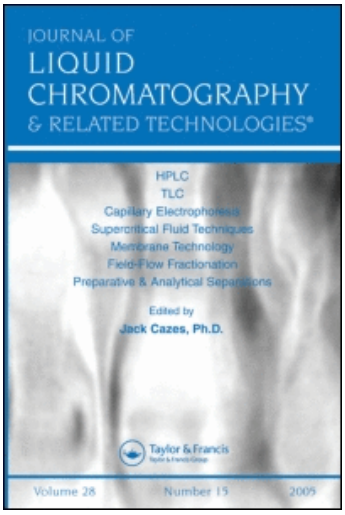

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□ *Hyphenation of mass spectrometry (MS) to liquid chromatography (LC) represents a powerful tool for qualitative and quantitative characterization of target compounds in very complex matrixes of biological origins. In spite of many advantages due to recent advances and innovations in the area of instrumentation and dedicated software support, some difficulties are still encountered in its current applications. The large variety of functional principles and technical solutions applied for hyphenation of the two techniques, for ion sources, ion extraction and focusing, mass analysis, and ion counting makes it more difficult to obtain perfect agreement between the intrinsic characteristics of the laboratory-available instrumentation and the declared goals of specific determination. This review covers a part of the literature data dealing with the shortcomings of LC/MS in bioanalysis. The following topics are discussed: structural identification and confirmation in LC/MS; precision of the instrumental response over short and long term periods; non-linear response functions; adduct formation in atmospheric pressure ion sources; and carryover effects. Most of the problems arising in LC/MS are related to phenomena occurring during ionization. Obviously, the structural characteristics of the analyzed compounds play an important role, although the principles of ionization within the source and the supporting technical solutions and constructive designs add their own particular features. The complex influence of residual sample matrixes over ionization yields of target compounds and internal standards needs to be studied through proper experimental procedures, in order to control both precision and instrumental response function in analysis of biological samples.*

Keywords adduct formation, carryover effects, instrumental response functions, instrumental variety, LC/MS hyphenation, matrix effects, precision, related drawbacks, structural identification and confirmation

INTRODUCTION

Bioanalysis is more often addressed as the process of analyzing (meaning assaying/structural characterization/structural confirmation)

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molecular entities (small molecules or biomolecules) in biological matrices (i.e., fluids, tissues).[1–3] This is applicable to drugs, drug metabolites, or other chemicals (of exogenous or endogenous nature) and relates to drug discovery and development processes, pharmacokinetics, biomarkers, therapeutic drug monitoring, drugs of abuse, and forensic science.

Hyphenation between liquid chromatography (LC) and mass spectrometry (MS) is based on their intrinsic complementary features: the separating ability and the structural identification/confirmation capability.[4] More specifically, LC handles with molecules having reduced volatility and relatively low thermal stability, usually existing in aqueous media. Mass spectrometry acts as a reliable detection system, switching between universal and specific behaviors, producing structural information and allowing high sensitivity. It is important to mention that extreme sensitivities are predominantly produced through the specificity of the response (drastically reducing the noise level), the process being reversely related to the resulting amount of structural information. The tandem between LC and MS strongly depends upon the characteristics of the transfer of the analytes from the liquid mobile phase in the gas phase and their ionization with specific yields. In time, instrumentation designed as interfaces for achieving ionization in the gas phase, resulting through mobile phase evaporation and selective solvent vapor elimination,[5] evolved as stand-alone ion sources.

The main problem relating to LC-MS analysis results from the diversity of the existing instrumentation (based on different constructive and functioning principles) as well as the intrinsic versatility of the technique

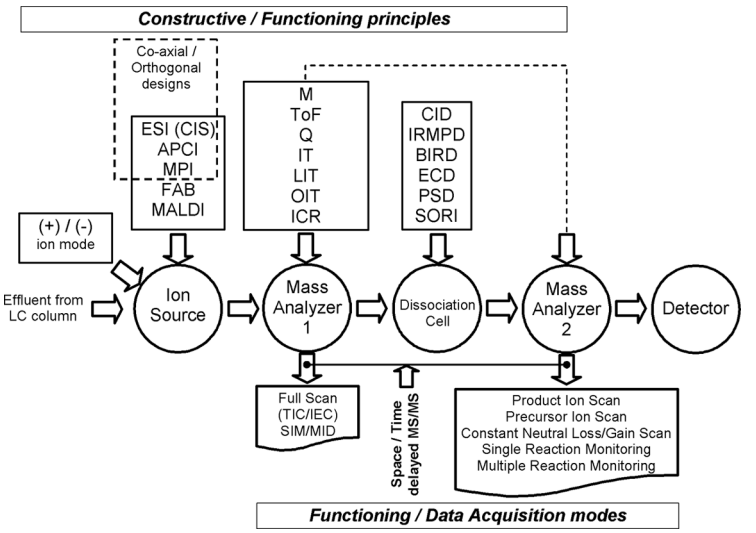

FIGURE 1 Variety of the available mass spectrometry instrumentation.

