.[28,29,47,48]

In the case of the presence of coeluted phospholipids, ESI is a preferred ionization technique rather than APCI because the former prevents ejection of analyte ions either by entrapping inside the droplets or by enriching at the surface of the droplets during liquid to a gas phase transition. Moreover, when a high concentration of polar analyte enters, ESI loses response either due to ionization saturation or an increase in the surface tension or viscosity of the droplets. It also reduces the ability of the analyte to reach the gas phase transition state during the ionization process and ultimately results in suppression of the signal intensity.[13,14,16,21,29,49] Matrix components such as proteins, amino acids, *etc.* may also induce co-precipitation of the target analytes with nonvolatile materials. In the case of multicomponent analysis of the sample by APCI, less interference by matrix components leads to a highly specific analysis.[36]

Comparing these two ionization methods, Kebarle and Tang *et al.* reported that the ion signal suppression phenomenon in ESI may diminish responses of organic bases with the increases in the level of other organic phases.[16] During the analysis of non-volatile materials in APCI/APPI, there may be a chance of salt deposition in gaseous phase droplets, which in turn negatively affects the number of ions reaching the mass spectrometer.[33,34] Moreover, internal standard may attenuate the ionization of the analyte.[35] It has been reported that when ion spray interface is employed, a matrix effect is observed but it is not at all observed when heat nebulizer interface is utilized.[5,17,34] Besides ion suppression or enhancement in mass spectrometer, other factors like sample clean-up, physicochemical characteristics of analyte or internal standard, chromatographic parameters, *etc.* may also contribute to the negative intensity of signals.[50,51] The matrix effect can effectively be minimized by modifying the sample extraction method or by preconcentrating a sample, which might have a great impact on removing unwanted endogenous impurities. It becomes necessary to take early preventive action and modify the sample extraction procedure to establish a highly sensitive and specific LC-MS based bioanalytical method.

4·2 Physicochemical properties of drug and pH control

Bioanalytical sample extraction method is affected by distribution ratio, pH and stability of analyte in biological matrices in order to develop a cost-effective method. Physicochemical properties can have a great impact on the quality of bioanalytical results and it also alters the stability of the molecule in bioanalysis. The sample preparation procedure of bioanalyses, including collection method, processing technique, storage condition, extraction time, and reconstitution volume, help in preventing the interconversion of the molecular isomeric forms or pairs.[52] The goal of pH adjustment in sample analysis is to extract the target analyte from the complex sample and to remove unwanted compounds. However, extreme pH may lead to chemical instability of the analyte.[53-55] It is very tedious to analyze several groups of compounds, especially glucuronides (acyl-glucuronides) and other conjugates like esters, amides, oxides, lactams, and lactones, which are usually prone to inter-conversion or *ex vivo* degradation.[12] Furthermore, various endogenous compounds are unstable and are prone to hydrolysis at physiological pH condition. For example, most of the acyl glucuronides are usually found to be more stable at an adjusted pH of 5 in a biological matrix.[36,56-58] To achieve better sensitivity, attention should be paid to pH control of the sample prior to LLE. For ionizable compounds, sample pH should be low enough for acidic compounds and high enough for basic compound to get a satisfactory recovery.[58] A key aspect of any LLE to get a good recovery at a lower level of quantification is selection of solvent, which will reduce unspecific extraction and optimization of that extraction procedure. Every analyte has a polarity or solubility difference between the two phases, which allows the analyte to be effectively distributed in some specific liquid solvents over others facilitating optimum recovery. Neutral analytes can more efficiently be extracted by organic solvents. On the other hand, halogenated solvents like chloroform or dichloromethane in combination with hydrophilic solvents can be used for the extraction of polar compounds.[12,25,59,60]

Another method of sample extraction is SPE in which extraction mainly depends upon the pH, polarity of the analyte, solvents and physicochemical property of the analyte. To achieve better retention on the hydrophobic SPE cartridge, the pH must be accurately adjusted to suppress ionization of the analyte. In the case of ion exchange-SPE, pH should be adjusted to maximize the ionization of the analyte, which ensures interaction with the charged groups of the stationary phase. The pH can be adjusted by mixing the main aliquot of the sample with a certain volume of acid (formic acid, acetic acid) or base (sodium hydroxide, trimethyl amine) or a buffer solution (Tris buffer) with a suitable pH at different concentration range for effective SPE extraction.[52,54]

4·3 Stability issue of small molecules in biological matrices

The stability of a drug in any biological sample is considered as an important parameter to be evaluated to identify the possible instabilities in qualitative LC-MS/MS bioanalysis.[61] Inter-conversion of sensitive endogenous molecules due to their poor stability can adversely result in matrix interference and make a method less specific. These unstable moieties may be either hydrolyzed in the presence of esterases or under other physiological changes like temperature and pH.[62] Primarily, esterase enzyme may give rise to erroneous results *via* catalysis of the unwanted hydrolysis of conjugated phase II metabolites.[56,57] Changes in structure and chemical properties may hamper the actual quantitation of those molecules, which are unstable in the biological matrix and prone to an interconversion. Again, at the time of bioanalytical method development, it is essential to assess stability of the analyte in sample preparation, storage, extraction procedure, freeze-thawing, on bench-top and inside the autosampler.

5 Matrix Effects in LC-MS

The matrix effect has a negative impact on recovery and quantification of the target analyte during bioanalysis. It is an important prerequisite to assess the inter-sample variation during validation as each biological matrix has its own unique properties affecting the analysis. The extent of matrix influence needs to be evaluated to acquire more precise and accurate bioanalytical data.[3,20] Matrix effects can be compensated or reduced by applying a combination of strategies regardless of instrumental parameters. Various specific approaches include the use of hybrid mass analyzer and modification in sample extraction procedure by derivatizing the compound of interest.[5,20,63]

The exact mechanism underlying this matrix effect is still unclear. In the LC-MS method, it can be connected to the degree of ion suppression or enhancement of analyte or internal standard.[56] King *et al.* performed a series of experiments to find out the exact mechanism of matrix effects and its interferences related to competition between non-volatile matrix components and analyte ions.[21] The matrix effect can be evaluated by monitoring the variation in response between the sample containing matrix and the drug solution devoid of the matrix.[12] Absolute matrix effects usually can be calculated at a given concentration of target analyte as the difference in response of the post-extraction spiked samples to the mean peak area response of the drug in a solution state.[12,59] Relative matrix effect of an analyte is the difference in response at the same concentration in different lots of biofluids.[25] Assessment of the matrix effect during bioanalytical method optimization in biological matrices play a key role in reproducibility of results in the prediction of selectivity and sensitivity of the method.[64] Researchers introduced the terms "process efficiency", "extraction efficiency", and "ion suppression" related to the matrix effect, which may be represented as the combined matrix effects and recovery during the sample extraction process. Extraction or process efficiency can be illustrated by a comparison of validated results of an experiment performed in a single lot of biological sample *versus* in at least five different lots of biofluids. The absolute matrix effect results in the alteration of accuracy, whereas the relative matrix effect alters accuracy as well as the precision of a bioanalytical method.[64]

The matrix effect is highly dependent on the approaches of sample clean-up procedures and degree of chromatographic separation. However, there is no universal strategy for the elimination of matrix effect. Matuszewski *et al.* proposed the detection of the matrix effect by simplified and alternative approaches. The post-column infusion method used to assess possible matrix effect reported as the most preferred method. It is carried out by continuous injection of the analyte after the injection of an un-spiked matrix (blank control) into the chromatography column.[5] Bonfiglio *et al.* proposed the qualitative procedure to assess matrix effects by injecting a constant infusion of analyte into the LC-MS system *via* T-connector between the analytical column and mass analyzer. The endogenous matrix interfering substance coeluted with the analyte through the column can be observed as suppression or enhancement of the infused analyte signal. The matrix effect can be determined by comparing the changes in baseline signal intensity at or near the retention times of the target analyte by the post-infusion method.

Flow injection analysis, an identical method to the post-column infusion method, is also used to determine the matrix effects by injecting the sample into the MS using a sample loop. In this approach, the response of the analyte of a spiked biological matrix after the sample extraction (matrix-matched standard) is compared to a response of the same analyte of interest containing a matrix free neat solution. On the other hand, pre-extraction sample is used to calculate extraction efficiency. Hence, the evaluation of matrix effect requires a comprehensive strategy that relies on different kinds of biological matrices.[65]

6 Strategy for Development and Optimization of Sample Extraction Method

Sample extraction process purifies the sample and makes it a homogeneous solution that is suitable for injection into the column. Sample preparation techniques always aid in analytical method development by removing unwanted salts, cellular components, lipids, and proteins from the complex biological sample and concentrate the analyte before injection into the analytical system. Development of an efficient sample extraction technique is required to ascertain certain distinct objectives. It increases the lifespan of the analytical column and the mass spectroscopic ion source. In LC-MS analysis, it decreases the ion suppression or enhancement in order to ascertain good reproducibility, accuracy, and precision. Effective sample extraction can prevent column overloading with hydrophilic matrix components that may get deposited on the column and decrease column life.[52]

Protein precipitation (PPT), liquid–liquid extraction (LLE), and solid-phase extraction (SPE) are the most frequently used conventional sample extraction techniques. In addition to these general sample extraction methods, solid phase microextraction (SPME), monolithic spin column extraction, microextraction by packed sorbent (MEPS), supported liquid extraction (SLE) and micro-solid phase extraction techniques are some advanced techniques reported in the literature.

6·1 Protein precipitation

The conventional protein precipitation technique is based on the denaturation and precipitation of proteins and solubilization of the analyte in the solvent. Several organic solvents like acetonitrile, ethanol, methanol, and acetone are used to clear out plasma proteins. However, perchloric acid and trichloroacetic acid are also used as precipitating agents. Dilution of the sample matrix is carried out by one part sample with three to four parts precipitating agent followed by centrifugation process. Following centrifugation, the supernatant can be directly analyzed after filtration.[66] Protein precipitation has been extensively used to extract the analyte from biological matrices over the years in pharmacokinetic studies.[67,68] Quantification of bioactive flavonoid, alpinetin in rat plasma using UHPLC-MS/MS has been carried out by Ye *et al.* Protein precipitation together with dilution approach using acetonitrile and water (50:50, v/v) was used to eliminate the matrix components.[69] Mohammed *et al.* published a fast and sensitive validated bioanalytical method for quantification of Linezolid using a simple protein extraction procedure with methanol.[70] Serum profiling of metabolites with the help of mass spectrometry also utilizes protein precipitation followed by centrifugation. The study reported by Want *et al.* suggested that the organic solvents are the most effective and reproducible for protein precipitation as well as metabolite recovery. Observations from this study suggested an extraction method with the help of 100% methanol.[71]

Tsakelidou *et al.* reported a study in which, the various sample extraction techniques were investigated for the elimination of

Table 1 Bioanalysis using protein precipitation as the sample preparation technique

Analyte	Chromatographic conditions		Matrix	Sample extraction method (protein precipitant used)	Quantitation level	Mass spectrometric condition		Ref.
	Stationary phase	Mobile phase				Ionization mode	Instrument	
Metaxalone	Sapphire C18 (150 mm × 2.1 mm, 5 µm)	5 mM ammonium acetate containing 0.01% formic acid: acetonitrile (45:55, v/v)	Human plasma	Acetonitrile	30.24 – 5040 ng/mL	ESI, positive	Agilent Technologies Series LC/MSD SL (Agilent Technologies, USA)	129
Acetaminophen and oxycodone	Venusil ASB C18 column (50 mm × 2.1 mm, 3 µm)	2 mM ammonium acetate containing 0.1% (v/v) acetic acid (pH 3.6) and acetonitrile Gradient elution	Human plasma	Acetonitrile	Acetaminophen: 40.0 – 8000 ng/mL, oxycodone: 0.20 – 40.0 ng/mL	ESI, positive	AB SCIEX Triple Quad™ 6500+ (Applied Biosystems/Sciex, USA)	130
Clonidine hydrochloride	ZORBAX-XDB-ODS C18 column (30 mm × 2.1 mm, 3.5 µm)	Acetonitrile–water 60:40 (v/v), and 0.2% formic acid	Human plasma	Methanol and perchloric acid	0.01 – 10 ng/mL	ESI, positive	Triple-quadruple mass spectrometer (Agilent Technologies, LCMS-6410, Englewood, USA)	131
Bupivacaine and Meloxicam	Acquity HSS T3 column (50 mm × 2.1 mm, 1.8 µm)	10 mM ammonium formate and acetonitrile: water:formic acid (96:5:0.2, v/v/v)	Human plasma	Water:acetonitrile: formic acid (76:24:0.1,v/v/v)	10 – 4500 ng/mL	ESI, positive	API 4000 triple quadrupole mass spectrophotometer	132
Fluconazole	Synergi Max-RP HPLC column (50 mm × 4.6 mm, 4 µm)	Methanol and 0.1% v/v formic acid Gradient elution	Human plasma	Not reported	50 – 4000 ng/mL	APCI, positive	Not reported	133
Levetiracetam	Kinetex C18 column (100 mm × 2.1 mm, 5 µm)	Methanol, water and 100% formic acid at a ratio of 97:3:0.25 (v/v/v) Isocratic elution	Human plasma and saliva	Acetonitrile	1 – 50 µg/mL for plasma and 0.5 – 30 µg/mL for saliva	ESI, positive	LCMS-8030 Triple Quadrupole Liquid Chromatography Mass Spectrometer	134
Meclizine	Zorbax SB-C18 column (150 mm × 2.1 mm, 5 µm)	Acetonitrile and 0.2% formic acid containing 2 mM ammonium acetate	Human plasma	Acetonitrile	0.5 ng/mL	ESI, positive	API 3200 LC/MS/MS system (Applied Biosystems, Foster City, CA, USA)	135
Mesoridazine	Hypersil GOLD C18 column (50 mm × 2.1 mm, 3 µm)	10 mM ammonium formate in water and ACN (v/v) Gradient elution	Rat plasma	Acetonitrile	1 ng/mL	ESI, positive	API 4000 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer (AB Sciex, Foster City, CA, USA)	136
Metoclopramide	Atlantis HILIC silica column (50 mm × 3.0 mm, 3 µm)	Acetonitrile and ammonium formate (100 mM, pH 6.5) (85:15, v/v)	Human plasma	Dichloromethane	2 – 150 ng/mL	ESI, positive	Tandem quadrupole mass spectrometer (TSQ Quantum Access, ThermoFisher Scientific, CA, USA)	137
Metoprolol	An Ultimate XB-C18 column (150 mm × 2.1 mm, 5 µm)	Methanol and water containing 0.2% formic acid (65:35, v/v)	Dog plasma	Methanol	3.03 – 416.35 ng/mL	ESI, positive	Triple quadrupole tandem mass spectrometer (API 4000TM LC-MS/MS system, Applied Biosystems Company, USA)	138
Tramadol	TSKgel ODS-100 V, (150 mm × 2 mm, 3 µm)	Methanol and 0.15% formic acid in water (35:65, v/v)	Human plasma	Acetonitrile and methanol under basic conditions	12.5 ng/mL	ESI, positive	Triple quadrupole mass spectrometer (3200 QTRAP®, AB Sciex, Foster City, CA, USA)	139
Naloxone and its metabolites	Aquasil C18 column (50 mm × 2.1 mm, 5 µm)	Formic acid (0.1% in water) and 0.1% formic acid in acetonitrile Gradient elution	Mouse plasma	Acetonitrile	Naloxone: 0.2 – 100 ng/mL, Naloxol: 0.4 – 200 ng/mL and Naloxone-3-D-glucuronide (NLG): 0.5 – 250 ng/mL	TIS (total ion spectrum), positive	An API 5000 triple quadrupole mass spectrometer (MDS-Sciex, Concord, Canada)	140
Propranolol	Imtakt Unison 50 mm × 2 mm, 3 µm)	Methanol and 10 mm ammonium formate (70/30, v/v) Isocratic elution	Rat plasma	Acetonitrile	2 – 800 ng/mL	ESI, positive	AB Sciex 400 QTRAP™ mass spectrometer system (Applied Biosystems, Foster City, CA, USA)	141
Trimipramine maleate	ZORBAX ECLIPSE XDB-C18 column (150 mm × 4.6 mm, 5 µm).	5 mM ammonium formate with 0.1% formic acid and methanol 25:75(v/v) Isocratic elution	Human plasma	Methanol	0.1 – 100.1 ng/mL	ESI, positive	Sciex Triple Quadrupole Mass Spectrometer (API 4000, MDS, Sciex, Ontario-Canada)	142
Venlafaxine hydrochloride	Kromasil C18 column (100 mm × 4.6 mm, 5 µm)	Acetonitrile and water (90:10%v/v)	Human plasma	Acetonitrile	5 – 250 ng/mL	APCI, positive	Single Quadrupole Mass Spectrometer (Shimadzu, Japan)	158

phospholipids before quantification of hydrophilic endogenous metabolism products in serum by mass spectroscopy. Simple protein precipitation procedure has been compared with hybrid solid phase extraction, modified dispersive solid phase extraction, a combination of LLE and protein precipitation in order to obtain satisfactory recoveries for a different set of analytes.[59] Bioanalysis of different drugs reported using protein precipitation as the sample preparation technique has been summarized in Table 1.

6·2 Liquid-liquid extraction

The LLE technique based on the principle of partitioning is the most widely employed for extracting the analyte from a biological matrix. LLE involves the use of two immiscible solvents, one of which is aqueous while the other is organic. Analytes distribute themselves according to their respective solubility in either phase. The whole success of the extraction procedure mainly depends on the choice of organic solvent. However, other factors that need to be taken into consideration are volatility, selectivity, toxicity, immiscibility, and cost of the solvents. A large volume of fresh solvents may be required for the repetitive extraction of an analyte from the same sample to achieve highest recovery. Common solvents used in this extraction procedure are ethyl acetate, tertiary butyl methyl ether (TBME), methylene chloride, hexane and dichloromethane.[72]

Substantial efforts have been made in past decades to adopt the existing methods of extraction and in the development of new approaches to save material, labour, and time. Although the strategies for extraction and purification of the drug from biological fluid were not widely explored in the analysis of biomarkers in terms of sample preparation, liquid-phase microextraction (LPME) now has been widely demonstrated in the area of metabolite profiling and biomarker studies.[73] In the

middle to late 1990s, LPME had emerged as a better extraction procedure over conventional method.[74,75] The LPME procedure utilizes a very small volume of water-immiscible solvents (usually in microliters) in order to concentrate drugs from the aqueous phase.[76] It is advantageous over LLE and SPE techniques as it is fast, requires a simple set-up, and is not dependent on a commercial device. The established form of solvent-based microextraction techniques are single drop microextraction, headspace single drop microextraction, and dispersive liquid–liquid microextraction.[77]

Since long ago, LLE has suffered from the disadvantage of a lack of automation. However, various recent studies demonstrated the possibility of automation of LLE using 96 well plates.[78] Various semi-automated LLE procedures using different biological matrices have been reported in the literature for a variety of analytes, such as methotrexate and its major metabolite 7-hydroxymethotrexate in human plasma using Quadra 96 well plate,[79] carboxylic acid containing analyte in human plasma,[80] diphenhydramine, chlorpheniramine, desipramine and trimipramine in rat plasma using Tomtec Quadra 96 well plate[78] and paclitaxel in human plasma using Packard Multiprobe II.[81] Riffel *et al.* described the development and validation of a bioanalytical method with two fully automated LLE techniques for determining a novel insulin sensitizer in human plasma.[82]

Song *et al.* reported the LC-MS method using the salting out-assisted LLE (SALLE) procedure for the determination of multi-mycotoxin biomarkers in pig urine. Because of the wide range of polarity of different analytes, it was relatively difficult to get good recoveries for all the analytes.[83] Some mycotoxin biomarkers such as deoxynivalenol, fumonisin B1 (FB1), and neosolaniol (NEO) are highly hydrophilic ($\log P < 0$) and therefore, LLE techniques using water immiscible solvents failed to achieve satisfactory recoveries in the previous studies.[84] Very few articles published and reported the sample extraction methods for this group of analytes and are confined to blood matrix. Few methods reported using the SALLE approach for which the effect of different salts was compared and various other parameters, such as volume of urine, extraction solvent and $MgSO_4$ solution were evaluated toward the recoveries of each biomarker. It is interesting to note that obtaining the satisfactory recovery of FB1 was a major challenge in mycotoxin research. However, using the SALLE approach, more than 80% recovery was achieved performing two consecutive LLE steps using ethyl acetate followed by acetonitrile. Bioanalysis of drugs in different biological matrices reported using LLE as the sample preparation technique is summarized in Table 2.