TABLE 1. Types and Characteristics of Ion Sources Designed for Condensed Phases[6,7]

Characteristics	Ion Source*					
	Thermo Spray	Atmospheric Pressure Chemical Ionization	Atmospheric Pressure Photon Ionization	Atmospheric Pressure Electrospray	Fast Atom Bombardment	Matrix Assisted Laser Desorption Ionization
Acronym	**TSI**	**APCI**	**APPS**	**AP-ESI**	**FAB**	**MALDI**
Sample phase	liquid	liquid	liquid	liquid/solid	liquid/solid	solid
Interfacing with LC	direct	direct	direct	direct	interface needed	transport interface needed
Working pressure	Low vacuum	Atmospheric pressure	Atmospheric pressure	Atmospheric pressure	High vacuum	High vacuum/Atmospheric pressure
Ionization from phase	liquid	gas	gas	liquid/solid	liquid/solid	solid
Flow rates	0.5 ÷ 2 mL/min	<2 mL/min	<1 mL/min	µL range or <0.8 mL/min	µL range	–
Mw of analytes	low to medium	<1500 amu	low	low to high	<5000	medium to high
Geometry design	orthogonal	coaxial/orthogonal	coaxial/orthogonal	coaxial/orthogonal/ Z shaped	orthogonal	orthogonal
Operating alternatives	• Plasma spray ionization	• Electron capture negative ionization	• Dopant assisted	• Coordination ion spray (CIS) • Desorption electrospray ionization (DESI) • Nanoelectrospray	• Continous flow (CF-FAB) • Liquid SIMS • Direct Analysis in Real Time (DART)	• Atmospheric pressure (AP-MALDI) • Surface enhanced (SELDI) • Surface assisted (SALDI) • Desorption ionization on silicon (DIOS) • Material enhanced (MELDI)
Ionization agent	Thermal assisted nebulization + electric discharge + electron beam impact	electric discharge	photons	nebulization within an electric field	atom/ion impact	LASER energy absorbed by matrix
[Ref.]	[8], [9]	[10]–[17]	[18]–[23]	[24]–[34]	[35]–[38]	[39]–[52]

*Other ionization sources are also commercially available or described in literature, as the Sonic Spray Ionization (SSI)[53] and the Laser Spray Ionization (LSI).[54]

TABLE 2. Types and Characteristics of Mass Analyzers[55,56]

Characteristics	Mass Analyzer						
	Magnetic Sector	Time of Flight	Quadrupole	Ion Trap	Linear Ion Trap	Orbital Ion Trap	Ion Cyclotron Resonance
Acronym	**B**	**ToF**	**Q**	**IT**	**LIT**	**(FT)-OIT**	**(FT)-ICR**
Separation principle	Momentum dispersion	Velocity	m/z trajectory stability	m/z resonance frequency	m/z resonance frequency	m/z resonance frequency	m/z resonance frequency
Upper m/z amenable to analysis	15,000	1,000,000	4,000	5,000	4,000	<50,000	30,000
Mass resolution*	$10^2 \div 10^6$	10^4	$\sim 10^3$	$10^3 \div 10^4$	$10^3 \div 10^4$	10^5	10^6
Mass accuracy (ppm)	<10	<50	~100	$50 \div 100$	$50 \div 100$	$2 \div 5$	$1 \div 5$
Ion sampling	Continuous	Pulsed	Continuous	Pulsed	Pulsed	Pulsed	Pulsed
Speed (Hz)**	$0.1 \div 20$	$10 \div 10^6$	$1 \div 20$	$1 \div 30$	$1 \div 30$	~50	$10^{-2} \div 10$
Dynamic range	10^9	$10^2 \div 10^6$	10^7	$10^2 \div 10^5$	$10^2 \div 10^5$	$>10^7$	$10^2 \div 10^5$
Abundance sensitivity***	$10^6 \div 10^9$	$<10^6$	$10^4 \div 10^6$	10^3	$10^3 \div 10^5$	$10^3 \div 10^5$	$10^2 \div 10^5$
Constructive variety/ operation modes	• Single focusing • Double Focusing *Nier-Johnson* *Mattauch-Herzog*	• Ion delayed Extraction • Reflectron • Orthogonal acceleration	–	• mass selective instability • mass selective stability • Resonant excitation • Ion detection	• Axial extraction (AREX) • Rectiliniar	–	• Fourier transform • Stored waveform inverse FT (SWIFT)
MS/MS functioning ability	Very high	High	High	Very high	Very high	Fair	High
LC/MS hyphenation ability	Poor	High	Very high	Very high	Very high	High	Fair
Cost	Very High	High	Low	Low	Fair	High	Very high
[Ref.]	57–59	60–63	64–67	68–71	72–78	79–82	83,84

*considered at 10% valley; **number of spectra acquired per unit of time; ***ratio between abundance of a background peak situated ±one unit m/z value and the abundance of a target signal.

(issuing from different functioning and data acquisition modes). Figure 1 illustrates the diversity of the technical solutions in mass spectrometry designed as detection tools for liquid chromatography.

Although it is not the aim of the present work to discuss functioning principles, construction and related applications of the ion sources, and mass analyzers used in modern mass spectrometry, we considered it useful to provide basic information on these features, as the choice of the best suited instrumentation with respect of the major goals of an analytical challenge may be considered a difficult task. Table 1 deals with ion sources commonly used in bioanalysis, for hyphenation with LC, or as stand-alone devices allowing specific direct bio-sample investigation.[6–54] Table 2 resumes the performances of the commercially available mass analyzers and their adaptability to hyphenation with the LC technique and ion source functional requirements.[55–84] Both tables also provide the acronyms used in Figure 1.