6·3 Solid-phase extraction

The solid-phase extraction (SPE) procedure uses solid packing material usually in a cartridge type of device to separate the different components of the sample according to their physicochemical nature.[85,86] Extraction by SPE technique is obtained following a few processing steps such as conditioning the sorbent, equilibration, application of the sample, washing, and elution. Conditioning is performed to avoid sorbent bed drying, to remove moisture, dust particles, or contaminants and to activate the stationary bed sites. Methanol, dichloromethane, TBME, *etc.* are the widely used conditioning solvents. The second step is the sample loading from the top of the cartridge at a slower rate so that the analyte will get enough time to interact with adsorbent and retention can be achieved. Washing of the cartridge with solvent mixtures or weak dilute solvents is required to get rid of weakly retained matrix components and contaminants. Drying step is carried out applying vacuum to avoid any possibility of cartridge blockage due to the formation of air bubble during elution. Final step is the elution of analyte, which is performed by passing the solvents having high elution strength such as acetonitrile or methanol, TBME, dichloromethane, or a mixture of suitable solvents to achieve the maximum extraction recovery.[55,87] SPE can be performed off-line or coupled on-line directly to a chromatograph.[88,89] SPE has become the method of choice for sample extraction for complex mixtures as it is highly selective, offers a wide separation mechanism, is suitable for automation, has greater potential to minimize the matrix effects, and reduces organic solvent consumption.[85] However, it has certain demerits, like risk of clogging, need of expensive cartridges, long method development time and greater complexity.[55,90]

Solid phase extraction has experienced many developments in the recent past, such as dispersive SPE, molecularly imprinted polymer SPE, disposable pipette extraction, microextraction by packed sorbents, solid phase microextraction, stir bar sorptive extraction and online solid phase extraction. These techniques are reported to be used for sample extraction in pharmacokinetics studies, biomarker discovery, and pharmacodynamic studies.[68,87,91]

Capka and Carter reported the development and validation of an LC-MS/MS method for determining salmeterol in human plasma using mixed mode anion exchange SPE for sample clean up combined with the column switching approach to reduce matrix interference. Salmeterol is a basic drug having pK_a about 9.3. Based on its structure and pK_a, authors selected cation exchange or reverse phase extraction technique for extracting the drug from biological matrix and they screened out many SPE components depending on the difference in retention chemistries under different pH and loading. As expected, using HLB reverse phase and Waters Oasis MCX mixed-mode cation exchange, they achieved high recoveries of salmeterol but failed to reach the desired level of quantification with these materials. The likely cause they found was basic elution and highly organic conditions with MCX sorbent and low selectivity of HLB that resulted in higher co-elution of matrix components. In addition to these two sorbents, they used Oasis MAX mixed-mode anion exchange sorbent, which also resulted in high recovery under elution conditions (pH ~6.9) and neutral sample loading. Under these conditions, protonation of the secondary amino group of salmeterol takes place and better retention of the molecule occurs due to weak reverse phase interactions with packing material and weak elution strength solvent, which served the purpose of achieving the required sensitivity for the method. Besides this, the developed SPE procedure also offered the advantages of direct injection of the sample extract into LC-MS/MS, eliminating the time-consuming evaporation step. The extraction based matrix effects were minimized due to the use of less elution volume.[92]

Recently, SPE has gained more attention in the field of biomarker discovery as it offers the benefits of suitability to automation and versatility, which is very essential. Generally, concentration level of the biomarkers present in biological matrices is very low. In order to avoid the loss of target analyte during analysis, a suitable sample extraction method should be used for the quantification of biomarkers.[90,93,94] Serum and plasma are among the most widely used matrices as they contain the elements of all the proteins produced in the body.[95] Nevertheless, some proteins like serum immunoglobulin and albumin are so dominant and lead to mask the other low abundant proteins. Therefore, optimization and evaluation of sample extraction procedures for serum/plasma profiling are very much critical in order to obtain trustworthy biomarkers as well as for building biomarker patterns. Minor variations in a

Table 2 Bioanalysis using liquid–liquid extraction as the sample preparation technique

Analyte	Chromatographic conditions		Matrix	Sample extraction method (extraction solvent used)	Quantitation level	Mass spectrometry condition		Ref.
	Stationary phase	Mobile phase				Ionization mode	Instrument	
Aflatoxin B1, deoxynivalenol, fumonisin B1, ochratoxin A, zearalenone and T2 toxin	Symmetry C18 (150 mm × 2.1 mm, 5 μm)	Water, 0.3% formic acid, 5 mM ammonium formate) and (methanol, 0.3% formic acid, 5 mM ammonium formate Gradient elution	Pig urine	Salting out assisted liquid–liquid extraction using ethyl acetate/ formic acid (99/1, v/v)	Ochratoxin A: 0.07 ng/mL and deoxynivalenol: 3.3 ng/mL	ESI, positive	Micromass Quatro Micro triple quadrupole mass spectrometer	83
Methotrexate and 7-hydroxy methotrexate	Kromosil C8	Acetonitrile:0.1% formic acid (20:80) Isocratic elution	Human plasma	Protein precipitated using acetonitrile followed by chloroform	Methotrexate: 0.5 ng/mL and 7-hydroxy methotrexate: 0.75 ng/mL	TIS, positive	PESCIEX API-365 triple quadrupole mass spectrometer (Concord, Canada)	79
Diphenhydramine (I), desipramine (II), chlorpheniramine (III) and trimipramine (IV)	YMC basic (50 mm × 2 mm, 3 μm) Phenomenex C-18 (50 mm × 2 mm, 5 μm)	50% methanol, 50% 10 mM ammonium acetate (pH 4)	Rat plasma	Ethyl acetate	2.5 ng/mL	ESI, positive	A Quattro II triple-quadrupole LC:MS system (Micromass, Beverly, MA)	78
Paclitaxel	SB C18 Zorbax column (150 mm × 4.6 mm, 5 μm)	Acetonitrile:2 mM ammonium acetate, pH 5 (65:35)	Human plasma	Tertiary butyl methyl ether	1 – 1000 ng/mL	TIS, positive	API 3000 triple quadrupole mass spectrometry (Applera, Monza, Italy)	81
Carboxylic acid compound	BDS Hypersil C8, (50 mm × 2 mm, 5 μm)	Acetonitrile and water, with or without formic acid, ammonium formate, acetic acid and ammonium acetate Isocratic elution	Human Plasma	Methyl tertiary butyl ether in acidic condition	Not reported	ESI, negative	TSQ-7000 mass spectrometer	80
Amitryptiline and its metabolites	Gemini C18 (50 mm × 4.6 mm, 5 μm)	1% formic acid in water and methanol (10:90, v/v) Isocratic elution	Rat plasma	Ethyl acetate	Amitriptyline: 0.1 – 500 ng/mL Nortriptyline: 0.08 – 500 ng/mL	ESI, positive	PE SCIEX API2000 (triple-quadrupole) system (Applied Biosystems, Foster City, CA, USA)	143
Citalopram	Gemini C18 (50 mm × 2 mm, 3 μm)	0.1% formic acid in water and 0.1% formic acid in acetonitrile Gradient elution	Mice plasma and hair	Methanol	32.4 – 973.2 ng/mL	ESI, positive	4000 QTRAP® mass spectrometer (ABSciex)	144
Cocaine, metabolites and a crack cocaine biomarker	Core shell biphenyl UHPLC (100 mm × 2.1 mm, 1.7 μm)	10 mM ammonium formate buffer pH 3.1 and methanol Gradient elution	Whole blood	Methyl tertiary butyl ether/2-propanol (70:30, v:v) in basic condition obtained using 0.2 M carbonate buffer	0.7 – 1.5 ng/mL	ESI, positive	Xevo TQ-S MS/MS from Waters	145
Fentanyl and nor-fentanyl	Phenomenex Luna C18 (30 mm × 2 mm, 5 μm)	0.1% formic acid in water and methanol (90:10, v/v) Isocratic elution	Human plasma	*n*-Butyl chloride/acetonitrile (4:1, v/v) in basic condition obtained using conc. ammonium hydroxide	Not reported	ESI, positive	Thermo Quest TSQ tandem MS	146
Cyclobenzaprine	Agelant C18 (50 mm × 2.1 mm, 3 μm)	Acetonitrile: 5 mM ammonium acetate: formic acid (90:10:0.01, v/v/v)	Dog plasma	*n*-Hexane:dichloromethane: isopropanol (2:1:0.1, v/v/v)	0.02 ng/mL	ESI, positive	An API 4000 triple quadrupole tandem mass spectrometer (Applied Biosystem/MDS SCIEX, CA, USA)	129, 146
Methylprednisolone	Kromasil C18, (100 mm × 4.6 mm, 5 μm)	10 mM ammonium formate buffer and acetonitrile (35:65, v/v)	Human plasma	Tertiary butyl methyl ether	10.1 – 804 ng/mL	TIS, positive	AB Sciex API-4000 (Foster City, CA, USA) triple quadrupole mass spectrometer	147
Paroxetine and 4-hydroxy-3-methoxy metabolite	Synergi MAX-RP 80A (150 mm × 2 mm, 4 μm)	Acetonitrile/0.02% formic acid (66:34, v/v) Isocratic elution	Human plasma	Acid hydrolysis using 0.5 M hydrochloric acid followed by adjusting pH with 10 M sodium hydroxide and extraction with ethyl acetate	Paroxetine 0.70 μg/L	ESI, positive	Esquire 3000 ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany)	148
Sertraline	Acquity UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 μm)	Acetonitrile and 1% formic acid. Gradient elution	Human plasma	Ethyl acetate in basic condition obtained using 0.1 M sodium hydroxide	1.0 – 100.0 ng/mL	ESI, positive	XEVO TQD triple quadruple mass spectrometer	149
Amphetamine	Hypersil BDS C18 (100 mm × 2.1 mm, 3.5 μm)	10 mM ammonium acetate and acetonitrile (75:25) Isocratic elution	Human plasma and oral fluids	Methanol	2 pg/L	ESI, positive	Quattro Ultirna triple quadrupole MS (Micromass UK, Ltd., Manchester, UK)	150
Docetaxel and paclitaxel	Merck Purospher Star, RP-18 (55 mm × 2 mm, 3 μm)	2 mM acetic acid/0.2 mM ammonium acetate in water and 2 mM acetic acid/0.2 mM ammonium acetate in methanol Gradient elution	Human plasma and oral fluids	Methyl tertiary butyl ether	Docetaxel: 2 ng/mL, Paclitaxel: 1000 ng/mL in plasma and docetaxel: 0.125 ng/mL, paclitaxel 62.5 ng/mL in oral fluid	ESI, positive	API 4000 triple quadrupole mass spectrometer from Applied Biosystems (Foster City, CA)	151
Levamisole	Agilent HC-C8 (150 mm × 4.6 mm, 5 μm)	Acetonitrile:10 mM ammonium acetate (70:30, v/v)	Human plasma	Ethyl ether	0.1 – 30 ng/mL	ESI, positive	Agilent G6410 B tandem quadrupole mass spectrometer (Agilent Technologies, USA)	152
Lidocaine	Phenomenex Luna C18 (75 mm × 4.6 mm, 3 μm)	Acetonitrile-26 mmol/L ammonium acetate at pH 4.5 (70:30, v/v)	Human plasma	Tertiary butyl methyl ether	0.2 ng/mL	APCI, positive	Triple-quadrupole API 365 LC-MS-MS	153
Metoprolol	Purospher Star C 18 (150 mm × 4.6 mm, 5 μm)	10 mmol/L ammonium acetate (pH 5.0)/ acetonitrile (15:85, v/v)	Human plasma	Ethyl acetate	1 – 200 ng/mL	APCI	API 3200 (triple quadrupole) instrument from Applied Bio Systems (MDS SCIEX, Canada)	154
Mirtazapine and demethyl-mirtazapine	XTerra MS C8 (150 mm × 4.6 mm, 3.5 μm)	Mixture of 0.010 M ammonium formate (pH 7.8) and acetonitrile 35:65, (v/v) Isocratic elution	Human plasma	Mixture of 1-chlorobutane/ isopropanol/ethyl acetate (88:2:10, (v/v/v))	0.10 – 200 ng/mL	ESI, positive	Finnigan LCQ advantage ion-trap mass spectrometer (San Jose, CA, USA)	155
Topiramate	Gemini C18 (150 mm × 4.6 mm, 5 μm)	Acetonitrile:2 mM ammonium acetate (85:15, v/v)	Human plasma	Tertiary butyl methyl ether	15 – 3000 ng/mL	ESI, positive	Triple quadrupole mass spectrometer	156

(Continued)

(Continued)

Analyte	Chromatographic conditions		Matrix	Sample extraction method (extraction solvent used)	Quantitation level	Mass spectrometry condition		Ref.
	Stationary phase	Mobile phase				Ionization mode	Instrument	
Scopolamine butylbromide	C18 analytical column (50 mm × 4.6 mm)	Acetonitrile and a buffer of 5 mM ammonium acetate and 0.1% formic acid (60:40, v/v)	Human plasma	Dichloromethane	0.1 – 40 ng/mL	ESI, positive	Triple quadrupole mass spectrometer	157
Venlafaxine	Diamonsil C18 (150 mm × 4.6 mm., 5 µm)	Methanol–water containing 10 mmol/L ammonium acetate, pH 7.9 (adjusted with aqueous ammonia; 80:20, v/v)	Human plasma	Ether in basic condition obtained with 0.1 M sodium hydroxide	1 – 200 ng/mL	ESI, positive	Triple quadrupole mass spectrometer	158
Xylazine and 2,6-xylidine	Atlantis T3 column (100 mm × 2.1 mm, 3µm)	0.1% formic acid in water and 100% acetonitrile Gradient elution	Animal tissues (liver, fat and kidney)	Meat, kidney and liver samples extracted using 10 mL acetonitrile and fat samples using 10 mL ammonia modified acetonitrile	Xylazine: 0.06 µg/kg and 2,6-xylidine: 1.5 µg/kg	ESI, positive	Tandem MS was API 5000 Triple quadrupole from Applied Biosystem (Canada)	159
Benzodiazepines and hypnotics	XTerra MS C18 (100 mm × 2.1 mm, 3.5µm)	5% acetonitrile-95% formic acid 0.1%	Human hair	Overnight incubation of hair sample in 1 mL phosphate buffer followed by extraction with 5 ml methylene chloride/diethylether (90/10, v/v)	0.5 – 5 pg/mg	ESI, positive	Micromass Quattro Micro tandem mass spectrometer	160

certain sample preparation procedure can lead to vary the diverse protein profile.[96] Different strategies are investigated in order to remove high abundant proteins such as centrifugal ultrafiltration,[97-99] use of a variety of solid phase extraction columns,[100] disk plates,[101,102] and organic solvent extraction.[103]

Bjorhall *et al.* reported an interesting proteome analysis employing five different columns for better resolution of human serum samples. Amongst the five, multiple affinity removal columns (MARC) showed depletion about 90 – 95% of total serum proteins in plasma/serum. Removal of highly abundant proteins, specifically albumin, IgA, transferrin, IgG, a1-antitrypsin and heptoglobin undoubtedly improved the intensity of low abundant proteins.[100] The high abundant protein depletion method increases protein identification but it also has certain demerits. Removal of the high abundant proteins leads to the removal of their interacting partner proteins, which would be the potential biomarkers.

Duarte *et al.* examined different SPE sorbent materials to specifically extract the alcohol abuse biomarker phosphatidyl-ethanol (PEth) from spiked plasma and blood of human, in order to determine the surface characteristics those have an effect on recovery and retention of PEth. The library of these sorbent materials was then employed for evaluation with the developed method and analyzed by LC-MS/MS. A newly synthesized polymeric sorbent material containing quaternary heterocyclic groups (quaternized 1-vinyl imidazole) with a backbone poly-trimethylolpropane trimethacrylate was found more appropriate for extraction of PEth in spiked blood reflecting the maximum analyte recovery.[104] Some other publications have extensively reviewed the strategies used for serum/plasma fractionation in biomarker discovery in various diseases.[105–107] Bioanalysis of different drugs reported using SPE as the sample preparation technique has been summarized in Table 3.

6·4 Solid phase microextraction

Solid phase microextraction (SPME) technique of sample extraction is a non-exhaustive technique, adopted as an alternative to the present sample preparation methods.[108,109] Nowadays, it has various fields of applications for the extraction of a variety of analytes. Initially, it was used for the analysis of the fatty acid and flavour volatiles.[110]

The principal mechanism behind the SPME is the partitioning of the analyte between the matrix and the coated silica fiber (sorbent phase immobilized on a solid support).[111] The small volume of extractive phase makes contact with the sample analyte for a pre-defined time to achieve the equilibrium between sample matrix and extractive phase.[112] For the extraction of a volatile and semi-volatile analyte, SPME coating is exposed to headspace above the sample matrix.[113] For non-volatile or low-volatile analytes, SPME extractive phase is directly immersed into the sample matrix. Thermal or solvent desorption is then carried out to isolate the extracted analyte on SPME coating before the analysis. Equilibration time is possibly influenced by numerous factors, including pH, temperature, stirring, and salt concentration.[108,114] The sample solution is neutralized with the adjustment of pH. Basic pH improves the recovery of basic analytes, while acidic analytes are better recovered at acidic pH. Addition of salt like sodium chloride can give rise to maximum recovery, as it is a source of strong ionic effects.

Mirnaghi *et al.* showed that though the volume of extractive phase is reduced in SPME, the magnitude of the extracted analyte with high distribution coefficient and different polarity at optimum conditions could be greater than those attained by exhaustive SPE.[115] The appropriate geometry of SPME, as well as the flexibility in sample volume, offers additional opportunities such as *in vivo* micro sampling based on a solid microextraction integrated system.[116] Zhou *et al.* expanded the SPME method to semisolid tissues, such as muscles of living fish under laboratory and field conditions to determine free and total analyte concentration simultaneously in living tissue.[117]

To achieve the maximum recoveries of polar compounds from the biological matrix, derivatization procedures are implemented in a solid state or solution in analytical methods. Derivatization in SPME has made it possible to achieve low detection levels of substances having issues like high reactivity or volatility, poor chromatographic behavior, and thermal instability.[111] Several techniques for derivatization have been used and described in the literature.[114] The derivatization process comprises the addition of appropriate reagents to the sample matrix, and then extraction or derivatization on the fiber. Although several studies on SPME using drugs and their metabolites in plasma or human urine have been published,[118] only a few of them reported SPME optimization procedures. In most of the reported studies, SPME connected on-line with GC-MS and LC-MS has been applied. Sharma *et al.* used the SPME for on-fibre derivatization and GC-MS for the development of a sensitive and solvent-free

Table 3 Bioanalysis using solid phase extraction as the sample preparation technique