RESULTS AND DISCUSSIONS

Structural Identification/Confirmation

Fragmentation in MS sources designed for LC/MS hyphenation differs tremendously in comparison to classical gas phase EI/CI sources used in GC/MS. Ionization in LC/MS is considered a soft approach (mild ionization techniques), whereas, EI ionization in GC/MS leads to advanced fragmentation due to the high energy transfer between primary electrons and target molecules. However, if fragmentation occurs in LC/MS ion sources, the fragmentation routes are generally very simple ones,[85,86] consisting in elimination of water, carbon dioxide, carbon monoxide, ammonia, inorganic acids, or even carbamates. More complex fragmentation routes rely on de-alkylation, de-acetylation, or a retro-synthesis pathway. For these reasons, the LC/MS spectra are poor with respect to signals attributed to fragments and, from this point of view, in many applications of LC-MS^n, the fragmentation is used as a confirmation tool rather than for structural identification. Multiple stage MS may reveal, through the different available dissociation techniques (see Figure 1), some unusual mass fragmentation pathways and, meanwhile, permits identification of single drugs with high selectivity. Some unexpected pathways could, however, weaken the entire process.[87] When combined with other experimental or theoretical approaches, MS^n becomes useful in certain identification of species in complex samples.[88] As an example, an unreported metabolite of norfloxacin was identified during a BE study by means of MS and MS^2 detection and quantum computation, bringing additional insights on the different pathways of metabolization of the parent active ingredient.[88] A

sensitive and specific LC/MS method based on the combination of constant neutral loss scans with product ion scans was developed for the detection and identification of analytes with identical chemical substructures, such as conjugates of xenobiotics formed in biological systems (i.e., thioethers of N-acetyl-L-cysteine).[89] A paper describing a tandem LC/MS/MS method specifically designed for the screening of synthetic gluco-corticosteroids in human urine was recently reported. The method is designed to recognize a common mass spectral fragment formed from a particular portion of the molecular structure that is common to all synthetic gluco-corticosteroids supporting the specificity of their pharmacological activity.[90]

Although the determination of the chemical identity or molecular structure for related substances in bioanalysis has continuously benefited from the availability and evolution of modern instrumentation, fundamental knowledge about solution phase chemistry, ionization, and gas-phase processes is still vitally important for achieving success in this endeavor.[91] Gas-phase ion/ion reactions involving either multiply-charged analyte ions, multiply-charged reagent ions, or both, exhibit all the characteristics of an analytically useful reaction. They can be highly efficient, fast, and informative and can be readily implemented in MS. Experiments conducted in electrodynamic ion traps capable of executing MS^n procedures can employ multiple ion/ion reaction steps, possibly involving distinct reaction mechanisms. The main barrier for most chemists to use the ion/ion reactions approach consists of the lack of access to appropriate instrumentation. This situation is changing quickly with the growing commercial availability of instruments capable of executing ion/ion reaction experiments along with software to support them.[92]

Unlike MS spectra obtained by EI that have been gathered in many spectral libraries, the MS spectra obtained in LC/MS technique are not entirely reproducible and affordable as data bases. The main drawback of LC/MS as a universal identification tool is the high variability in the degree of fragmentation of the examined compounds, observed for different instruments or even for identical instruments used in different labs. In the study achieved in three laboratories, mass spectra of identical substances, analyzed on the same instruments in nominally identical conditions, showed large differences in the degree of fragmentation.[93] This is caused by the intrinsic mechanisms involved in molecular fragmentation, which are strongly influenced by the surrounding environment produced by mobile phase composition. Some attempts to elaborate a MS spectral library within the laboratory have been reported by literature and briefly discussed here. An overview and comparison between GC/MS and LC/MS, the two major hyphenated techniques used for the metabolic profiling that complement direct “fingerprinting” methods such as

APCI/Q-TOF, APCI/FT-ICR, and NMR, is available in literature.[94] Chemical derivatization can increase the sensitivity and specificity of LC/MS ionization methods for less polar compounds (through CIS and/or or electron capture negative ionization) and provides additional structural information. The important role of a mass spectral library creation and usage in these techniques is illustrated by the following examples.

β-Lactam antibiotics are among the most frequently used in clinical therapy. A single-quadrupole mass analyzer was used for their rapid identification. The product ions of 33 cephalosporin and 11 penicillin antibiotics were assigned to establish the fragmentation patterns and a standard ESI/MS library. The procedure for identification using a LC/ESI/MS library combined with retention data has been proposed in order to overcome difficulties of similar MS spectra of the investigated compounds.[95]

The possibility of creating a robust mass spectral library by means of LC/AP-ESI/MS for the identification of drugs misused in cases of clinical toxicology has been examined by Lips et al.[96] Experimental factors (solvent, pH, different acids, or buffer salts and their concentrations, different organic modifiers, and modifier concentrations) reported to influence the fragmentation have been tested. The large number of experimental mass spectra appears to be affected by the mobile phase compositions by only a minor extent. The search according to the MS spectral pattern made for the major peaks in the LC chromatograms by application of the developed mass spectral library produced a positive identification in a proportion of more than 95%.