Analyte	Chromatographic conditions		Matrix	Sample extraction method (extraction cartridges used)	Quantitation level	Mass spectrometry condition		Ref.
	Stationary phase	Mobile phase				Ionization mode	Instrument	
Salmeterol	Betasil C18 (100 mm × 2.1 mm, 5 µm)	0.1% formic acid in water: methanol	Human plasma	Oasis MAX mixed-mode polymeric anion-exchange sorbent cartridges	2.5 pg/mL	TIS, positive	Sciex API5000 triple quadrupole mass spectrometer	161
Phosphatidylethanol	Hypersil HYPURITY C4 (100 mm × 4.6 mm, 5 µm)	Solvent A 20% 2 mM ammonium acetate + 80% methanol, solvent B:100% isopropanol Gradient elution	Plasma and blood	Not reported	Not reported	ESI, negative	Applied Biosystems mass spectrometer (API3200)	104
Urinary conjugates of bisphenol A (BPA), 2,5-dichlorophenol (2,5-DCP), and 2-hydroxy4-methoxy-benzophenone, benzo-phenone-3 (BP-3)	Chromolith Performance RP-18 (100 mm × 4.6 mm)	10 mM ammonium acetate (pH 6.5) and MeOH: acetonitrile (50:50) Gradient elution	Human urine	LiChrospher RP-18 ADS SPE column (25 × 4, 25 µm particle size, 60 Å pore size)	BPA: 0.3 µg/L, 2,5-DCP: 0.4 µg/L, and BP-3: 0.5 µg/L	APCI, negative	API 4000 triple quadrupole mass spectrometer	162
Bupropion and its metabolites	Zorbax Eclipse XDB C18 (150 mm × 4.6 mm, 5 µm)	20 mm ammonium acetate methanol (10:90, v/v) Isocratic elution	Human plasma	Waters Oasis HLB (1 cm^3, 30 mg)	Bupropion: 0.1 – 350 ng/mL and hydroxy bupropion: 0.1 – 600 ng/mL	TIS, positive	MDS SCIEX API-4000 (Toronto, Canada)	163
Chlorpromazine, haloperidol, levome-promazine, olanzapine, risperidone, and sulpiride	Restek PFP Propyl C18 (50 mm × 2.1 mm, 5 µm)	Ammonium formate 2 mM, pH 2.7, and acetonitrile	Human plasma	Oasis HLB cartridges	Chlorpromazine: 13.17 ng/mL Haloperidol: 1.19 ng/mL Levomepromazine: 4.99 ng/mL Olanzapine: 2.89 ng/mL, risperidone: 4.59 ng/mL, and sulpiride: 7.04 ng/mL	TIS, positive	API BioSystem 3200 tandem mass spectrometer	164
Paliperidone	Thermo Betabasic-8, (100 mm × 4.6 mm, 5 µm)	Methanol:ammonium acetate solution (70:30v/v)	Human plasma	Oasis HLB SPE cartridge of 1 cc (30 mg)	0.2 ng/mL	ESI, positive	Triple quadrupole mass spectrometer API 5500	165
Phencyclidine	Pursuit C8 (100 mm × 2 mm, 3 µm)	20 mM ammonium formate (pH 2.70):acetonitrile (72:28%) and mobile phase B consisted of 20 mM ammonium formate (pH 2.70): acetonitrile (5:95%) Gradient elution	Human blood and serum	Strata X-C Cation mixed-mode polymer (60 mg/3 mL) SPE column	0.06 ng/mL	ESI, positive	A Quattro Premier triple-quadrupole mass spectrometer (Waters, Milford, MA)	166
Olanzapine	ACE 5C18-300 (100 mm × 4.6 mm, 5.0 µm)	Acetonitrile: 0.01% ammonia in 2 mM ammonium formate (85:15, v/v, pH 6.6) Isocratic elution	Human plasma	Waters Oasis HLB cartridges	0.10 – 40 ng/mL	ESI, positive	API-4000 triple quadrupole mass spectrometer	167
Olanzapine and fluoxetine	Thermo Hypersil Gold C18 (50 mm × 4.6 mm, 5 µm)	Methanol:2 mM ammonium acetate buffer (90:10) Isocratic elution	Human plasma	Waters Oasis HLB cartridges	Olanzapine: 0.10 – 20 ng/mL Fluoxetine: 0.50 – 50 ng/mL	ESI, positive	Triple quadrupole mass spectro-meter, API-4000, (MDS SCIEX)	168
Quetiapine	Inertsil ODS-3, RP C18 (33 mm × 4.6 mm, 3 µm)	Mixture of ammonium formate buffer solution (5 mM, pH 4.5) and acetonitrile and methanol 10:75:15 (v/v) Isocratic elution	Human plasma	HLB SPE cartridges (1 cc, 30 mg, Waters Oasis®)	5.01 – 2501.04 ng/mL	ESI, positive	MDS Sciex (Foster City, CA, USA) API-3000 mass spectrometer	169
Multiple hallucinogens, chlorpheniramine, ketamine, ritalinic acid, and metabolites	Sunfire C8 column	Ammonium formate buffer 10 mM pH 3.5 and acetonitrile/methanol (2:1, v/v) Gradient elution	Human urine	Not reported	Lysergic acid diethylamide (LSD): 0.05, 2-oxo-3hydroxy-LSD: 1 ng/mL	ESI, positive	A Quattro ultima tandem MS (Waters)	170
Scopolamine	ACQUITY UPLC CSH C18 (50 mm × 2.1 mm, 1.7 µm)	Not reported	Human plasma	Oasis HLB Micro-Elution Plates	5 – 640 pg/mL	ESI, positive	Xevo TQ-S	171
Venlafaxine	Phenomenex Luna RP8 (2) (150 mm × 4.6 mm, 5 µm)	35% ACN/10% methanol/55% acetic acid 20 mM pH 3.0	Human plasma	Not reported	0.25 – 200 ng/mL	ESI, positive	Quattro LC triple-quadrupole mass spectrometer (Waters, Milford, MA, USA)	172
Benzodiazepines	Gemini C18 (150 mm × 2 mm, 5 µm)	3 mM ammonium formate/0.001% formic acid in water and acetonitrile	Human hair	Not reported	0.05 – 1.02 ng/30 mg	ESI, positive	LCQ Deca XP Plus ion trap MS	173

method to determine aromatic amines in food and environmental samples. Derivatization directly performed on SPME fiber to avoid susceptibility to side reaction and interference occurs during the reaction in solution. Impregnation of fiber with derivatization reagents like allyl iso-thiocyanate followed by pyrolysis in the injection port of the GC leads to the formation of aryl iso-thiocyanate. The optimal extraction was observed with a divinylbenzene/carboxen/polydimethylsiloxane fiber placed for 20 min in the headspace of the sample heated to 70°C. A limited detection was found in the range 6 – 180 ng/L.[119]

6·5 Monolithic spin column extraction

Monolithic C18 silica column contains silica rods in place of spherical particles. Monoliths offer high surface area, which helps to achieve the good adsorption of analytes. Monolithic silica packed into spin columns has become a new tool for extraction of an analyte from biological samples.[120,121] Based on the concentration, variable porosity and pore size can be obtained. Several working steps during sample preparation such as sample loading, washing, and elution of analyte of interest can be obtained by centrifugation of spin column. Additionally,

Table 4 Bioanalysis using monolithic silica spin column extraction technique

Analyte	Chromatographic conditions		Matrix	Sample extraction method	Quantitation level	Mass spectrometry condition		Ref.
	Stationary phase	Mobile phase				Ionization mode	Instrument	
Amitraz and metabolites	XTerra MS C18 (150 mm × 2.1 mm, 3.5 µm)	10 mM formic ammonium and acetonitrile Gradient elution	Human serum	Spin column conditioned with acetonitrile followed by centrifugation at 2500 rpm for 1 min	25 - 1000 ng/ml	APCI	A Shimadzu 8000 single quadrupole mass spectrometer	122
Dibucaine and naphazoline	XTerra MS C18 (150 mm × 2.1 mm, 3.5 µm)	10 mM ammonium formate: acetonitrile Gradient elution	Human serum	Spin column conditioned with acetonitrile followed by centrifugation at 2500 rpm for 1 min	10 and 25 ng/mL	ESI, positive	QP8000 quadruple single mass spectrometer (Shimadzu Corp., Kyoto, Japan)	123
Catecholamines	Inertsil ODS-4 (250 mm × 3 mm, 5 µm)	20 mmol/L sodium acetate-citrate buffer/ acetonitrile (100/16, v/v)	Human urine	Spin column conditioned with 1% acetic acid and 100 mmol/L phosphate buffer followed by centrifugation at 3000 rpm for 5 min	0.2 ng mL/ min	Electrochemical detection	Not reported	174
Ricin	L-column ODS	0.1% formic acid in acetonitrile-water Gradient elution	Crude extracts	Extraction on lactose immobilized monolithic silica by fixing into the spin column followed by tryptic digestion	200 ng/mL	ESI, positive	LTQXL-OrbitrapMS (ThermoFisher Scientific Inc.,Waltham, MA, USA)	175

with the help of this sample preparation technique, more than one sample can be prepared using only centrifugation and no further evaporation step is required.

Saito *et al*. reported a very sensitive and high throughput LC-MS method for simultaneous determination of amitraz and its metabolites in human serum using a monolithic silica column. Conditioning of the spin column was carried out using acetonitrile and then centrifuged at 2500 rpm for 60 s, followed by washing with distilled water and further centrifuging at 2500 rpm for 60 s. After conditioning, samples were loaded to the columns and centrifuged at 2500 rpm for 300 s followed by addition of distilled water to the columns and centrifugation at 2500 rpm for 60 s. After spinning, methanol was added and centrifuged at 2500 rpm for 60 s. Finally, acetonitrile was added to the column and the residual compounds were eluted after centrifuging at 2500 rpm for 60 s and injected into the LC system for quantification.[122] Similarly, simultaneous determination of dibucaine and naphazoline from human serum using monolithic spin column extraction procedure have been reported.[123] Different studies reported for the analysis of different drugs using monolithic spin column extraction procedure as the sample preparation technique has been summarized in Table 4.

6·6 Microextraction by packed sorbent

The implementation of new sample extraction techniques in bioanalysis is of great interest and value to the bioanalytical community.[124] Microextraction by packed sorbent (MEPS) is a relatively new technique of sample extraction originally designed and patented by AstraZeneca, Sweden. MEPS is an upgraded version over SPE towards a more efficient and sophisticated format that reduces the volumes of sample, washing and elution solvent.[125] MEPS resemble a miniaturized SPE packed bed device with a different range of volumes. In MEPS, the sample extraction, concentration, and clean-up steps are performed in a single device composed of a syringe and a MEPS barrel insert and needle (BIN). BIN is a very small tube containing 1 - 2 mg of the packed sorbent, which is similar to an SPE cartridge.[126] Abdel-Rehim *et al.* have demonstrated that the extraction recovery of ropivacaine has been increased by the use of selective packing material, molecularly imprinted polymer in syringe.[127] This extraction technique has also been implicated in the screening of drugs from saliva samples.[128]

7 Current Trend and Future Advancement

In the last few years, a great deal of sophistication in the field of chromatographic techniques has been reported. The advancement in column chemistries, such as different new types of columns has been introduced based on modification in silica gel, organic polymer, hybrid stationary heart and other types of supports that have a significant impact in bioanalysis. Hybrid stationary phases help to diminish the matrix effect to a significant extent. The use of updated versions of pumps, like direct gas pressure pumps, syringe pumps, reciprocating pumps, pneumatic intensifiers, *etc.*, allows precise sample quantification. Over the past few decades, the sophistication of analytical instruments equipped with highly sensitive detectors has proposed. These advanced techniques are reported to have advantages over other conventional analytical instruments in solving the riddles of the matrix effect. The conventional sample extraction techniques like LLE or PPT are being substituted gradually with the newer and much more accurate microextraction techniques involving improved resolving powers of column cartridges and microfiber loops. Today, the advancements in instruments has made it possible to separate the undesirable influences on qualitative analysis, including post and pre-spiked column infusions and other sensitive modes, especially in the mass spectroscopy. The hyphenated MS techniques almost changed the way of analysis of biological specimens. This became only possible with the application of the powerful ionization techniques along with highly sensitive and precise detection powers of MS, Q-TOF and Orbitrap. Other techniques like matrix-matched external calibration and proper internal standardization techniques are undergoing continuous advancements. With the paradigm shift in the arena of sample preparation techniques, the methods of biological sample analysis are being shaped into more effective approaches primarily lesser matrix effect, higher analyte recovery and improved analytical sensitivity.

8 Conclusion

In spite of the development of the high-end, highly sensitive and robust instruments, the effect of matrix components remains a challenge in bioanalysis. Interference of matrix components in bioanalysis leads to faulty interpretation of results. Reliability of a quantitative method can be affected due to false positive or negative responses derived from matrix components present in biological samples. Hyphenated analytical techniques including LC-MS/MS are considered the first line methods in the qualitative and quantitative analysis of small molecules present in biological samples. In this review, we have highlighted the implications of advanced sample extraction techniques,

which ensure good extraction recovery along with less matrix interference. The emerging new concepts and applications of effective sample extraction techniques have been scrutinized. Despite the advancements, effectiveness in sample preparation is still a major concern. In conclusion, bioanalysis requires a strategic plan in using the appropriate sample extraction technique based on the type of the matrix and expected analyte concentration level for detection. Significant advancements in the field of sample preparation techniques nowadays widen the scope for a bioanalyst in selecting the appropriate approach to achieve highly pure and concentrated analytes from biological samples.

9 Conflict of Interest

We do not have any conflict of interest to declare.

10 Acknowledgements

We are thankful to NIPER-Ahmedabad and Department of Pharmaceuticals, Ministry of Chemicals and Fertilizers, Govt. of India for providing the necessary facilities and supports.

11 References

1. C. Bylda, R. Thiele, U. Kobold, and D. A. Volmer, *Analyst*, **2014**, *139*, 2265.
2. G. N. Reddy, A. D. Zagade, and P. Sengupta, *Bioanalysis*, **2018**, *11*, 4.
3. S. Jain, T. Jadav, A. Sahu, K. Kalia, and P. Sengupta, *Anal. Sci.*, **2019**, *35*, 719.
4. A. Cappiello, G. Famiglini, P. Palma, E. Pierini, V. Termopoli, and H. Trufelli, *Anal. Chem.*, **2008**, *80*, 9343.
5. B. Matuszewski, M. Constanzer, and C. Chavez-Eng, *Anal. Chem.*, **2003**, 75, 3019.
6. E. Chambers, D. M. Wagrowski-Diehl, Z. Lu, and J. R. Mazzeo, *J. Chromatogr., B*, **2007**, *22*, 852.
7. H. Mei, Y. Hsieh, C. Nardo, X. Xu, S. Wang, K. Ng, and W. A. Korfmacher, *Rapid Commun. Mass Spectrom.*, **2003**, *17*, 103.
8. A. Cappiello, G. Famiglini, P. Palma, E. Pierini, V. Termopoli, and H. Trufelli, *Anal. Chem.*, **2008**, *80*, 9343.
9. A. F. Aubry and J. X. Shen, "*Bioanalysis for Service Users*", in "*Future Medicine*", **2013**, DOI: https://doi.org/10.4155/ebo.13.283.
10. C. Prakash, C. L. Shaffer, and A. Nedderman, *Mass Spectrom. Rev.*, **2007**, *26*, 340.
11. S. W. Moore, *South. Med. J.*, **2003**, *96*, 1244.
12. W. Li, J. Zhang, and F. L. Tse, *Biomed. Chromatogr.*, **2011**, *25*, 258.
13. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, and C. M. Whitehouse, *Science*, **1989**, *64*, 246.
14. C. Côté, A. Bergeron, J.-N. Mess, M. Furtado, and F. Garofolo, *Bioanalysis*, **2009**, *1*, 1243.
15. H. Jiang, H. Cao, Y. Zhang, and D. M. Fast, *J. Chromatogr., B*, **2012**, *71*, 891.
16. L. Tang and P. Kebarle, *Anal. Chem.*, **1993**, *65*, 3654.
17. J.-P. Antignac, K. de Wasch, F. Monteau, H. De Brabander, F. Andre, and B. Le Bizec, *Anal. Chim. Acta*, **2005**, *129*, 529.
18. P. J. Taylor, *Clin. Biochem.*, **2005**, *38*, 328.
19. C. Viswanathan, S. Bansal, B. Booth, A. J. DeStefano, M. J. Rose, J. Sailstad, V. P. Shah, J. P. Skelly, P. G. Swann, and R. Weiner, *Pharm. Res.*, **2007**, *24*, 1962.
20. A. Cappiello, G. Famiglini, P. Palma, and H. Trufelli, *J. Liq. Chromatogr. Relat. Technol.*, **2010**, *33*, 1067.
21. R. King, R. Bonfiglio, C. Fernandez-Metzler, C. Miller-Stein, and T. Olah, *J. Am. Soc. Mass Spectrom.*, **2000**, *11*, 942.
22. L. L. Jessome and D. A. Volmer, *Lc Gc North America*, **2006**, *24*, 498.
23. H. Hotta and K.-i. Tsunoda, *Anal. Sci.*, **2015**, *7*, 31.
24. W. A. Korfmacher, "*Strategies and Techniques for Bioanalytical Assays as Part of New Drug Discovery*", **2009**, CRC Press,.
25. H. Mei, Y. Hsieh, C. Nardo, X. Xu, S. Wang, K. Ng, and W. A. Korfmacher, *Rapid Commun. Mass Spectrom.*, **2003**, *17*, 97.
26. S. Å. Gustavsson, J. Samskog, K. E. Markides, and B. Långström, *J. Chromatogr., A*, **2001**, *41*, 937.
27. C. R. Mallet, Z. Lu, and J. R. Mazzeo, *Rapid Commun. Mass Spectrom.*, **2004**, *18*, 49.
28. R. Dams, M. A. Huestis, W. E. Lambert, and C. M. Murphy, *J. Am. Soc. Mass Spectrom.*, **2003**, *14*, 1290.
29. T. M. Annesley, *Clin. Chem.*, **2003**, *49*, 1041.
30. R. Plumb, G. Dear, D. Mallett, D. Higton, S. Pleasance, and R. Biddlecombe, *Xenobiotica*, **2001**, *31*, 599.
31. A. M. Krstulovic, C. R. Lee, S. Firmin, G. Jacquet, C. N. Van Dau, and D. Tessier, *Lc Gc Europe*, **2002**, *15*, 31.
32. P. Marquet, *Ther. Drug. Monit.*, **2002**, *24*, 255.
33. A. U. Jackson, N. Talaty, R. G. Cooks, and G. J. Van Berkel, *J. Am. Soc. Mass Spectrom.*, **2007**, *18*, 2218.
34. H. Liu, D. Lin, and H. McCurdy, *J. Forensic Sci.*, **2013**, *25*, 65.
35. R. Ramanathan, M. Jemal, S. Ramagiri, Y. Q. Xia, W. G. Humpreys, T. Olah, and W. A. Korfmacher, *J. Mass Spectrom.*, **2011**, *46*, 595.
36. F. M. Lagerwerf, W. D. van Dongen, R. J. Steenvoorden, M. Honing, and J. H. Jonkman, *Trends Anal. Chem.*, **2000**, *19*, 418.
37. M. Thevis and D. A. Volmer, *Drug Test. Anal.*, **2012**, *4*, 242.
38. N. Kishikawa, *Anal. Sci.*, **2018**, *34*, 1109.
39. X. Xu, W. Li, and L. Francis, *Mass Spectrometry Handbook*, **2012**, *7*, 171.
40. M. R. Anari and T. A. Baillie, *Drug Disco. Today*, **2005**, *10*, 711.
41. G. Hopfgartner, D. Tonoli, and E. Varesio, *Anal. Bioanal. Chem.*, **2012**, *402*, 2587.
42. M. Scigelova and A. Makarov, *Proteomics*, **2006**, 1.
43. A. Makarov, E. Denisov, and O. Lange, *J. Am. Soc. Mass Spectrom.*, **2009**, *20*, 1391.
44. E. Virus, T. Sobolevsky, and G. Rodchenkov, *J. Mass Spectrom.*, **2008**, *43*, 949.
45. K. P. Bateman, M. Kellmann, H. Muenster, R. Papp, and L. Taylor, *J. Am. Soc. Mass Spectrom.*, **2009**, *20*, 1441.
46. M. Jemal and Y.-Q. Xia, *Curr. Drug Metab.*, **2006**, *7*, 491.
47. P. Panuwet, R. E. Hunter Jr, P. E. D'Souza, X. Chen, S. A. Radford, J. R. Cohen, M. E. Marder, K. Kartavenka, P. B. Ryan, and D. B. Barr, *Crit. Rev. Anal. Chem.*, **2016**, *46*, 93.
48. W. Niessen, *J. Chromatogr., A*, **2003**, *413*, 1000.
49. J. Antignac, K. D. Wasch, F. Monteau, H. D. Brabander, F. Andre, and B. L. Bizec, *Anal. Chim. Acta*, **2005**, *129*, 529.
50. D. A. Wells, "*Progress in Pharmaceutical and Biomedical Analysis*", **2003**, Elsevier Science & Technology Books.
51. A. T. Iavarone, O. A. Udekwu, and E. R. Williams, *Anal. Chem.*, **2004**, *76*, 3944.
52. G. Hendriks, D. Uges, and J. Franke, *J. Pharm. Biomed. Anal.*, **2008**, *47*, 126.