Applications of mass spectral library searches in pharmaceuticals have been reported in several papers, in both modes of ionization (AP-ESI or APCI).[97–100] Identification and control of impurities for drug substances are critical tasks in the pharmaceutical process for development of improved quality and safety. Several case studies were reported for the identification of unknown impurities or for leachables (impurities in pharmaceutical products whose origin is the pharmaceutical container closure system in either direct or indirect contact with the formulation) employing chromatographic techniques interfaced with mass spectrometry. The task of unknown identification was facilitated by complementary methodologies including tandem mass spectrometry (MS/MS), high resolution mass spectrometry (HRMS), preparative HPLC, and NMR.[101,102] A review focused on recent progresses reported in the literature from selected publications and that dealt with diverse qualitative and quantitative applications of LC/MS in the pharmaceutical industry (synthetic organic chemistry, combinatorial library parallel synthesis, bioanalysis in support of ADME, and proteomics) was published by Lee.[103]

An LC/MS/MS database, including 780 drug and toxic compounds, has been achieved and reported and features information-rich MS/MS

spectra derived from a novel fragmentation approach incorporating voltage ramping and a broadened mass window for activation. Coupled to effective sample preparation protocols, the database-searching process greatly improved the identification of drugs in postmortem specimens by the LC/ESI/MS/MS technique.[104]

Precision

The main concern frequently expressed when using MS detection in high routine work is related to precision. The variability of the equipment response over short (intra-day) or larger time periods (inter-day experiments) is well known, especially when loading "dirty" samples (samples resulting from preparation of biological matrixes). The origins of the response variability are undoubtedly related to phenomena arising in the MS source, directly influencing the ionization yields of the target compounds. A first approach deals with the influence of the residual matrix simultaneously reaching the source together with the target compounds. The influence on ionization leads to signal suppression or enhancement and may be responsible for poor precision over short periods of time. The phenomena are related to specific endogenous compounds existing in the initial biological sample, breaking through sample processing steps and chromatographic separation, or by exogenous compounds used during earlier steps of sample processing (i.e., anticoagulants). Such matrix effects are well known by MS practitioners and are widely discussed in the literature.[105–119] The study of the matrix influence on ionization is also recommended by the official guidances regulating the bioanalysis domain.[120,121] Another aspect lies in time accumulation within the source of a residual pattern. Such accumulation of residuals products, over longer periods, drifts in the detector response through progressively altering ionization yields within the source. These phenomena are directly affecting the intermediate precision of the method, by dynamically and additionally collecting influences from all residual sample or mobile phase matrixes loaded to the source.

In bioanalysis, the control on the response variability is usually obtained through use of the internal standard (IS) methodology. Historically, the IS role relates to the use of tedious sample preparation procedures often associated to biological matrixes, and its choice depends primarily on the similar chemical behavior with respect to the target analyte when subjected to preparation steps and chromatographic separation.

The use of deuterated labeled analytes as internal standards represents the best choice to ensure similar behavior over all steps of the analytical process (sample preparation, chromatographic elution, and ionization

yield) unless consideration of cost, commercial availability, and intrinsic purity are required.[122–133]

The choice of an IS as a compound structurally similar to the target analyte mainly focuses on the sample preparation steps and chromatographic behavior, while the dynamics of its ionization yield on residuals accumulating in the source is more often ignored. We should also take into consideration the inherent difficulty of developing experimental procedures that aim to emphasize the ionization behaviors of analytes and IS in source accumulation of residuals on functioning over longer time periods.

Actions focused on reduction of residuals co-eluting or accumulating in the MS source are obvious: a) development of sample preparation protocols (including clean-up steps) aiming to produce poor residual matrix; b) optimization of chromatographic elution conditions allowing increased separation selectivity combined with programmed column effluent orientation to waste outside the analyte detection windows for limitation of the accumulation of residuals in the source; c) the use of post separation gradients to remove residual matrix from the chromatographic column; d) the use of MS sources less sensitive to accumulation of residuals; e) the periodic cleaning of MS source; and f) the optimization of MS specific working conditions with pooled extracted samples for simulating non-ideal ionization conditions.

Each of these measures exhibits its own intrinsic limitations. Complex preparation protocols usually require large sample volumes, multiple manipulation steps (introducing variability through random errors), longer duration, additional efforts for optimization and validation, and implicitly higher costs. Automation of complex sample preparation protocols[134–136] may represent an interesting alternative but strongly impacts on costs through the use of complex and expensive equipment and the necessary implementation of Good Laboratory Automation Practices (GALP). Another approach relies on hyphenation between sample preparation procedures and the LC separation, often named on-line configurations.[137–140] Generically, solid phase extraction (SPE) is on-line coupled to LC separations, although it is quite difficult to place real borders between such applications and column switching techniques[141–145] and coupling of turbulent flow chromatography[146–161] (both cases belonging to bidimensional chromatography). The on-line use of SPE adsorbents combining the selectivity toward the target analytes to size exclusion features (restricted access materials [RAM])[162–174] fills the gap to the direct bio-sample loading on the separation system. Size exclusion regulates elimination of the protein fraction from the sample while surface chemistry within pores selectively involves target compounds by means of various mechanisms (i.e., hydrophobic, electrostatic, and steric modulated interactions).

Increasing chromatographic separation selectivity is equivalent to longer chromatographic runs. Increasing duration of the separation means a huge waste of time when dealing with thousands of samples to be analyzed as in bioavailability and bioequivalence studies (BA/BE). Post separation gradients lead to similar effects, through addition of the column re-equilibration periods. It is widely accepted that ionization under APCI conditions is less susceptible to accumulation of residual matrix compared to ESI,[117] although variation in ionization yields is related to structural characteristics of the target compounds. However, the shift from ESI to APCI is not always possible, when considering thermally labile compounds and particular sensitivity requirements. The periodical application of a cleaning procedure to the AP ion source may be effective in some cases, but the frequency of the operation should be carefully considered. Optimization of MS conditions with pooled extracted samples may offer additional insights for controlling ionization phenomena, although the residual pattern acting on the elution time of the analyte peak is not similar to the pattern of the residual matrix accumulating in the source under extensive operation.

The influence of the accumulation during larger time periods of a residual matrix within the MS source may be simply observed through the variation of the IS peak area values over a bioequivalence study. Plots in Figure 2 illustrate the procedure.

Figure 2A illustrates variation of the IS (fluoranthene) peak area values over 644 samples analyzed during a BE study for gliquidone containing pharmaceutical formulations, through use of fluorescence detection (FLD, excitation wavelength 230 nm, emission wavelength 415 nm). Separation was obtained in 1.8 min by using a fast gradient from 30 to 100% acetonitrile, on a Zorbax SB-C18 column, 50 mm L × 4.6 mm i.d. × 1.8 μm d.p., operated at 2 mL/min flow rate and 60°C. The variation of the monitored parameter is stationary (constant precision and constant accuracy); no trend being observable.

Data in Figure 2B were obtained over 560 samples analyzed during a BE study for tenoxicam containing pharmaceutical formulations; piroxicam was used as IS.[175] UV spectrometric detection (368 nm) was achieved, while separation conditions were very similar to those from the first example (column, temperature, gradient, flow rate). A chromatographic run took 4 min. Again, a stationary variation is observable (dispersion is, however, higher, probably due to the less selective character of UV compared to FLD detection modes).

Examples in Figures 2C–2F were all produced through using MS/MS detection. Plot in Figure 2C illustrates variation of nitrazepam (IS) peak area values in a BE study for bromazepam[176] containing 644 samples. A separation of 2 min was obtained on a Rapid Resolution Zorbax SB-C18 30 mm L × 2.1 mm i.d. × 3.5 μm d.p. column, at 0.8 mL/min and 25°C,

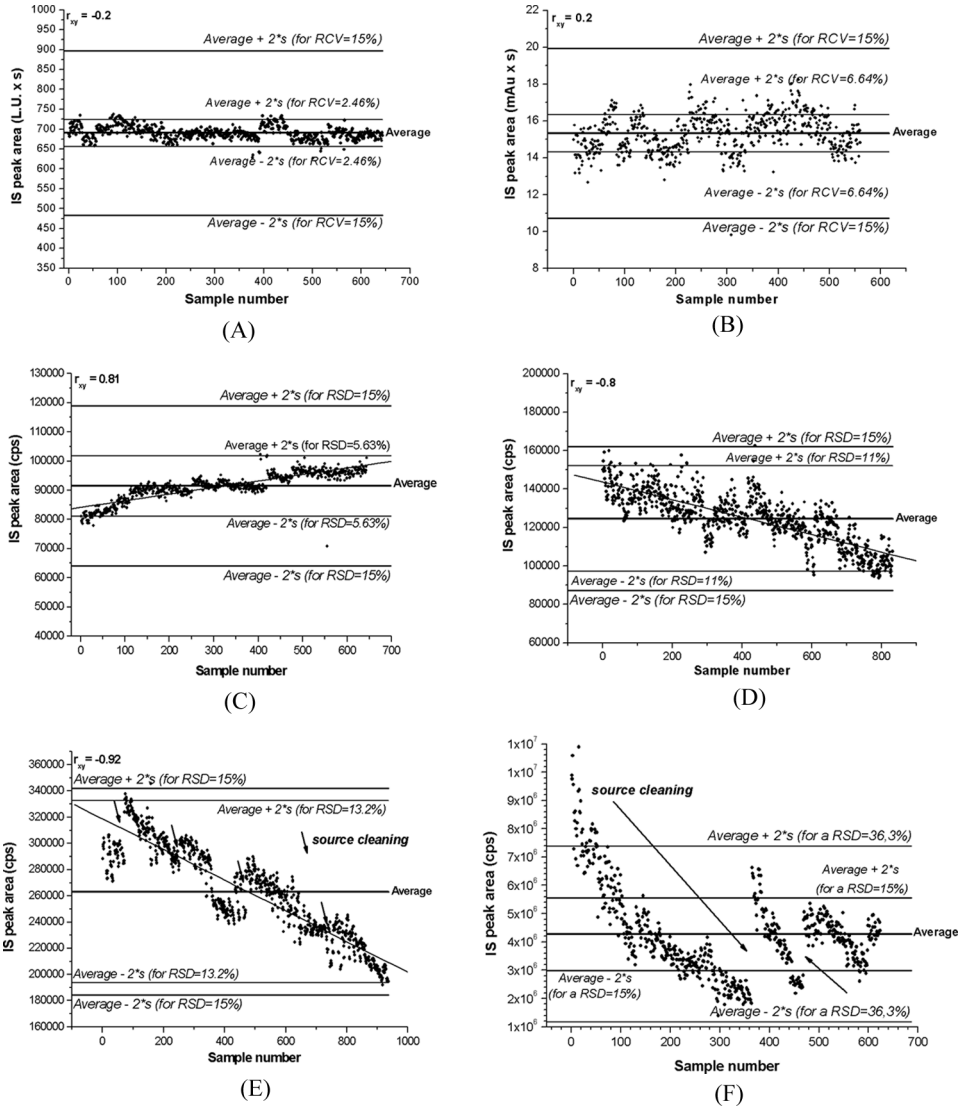

FIGURE 2 Variation and trends of internal standard (IS) peak areas values over long term period as effect of residual matrix accumulation in the MS ion source *(experimental conditions and discussions are made in text).*

under isocratic conditions. AP-ESI ionization was used together with a QQQ mass analyzer, operating under multiple reaction monitoring (MRM) conditions. Again, a stationary variation is observed. Precision is practically constant while a logarithmic profile is observable for the IS peak area values. The variability of the results is comparable to the dispersion obtained under UV detection conditions.