53. G. Hendriks, *J. Pharm. Biomed. Anal.*, **2009**, *49*, 1.
54. H. Kataoka, and D. A. Wells, *Anal. Bioanal. Chem.*, **2005**, *381*, 1313.
55. A. Vaghela, A. Patel, A. Patel, A. Vyas, and N. Patel, *Int. J. Sci. Technol. Res.*, **2016**, *5*, 6.
56. R. E. Hansen and J. R. Winther, *Anal. Biochem.*, **2009**, *147*, 394.
57. E. M. Hawes, *ASPET N-Glucuronidation of Xenobiotics Symposium*, ASPET, **1998**.
58. J. B. Soto, M.-J. Ruiz, L. Manyes, and A. Juan-García, *Food Addit. Contam.*, **2016**, *33*, 313.
59. E. Tsakelidou, C. Virgiliou, L. Valianou, H. G. Gika, N. Raikos, and G. Theodoridis, *Metabolites*, **2017**, *7*, 13.
60. D. M. Fast, M. Kelley, C. Viswanathan, J. O'Shaughnessy, S. P. King, A. Chaudhary, R. Weiner, A. J. DeStefano, and D. Tang, *AAPS J.*, **2009**, *11*, 238.
61. K. Cheng and L. Chasseaud, *Drug Metab. Pharmacokinet.*, **1998**, *13*, 382.
62. J. P. Scarth, C. Seibert, P. R. Brown, P. Teale, G. J. Beamon, C. M. Pearce, and R. A. Sams, *Chromatographia*, **2011**, *74*, 593.
63. T. Toyo'oka, *Anal. Sci.*, **2017**, *33*, 555.
64. S. Devanshu, M. Rahul, G. Annu, S. Kishan, and N. Anroop, *J. Pharm. Biomed. Sci.*, **2010**, *1*, 7.
65. C. Ghosh, S. Gaur, C. Shinde, and B. Chakraborty, *J. Bioequiv*, **2011**, *3*, 122.
66. K. S. Nemania, A. Shard, and P. Sengupta, *J. Chromatogr., B*, **2018**, *214*, 1096.
67. J. Guo, F. Meng, L. Li, B. Zhong, and Y. Zhao, *Biol. Pharm. Bull.*, **2011**, *34*, 877.
68. N. M. Biswas, A. Shard, S. Patel, and P. Sengupta, *Drug Dev. Res.*, **2018**, *79*, 391.
69. W. Ye, X. Lin, Y. Zhang, Y. Xu, R. Sun, C. Wen, X. Wang, S. Bao, and R. Chen, *J. Pharm. Biomed. Anal.*, **2018**, *152*, 242.
70. S. A. Mohammed, M. S. Eissa, and H. M. Ahmed, *J. Chromatogr., B*, **2017**, *232*, 1043.
71. E. J. Want, G. O'Maille, C. A. Smith, T. R. Brandon, W. Uritboonthai, C. Qin, S. A. Trauger, and G. Siuzdak, *Anal. Chem.*, **2006**, *78*, 743.
72. E. Boyacı, A. Rodriguez-Lafuente, K. Gorynski, F. Mirnaghi, E. A. Souza-Silva, D. Hein, and J. Pawliszyn, *Anal. Chim. Acta*, **2015**, *14*, 873.
73. S. Hamidi and N. Alipour-Ghorbani, *J. Liq. Chromatogr. Relat. Technol.*, **2017**, *40*, 853.
74. M. A. Jeannot and F. F. Cantwell, *Anal. Chem.*, **1996**, *68*, 2236.
75. H. Liu and P. K. Dasgupta, *Anal. Chem.*, **1996**, *68*, 1817.
76. P. Hashemi, F. N. Serenjeh, and A. R. Ghiasvand, *Anal. Sci.*, **2011**, *27*, 943.
77. M. Rezaee, Y. Yamini, and M. Faraji, *J. Chromatogr., A*, **2010**, *1217*, 2342.
78. N. Zhang, K. L. Hoffman, W. Li, and D. T. Rossi, *J. Pharm. Biomed. Anal.*, **2000**, *22*, 131.
79. S. Steinborner and J. Henion, *Anal. Chem.*, **1999**, *71*, 2340.
80. M. Jemal, Z. Ouyang, and D. S. Teitz, *Rapid Commun. Mass Spectrom.*, **1998**, *12*, 429.
81. G. Basileo, M. Breda, G. Fonte, R. Pisano, and C. James, *J. Pharm. Biomed. Anal.*, **2003**, *32*, 591.
82. K. A. Riffel, M. A. Groff, L. Wenning, H. Song and M.-W. Lo, *J. Chromatogr., B*, **2005**, *293*, 819.
83. S. Song, E. N. Ediage, A. Wu, and S. De Saeger, *J. Chromatogr, A*, **2013**, *111*, 1292.
84. M. Solfrizzo, L. Gambacorta, V. M. Lattanzio, S. Powers, and A. Visconti, *Anal. Bioanal. Chem.*, **2011**, *401*, 2831.
85. K . Yoshimatsu, F. Kiuchi, K. Shimomura, and Y. Makinoyo, *Chem. Pharm. Bull.*, **2005**, *53*, 1446.
86. M. Gunma, K. Sato, Y. Inoue, K. Ide, and H. Ohkawachi, *Anal. Chem.*, **1995**, *44*, 537.
87. K. Reddy and Y. Yachawad, *Int. J. Pharm.*, **2016**, *7*, 15.
88. O. Lerch, O. Temme, and T. Daldrup, *Anal. Bioanal. Chem.*, **2014**, *406*, 4443.
89. G. Maasz, J. Schmidt, P. Avar, and L. Mark, *J. Liq. Chromatogr. Relat. Technol.*, **2017**, *40*, 406.
90. C. F. Poole, *Trends Anal. Chem.*, **2003**, *22*, 362.
91. Y. Miki, H. Murakami, M. Omiya, and N. Teshima, *Bunseki Kagaku*, **2018**, *67*, 445.
92. V. Čápka and S. J. Carter, *J. Chromatogr., B*, **2007**, *285*, 856.
93. M. Duarte, K. K. Jagadeesan, J. Billing, E. Yilmaz, T. Laurell, and S. Ekström, *J. Chromatogr., A*, **2017**, *1*, 1519.
94. Z. Kuklenyik, J. A. Reich, J. S. Tully, L. L. Needham, and A. M. Calafat, *Environ. Sci. Technol.*, **2004**, *38*, 3698.
95. J. T. Lathrop, T. K. Hayes, K. Carrick, and D. J. Hammond, *Expert Rev Proteomic.*, **2005**, *2*, 393.
96. J. Villanueva, J. Philip, D. Entenberg, C. A. Chaparro, M. K. Tanwar, E. C. Holland, and P. Tempst, *Anal. Chem.*, **2004**, *76*, 1560.
97. B. L. Hood, D. A. Lucas, G. Kim, K. C. Chan, J. Blonder, H. J. Issaq, T. D. Veenstra, T. P. Conrads, I. Pollet, and A. Karsan, *J. Am. Soc. Mass Spectrom.*, **2005**, *16*, 1221.
98. H. R. Bergen, 3rd, G. Vasmatzis, W. A. Cliby, K. L. Johnson, A. L. Oberg, and D. C. Muddiman, *Dis Markers*, **2003**, *19*, 239.
99. E. Orvisky, S. K. Drake, B. M. Martin, M. Abdel-Hamid, H. W. Ressom, R. S. Varghese, Y. An, D. Saha, G. L. Hortin, C. A. Loffredo, and R. Goldman, *Proteomics*, **2006**, *6*, 2895.
100. K. Bjorhall, T. Miliotis, and P. Davidsson, *Proteomics*, **2005**, *5*, 307.
101. J. M. Koomen, D. Li, L. C. Xiao, T. C. Liu, K. R. Coombes, J. Abbruzzese, and R. Kobayashi, *J. Proteome Res.*, **2005**, *4*, 972.
102. J. M. Koomen, H. Zhao, D. Li, J. Abbruzzese, K. Baggerly and R. Kobayashi, *Rapid Commun. Mass Spectrom.*, **2004**, *18*, 2537.
103. K. Merrell, K. Southwick, S. W. Graves, M. S. Esplin, N. E. Lewis, and C. D. Thulin, *J. Biomol. Tech.*, **2004**, *15*, 238.
104. M. Duarte, K. K. Jagadeesan, J. Billing, E. Yilmaz, T. Laurell, and S. Ekstrom, *J. Chromatogr., A*, **2017**, *1*, 1519.
105. A. K. Callesen, J. S. Madsen, W. Vach, T. A. Kruse, O. Mogensen, and O. N. Jensen, *Proteomics*, **2009**, *9*, 1428.
106. J. L. Luque-Garcia and T. A. Neubert, *J. Chromatogr., A*, **2007**, *259*, 1153.
107. A. Navare, M. Zhou, J. McDonald, F. G. Noriega, M. C. Sullards, and F. M. Fernandez, *Rapid Commun. Mass Spectrom.*, **2008**, *22*, 997.
108. C. L. Arthur and J. Pawliszyn, *Anal. Chem.*, **1990**, *62*, 2145.
109. H. Yabuta, M. Fukushima, F. Tanaka, H. Ichikawa, and K. Tatsumi, *Anal. Sci.*, **2004**, *20*, 787.
110. A. Steffen and J. Pawliszyn, *J. Agric. Food Chem.*, **1996**, *44*, 2187.
111. H. Kataoka, *Anal. Sci.*, **2011**, *27*, 893.
112. T. Matsumoto, W. Yamazaki, A. Jo, S. Ogawa, K. Mitamura, S. Ikegawa, and T. Higashi, *Anal. Sci*., **2018**, *34*, 1003.
113. A. F. Biajoli and F. Augusto, *Anal. Sci.*, **2008**, *24*, 1141.
114. J. Pawliszyn, "*Applications of Solid Phase Microextraction*", **1997**, John Wiley & Sons.
115. F. S. Mirnaghi, K. Goryński, A. Rodriguez-Lafuente,