Figure 2D illustrates the variation of the peak area values of 1-methylbiguanide used as IS in 832 samples from a BE study designed for metformin formulations. Separation was made on a nitrile stationary phase (Zorbax CN 150 mm L × 4.6 mm i.d. × 5 μm d.p.) at 25°C and 0.8 mL/min under isocratic conditions (aqueous 10 mM acetate buffer at pH = 3.5 and acetonitrile in a volumetric ratio of 1:1). Electrospray ionization and QQQ mass analysis in the MRM mode were used. Duration of a chromatographic separation was 6.5 min. The MS source was not cleaned during the sample analysis period. This time, a negative trend is observable, attributed to a suppression effect brought by the residual matrix accumulating within the source. Method precision is conserved on study completion, while accuracy is continuously degrading in time. Dispersion of the IS peak areas is considerably higher (a RSD% of 11% was calculated) over the whole study period.

Figure 2E refers to a meloxicam BE study based on a LC-ESI/MS/MS method. 4-hydroxy-2-methyl-N-(pyridine-2-yl)-2-H-1,2-benzothiazine-3-carboxamide-1,1-dioxide was used as an internal standard in 936 samples. Chromatographic separation is obtained under RP conditions (Zorbax Eclipse XDB-C18, 150 mm L × 4.6 mm i.d. × 5 μm d.p.) and isocratic elution mode, at 25°C, 0.8 mL/min and a mobile phase composition of aqueous 0.1% formic acid and acetonitrile (3/7, v/v) in a total run of 4 min. AP-ESI ionization coupled QQQ mass analysis operating in the MRM mode were used. The MS source was periodically cleaned during sample analysis period (four cleaning operations). Dispersion is high, but still within the 15% RSD% limit. A continuous decreasing trend is observable. A non-stationary pattern became obvious, positive jumps in the response arising after each cleaning operation. On short time interval, precision is properly maintained.

Figure 2F illustrates results from a glibenclamide BE study[177] consisting of 624 samples. Gliquidone was used as the internal standard. Separation was achieved on a monolithic C-18 column (Chromolith Performance RP-18e, 10 mm L × 4.6 mm i.d.) at 1 mL/min, 40°C and isocratic elution conditions (aqueous 0.1% formic acid and acetonitrile in a volumetric ratio of 42:58). Ionization is made through an APCI source. The ion trap mass analyzer operated under MS/MS conditions, in the SRM mode. Surprisingly, despite the rational belief relating to APCI robustness with respect to residual matrix effects, the dispersion of the resulting IS peak area values was around 36%. Non-stationary conditions and a pronounced negative trend were observable. Source cleaning operations were producing positive step variations in the equipment response. It is worthwhile to note that same type of samples (human plasma) and sample preparation (protein precipitation by means of acetonitrile addition) were used for all examples formerly discussed. It seems

obvious that MS detection is prone to an increased variability of the response when compared to UV or FL detection modes, and that the structural properties of the target compounds with respect to ionization modes and specific conditions used by the method play a major role in controlling variation over short and long time intervals. In such conditions, the control of precision and accuracy over a BE study can be achieved only through a rational and correct choice of the analytical sequence length (number of samples associated to a calibration and a quality control—QC— set).

Significant variation of ionization yields, even on short time periods, may be produced by the MS source accumulation of additives used in the mobile phase, as illustrated in Figure 3.

Data were obtained from a study related to the assay of ephedrines (as doping agents) in urine through using a LC/(+)ESI/MS/MS method. The need of a resolution between the pairs of analytes cathine/norephedrine, and ephedrine/pseudoephedrine (analytes within the same pair exhibit identical MS/MS transitions) lead to two chromatographic solutions: (1) ion pair (IP) mechanism on a C-18 stationary phase, using

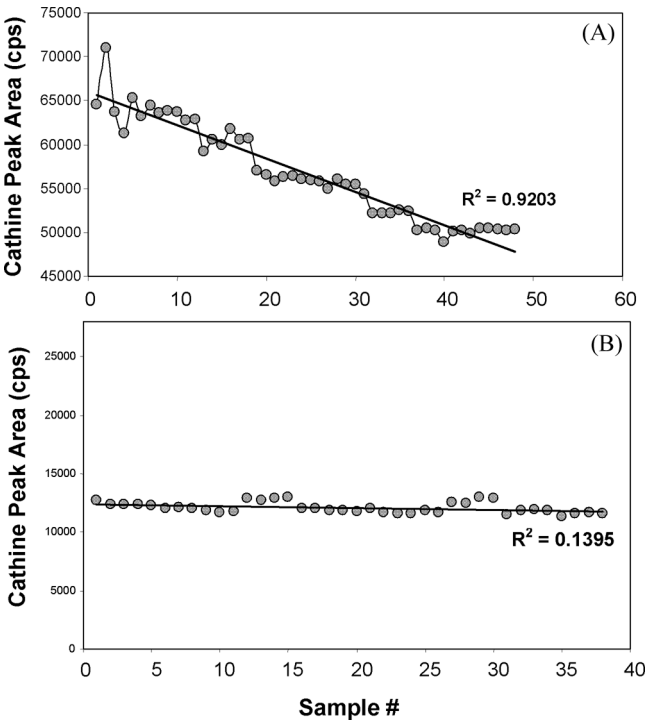

FIGURE 3 Influence of the mobile phase additive on the variation of the analyte peak area values over a short time period (*experimental conditions and discussions are made in text*).

heptafluorobutyric acid as ion pair agent (run time of 25 min); and (2) mixed RP and π–π interaction mechanism on a Phenyl stationary phase, using formic acid as the mobile phase additive (run time of 15 min). Short term variation of the cathine peak area values are illustrated in Figure 3A for the case of the IP separation, while the results of the mixed RP/π–π mechanism based separation are given in Figure 3B. The negative drift in the first case is obvious and was probably produced by accumulation of the per-fluorinated ion-pair agent in the MS source.

Non-Linear Response Functions

The response of a mass spectrometer should be proportional to the number of ions produced in the source after "filtration" through the mass analyzer(s). The various principles of ion production and extraction, mass analysis, and ion counting may basically explain the large differences with respect to the dynamic range covered by the different equipment (see also Table 2). However, based on theoretical considerations, the response of an MS equipment should be linearly related to the amount of analyte reaching the source (and, consequently, to the concentration of the analyte in the sample loaded to the chromatographic system) over at least two orders of magnitude domain.

A proper calibration design should be considered when establishing the univariate calibration function, defined as the functional relationship between the expected response of the detection equipment and the analyte concentration.[178,179] When the uncertainty of the concentration value (S_x^2) and the uncertainty related to the equipment response (S_y^2) are both negligible, the application of an unweighted least-squares regression procedure is advisable. When one of the two types of uncertainties ("making up solutions" and instrumental response) is non-negligible in respect to the other, a convenient approach for calibration should consist in a weighted least squares regression procedure with the weights containing the contributions of errors for x and/or y.[180] When both types of uncertainties are considerable, and the variance of the comprehensive contributions of y and x is too large, the use of the internal standard is strongly recommended and the calibration are based on plots between experimental peak area ratio (analyte/IS) against the known molar concentration ratios.[181]

Some recommendations reported in literature for supporting reliable calibrations are: a) the number of concentration levels to be considered ranges from 7 to 10;[182] b) the number of replicates at each of the concentration levels ranges from 8 to 10[183] (guidance in force advance a minimum of 6 replicates to be considered); c) the calibration design should

fit on the goal of the on-going procedure (i.e., estimation of detection limits requires calibration points near the hypothetical value; accurate quantitative analysis requires that concentration levels bracket the expected determined values interval); d) the calibrations measurements are to be run in blocks containing one replicate from each of the concentration levels and blanks; and e) the blank response has to be inserted in the regression procedure[184,185] (especially when determination of LOD is targeted).

Calibration data may be homoscedastic or heteroscedastic, according to uniformity or non-uniformity of their variance.[186] The scedastic character can be determined through a) plotting of the residuals of the un-weighted least squares regression versus the predicted values; b) comparison of the variances of the replicates at each concentration (Barlett's test); and c) the F-test applied between the largest and the smallest variance of the replicates. Homoscedastic data sets are calculated through un-weighted least squares regression, while heteroscedastic ones require weighted least squares regression models.

The relative low precision of the MS equipment (for the reasons already discussed in the previous section) leads to the conclusion that the uncertainty related to the instrumental response S_y^2 should be consistent. Depending on the complexity of the sample preparation procedure, the uncertainty S_x^2 related to the concentrations of the spiked solutions used for calibration may also be considered as non-negligible. Consequently, it is to be expected that weighted least squares regressions are the best fit models for the calibration designs in MS detection. The validity of such statement may be verified by taking a survey of the literature data.[187–203] Indeed, weighted least squares regression through $1/x$ and $1/x^2$ are the most used calibration designs for MS detection in bioanalysis.

However, unweighted linear design may also be applied, as illustrated in Figure 4A for the metabolite of nicergoline designated as LUOL (ergoline-8-methanol-10-methoxy-1,6-dimethyl). Data were produced during a BE study on IT mass spectrometer operateding in the MRM mode, through monitoring positive ions produced within an APCI ion source (mass transitions 287 to 255 + 269 amu).

Results, as those illustrated in Figure 4B, highlight the need for other options outside the linear regression for quantification of analytes in bioanalysis. Data belong to the assay of ephedrines in urine, more precisely the cathine congener. Chromatographic separation was achieved under RP conditions on a base deactivated stationary phase (Purosphere C18), the aqueous component of the mobile phase being buffered at pH = 10. MS detection was achieved on a QQQ mass analyzer operating in the MRM mode, through monitoring positive ions produced in an AP-ESI source. Obviously, the alkaline pH in the mobile phase allows the required chromatographic