E. Boyacı, B. Bojko, and J. Pawliszyn, *J. Chromatogr., A*, **2013**, *37*, 1316.
116. X. Zhang, A. Es-haghi, F. M. Musteata, G. Ouyang, and J. Pawliszyn, *Anal. Chem.*, **2007**, *79*, 4507.
117. S. N. Zhou, K. D. Oakes, M. R. Servos, and J. Pawliszyn, *Environ. Sci. Technol.*, **2008**, *42*, 6073.
118. J. S. Fritz, "*Analytical Solid Phase Extraction*", **1999**, Wiley-VCH.
119. N. Sharma, A. Jain, and K. K. Verma, *Anal Methods*, **2011**, *3*, 970.
120. T. Saito, T. Yamagiwa, Y. Yui, S. Miyazaki, A. Nakamoto, A. Namera, and S. Inokuchi, *J. Health Sci.*, **2010**, *56*, 598.
121. T. Saito, *Anal. Sci.*, **2011**, *27*, 999.
122. T. Saito, R. Yamamoto, S. Inoue, I. Kishiyama, S. Miyazaki, A. Nakamoto, M. Nishida, A. Namera, and S. Inokuchi, *J. Chromatogr., B*, **2008**, *99*, 867.
123. T. Saito, S. Morita, I. Kishiyama, S. Miyazaki, A. Nakamoto, M. Nishida, A. Namera, M. Nagao, and S. Inokuchi, *J. Chromatogr., B*, **2008**, *186*, 872.
124. N. Ozbek, G. T. Turan, B. F. Senkal, and S. Akman, *Anal. Sci.*, **2017**, *33*, 807.
125. Z. Khoshdel, P. Hashemi, M. Safdaryan, B. Delfan, M. Rashidipour, and A. Badiei, *Anal. Sci.*, **2013**, *5*, 29.
126. L. Yang, R. Said, and M. Abdel-Rehim, *J. Chromatogr., B*, **2017**, *33*, 1043.
127. M. Abdel-Rehim, L. Andersson, Z. Altun, and L. G. Blomberg, *J. Liq. Chromatogr. Relat. Technol.*, **2006**, *29*, 1725.
128. A. Abdel-Rehim and M. Abdel-Rehim, *Biomed. Chromatogr.*, **2013**, *27*, 1188.
129. W. Yu, X. Yang, W. Sui, H. Xu, X. Luan, X. Wang, Y. Jin, and B. Yuan, *Asian J. Pharm. Sci.*, **2014**, *9*, 117.
130. W. Lu, S. Zhao, M. Gong, L. Sun, and L. Ding, *J. Pharm. Anal.*, **2018**, *8*, 160.
131. H. Danafar, *Pharm. Biomed. Res.*, **2015**, *1*, 48.
132. K. Siddareddy, M. A. U. Reddy, B. Suresh, and J. Sreeramulu, *Pharm. Methods*, **2018**, *9*, 34.
133. J. Hillis, I. Morelli, D. Neville, J. Fox, and A. Leary, *Chromatographia*, **2004**, *59*, S203.
134. K. Karas, J. Kuczynska, H. Sienkiewicz-Jarosz, P. Bienkowski, and P. Mierzejewski, *J. Chromatogr. Sep. Tech.*, **2015**, *1*, 6.
135. Z. Wang, S. Qian, Q. Zhang, and M. S. Chow, *J. Chromatogr., B*, **2011**, *95*, 879.
136. S. H. Im, M. J. Park, H. Seo, S. H. Choi, S. K. Kim, and S.-H. Ahn, *J. Chromatogr., B*, **2014**, *117*, 958.
137. M. Wood, G. De Boeck, N. Samyn, M. Morris, D. Cooper, R. Maes, and E. De Bruijn, *J. Anal. Toxicol.*, **2003**, *27*, 78.
138. S. Li, X. Wang, K. Peng, Z. Ma, X. Zhang, S. Fu, X. Li, L. Li, A. Hong, and J. Jiang, *Molecules*, **2012**, *17*, 2663.
139. H. Tanaka, T. Naito, Y. Mino, and J. Kawakami, *J. Pharm. Health Care Sci.*, **2016**, *2*, 25.
140. H. Jiang, Y. Wang, M. S. Shet, Y. Zhang, and D. Zenke, *J. Chromatogr., B*, **2011**, *879*, 2663.
141. H.-K. Kang, M.-A. Kang, H.-J. Kim, Y.-S. Park, S.-H. Kim, D.-W. Kim, and J.-S. Kang, *Afr. J. Pharm. Pharmaco*, **2014**, *8*, 356.
142. V. Koganti, S. Reddy, J. Thejaswini, and G. Bannimath, *Pharm. Anal. Acta*, **2015**, *6*, 413.
143. J.-w. Chae, I.-h. Baek, J.-h. An, E. Kim, and K.-i. Kwon, *Bull Kor. Chem. Soc.*, **2012**, *33*, 2163.
144. J. Pinto, V. Mendes, M. Coelho, G. Baltazar, J. Pereira, M. Dunn, D. Cotter, and B. Manadas, *J. Chromatogr. Sep. Tech.*, **2015**, *1*, 6.
145. J. Takitane, V. Leyton, G. Andreuccetti, H. Gjerde, V. Vindenes, and T. Berg, *Forensic Sci. Int.*, **2018**, *289*, 165.
146. J. Day, M. Slawson, R. Lugo, and D. Wilkins, *J. Anal. Toxicol.*, **2003**, *27*, 513.
147. S. Siddiraju, M. L. L. Prasanth, and T. Sirisha, *Asian J. Pharm. Sci.*, **2016**, *11*, 459.
148. M. Segura, J. Ortuño, M. Farré, R. Pacifici, S. Pichini, J. Joglar, J. Segura, and R. d. l. Torre, *Rapid Commun. Mass Spectrom.*, **2003**, *17*, 1455.
149. X.-H. Yue, Z. Wang, D.-D. Tian, J.-W. Zhang, K. Zhu, and Q. Ye, *J. Chromatogr. Sci.*, **2015**, *54*, 195.
150. H. W. Lee, H. Y. Ji, H. Y. Kim, E.-S. Park, K. C. Lee, and H. S. Lee, *J. Chromatogr., B*, **2009**, *877*, 1716.
151. K. A. Mortier, V. Renard, A. G. Verstraete, A. Van Gussem, S. Van Belle, and W. E. Lambert, *Anal. Chem.*, **2005**, *77*, 4677.
152. J. L. Shea, *Clin. Chem. Lab. Med.*, **2013**, *51*, 205.
153. L. Dal Bo, P. Mazzucchelli, and A. Marzo, *J. Chromatogr., A*, **1999**, *3*, 854.
154. P. Venkateswarlu, B. Kumar, K. Seshaiah, and V. Prasad, *Acta Pharm.*, **2010**, *60*, 177.
155. C. Pistos, M. Koutsopoulou, and I. Panderi, *Anal. Chim. Acta*, **2004**, *15*, 514.
156. K. Thummar, D. Jethva, and N. Sheth, *WJPPS*, **2018**, *7*, 1016.
157. J. L. Manfio, M. B. Dos Santos, W. A. Favreto, F. I. Hoffmann, and A. C. Mertin, *J. AOAC Int.*, **2009**, *92*, 1366.
158. Z. Wei, X. Bing-ren, and W. Cai-yun, *Biomed. Chromatogr.*, **2007**, *21*, 266.
159. X. Zheng, X. Mi, S. Li, and G. Chen, *J. Food Sci.*, **2013**, *78*, 955.
160. M. Villain, M. Concheiro, V. Cirimele, and P. Kintz, *J. Chromatogr., B*, **2005**, *72*, 825.
161. V. Capka and S. J. Carter, *J. Chromatogr., B*, **2007**, *285*, 856.
162. X. Ye, Z. Kuklenyik, L. L. Needham, and A. M. Calafat, *Anal. Bioanal. Chem.*, **2005**, *383*, 638.
163. J. M. Parekh, D. K. Sutariya, R. N. Vaghela, M. Sanyal, M. Yadav, and P. S. Shrivastav, *Biomed. Chromatogr.*, **2012**, *26*, 314.
164. A. Khelfi, M. Azzouz, R. Abtroun, M. Reggabi, and B. Alamir, *Int. J. Anal. Chem.*, **2018**, *580*, 7218.
165. M. L. Ahmed Baig and S. A. Ali, *Anal. Lett.*, **2017**, *7*, 228.
166. K. C. Chimalakonda, C. Hailey, R. Black, A. Beekman, R. Carlisle, E. Lowman-Smith, H. Singletary, S. M. Owens, and H. Hendrickson, *Anal. Methods*, **2010**, *2*, 1249.
167. D. S. Patel, N. Sharma, M. C. Patel, B. N. Patel, P. S. Shrivastav, and M. Sanyal, *Acta Pharm. Sin. A*, **2012**, *2*, 481.
168. S. Bonde, R. Bhadane, A. Gaikwad, S. Gavali, D. Katale, and A. Narendiran, *J. Pharm. Biomed. Anal.*, **2014**, *64*, 90.
169. S. Ravinder, A. Bapuji, K. Mukkanti, M. Nagesh, and H. Ravikiran, *J. Chem.*, **2011**, *8*, 1802.
170. M. del Mar Ramirez Fernandez, M. Laloup, M. Wood, G. De Boeck, M. Lopez-Rivadulla, P. Wallemacq, and N. Samyn, *J. Anal. Toxicol.*, **2007**, *31*, 497.
171. P. Veeranjaneyulu, T. B. Rao, S. Mantha, and G. Vaidyanathan, *Waters Corporation*, **2012**, Bangalore, India.
172. E. M. Suenaga, D. R. Ifa, A. C. Cruz, R. Pereira, E. Abib, M. Tominaga, and C. R. Nakaie, *J. Sep. Sci.*, **2009**, *32*, 637.
173. E. I. Miller, F. M. Wylie, and J. S. Oliver, *J. Anal. Toxicol.*, **2006**, *30*, 441.
174. M. Tsunoda, C. Aoyama, S. Ota, T. Tamura, and T. Funatsu, *Anal. Methods*, **2011**, *3*, 582.
175. M. Kanamori-Kataoka, H. Kato, H. Uzawa, S. Ohta, Y. Takei, M. Furuno, and Y. Seto, *J. Mass Spectrom.*, **2011**, *46*, 821.