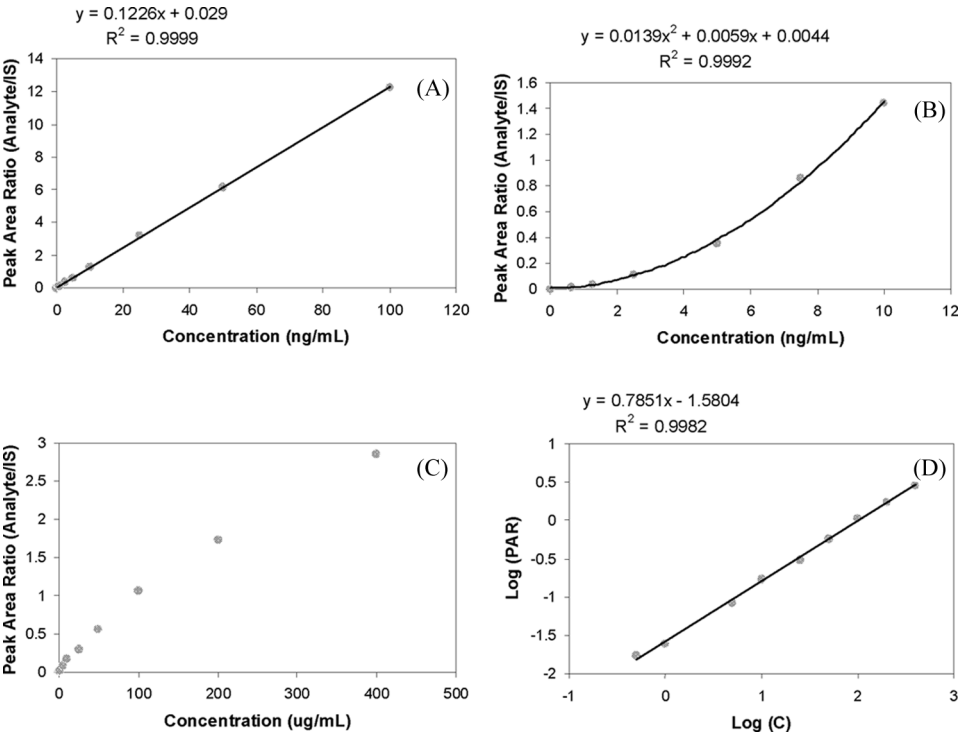

FIGURE 4 Different calibration models fitting to data obtained by means of tandem MS detection in liquid chromatography *(experimental conditions and discussions are made in text).*

selectivity of the ephedrine pairs' cathine/norephedrine and ephedrine/pseudoephedrine under pure reversed phase mechanism, but it is completely atypical for positive ionization under ESI conditions (the aliphatic hydroxyl group characteristic for ephedrines has no acidic character to support negative ionization). In such conditions, a self-enhancement effect seems to be produced (higher amounts of the analyte in the source enhance on ionization yield) leading to a response function modeled through a binomial regression.

Figure 4C illustrates a typical calibration obtained for diltiazem during a BE study.[204] Such profiles also appear for the related de-methylated and de-acetylated metabolites, simultaneously quantified over the respective study. The positive ions produced in an AP-ESI source were monitored by an IT mass analyzer operated under MRM conditions. Apparently, a weighted least squares regression is well suited to modulate the resulting response functions. Surprisingly, weighted linear regressions by $1/x$ or $1/x^2$ failed to produce expected results on back-interpolation. A linearization procedure was thus applied through log-log representation, followed

by application of an unweighted least squares regression (Figure 4D). This time, the source of the unusual calibration profiles is related to the choice of a large isolation window for the precursor ions of the target compounds in order to avoid time programming of the monitored mass transitions. This was leading to the trap saturation resulting in a reduced dynamic range of the response. Linearization through log-log representations were cited in literature[205–209] and produces interesting debates.[210]

Calibrations can be also difficult to produce during quantitative analysis of endogenous compounds (i.e., biomarkers) due to the lack of availability of blank matrixes. The following actions can be considered as practical solutions: a) the use of a surrogate matrix (a very similar matrix without containing the target analytes); b) the adoption of standard addition methods;[211] c) treatment of the matrix for removal of target endogenous compounds;[212] and d) making calibration through using a deuterated labeled analyte.[213] In the specific case of using the standard addition method, the knowledge about the response function of the detector is essential for the proper choice of the addition levels.

Last but not least, we have to mention that the adequacy of a calibration model can be verified in the following ways: a) through the evaluation of the correlation coefficient; b) though the use of an analysis of variance technique; c) by inspection of the behavior of the residuals versus the predicted values.

Adduct Formation Ability in MS Sources

Adduct formation in MS atmospheric pressure ionization sources is a well known phenomena for bioanalysts and a frequently discussed topic in literature.[214–216] One can hardly decide if adduct formation ability should be considered as a benefit or a nightmare in LC/MS applications, as both opinions are more often presented and sustained by experimental data.

The benefits of adduct ion formation may be summarized as following: a) makes possible the ionization of non-polar neutral analytes (representing the phenomenological substrate for the coordination ion spray—CIS—alternative of ESI) or enhance on the sensitivity of the determinations by increasing ionization yields;[217,218] b) may be used as a control tool for the in-source collisional induced dissociation phenomena and thermolysis effects;[219] c) improves CID behavior in MS/MS processes, with formation of specific product ions;[220] and d) represents an interesting option for monitoring clusters formed within crude bio-reaction mixtures.[221]

The adverse effects induced by adduct formation in MS sources are: a) overall sensitivity reduction as adduct formation opens a competitional

pathway to formation of the protonated molecular ions, more often preferred as precursor in MS/MS approaches;[222] b) improper precision of the quantitative results, as kinetics of protonated molecular ion formation and adduct formation may significantly vary in time according to local conditions within the MS source;[223] c) production of unusual mass fragmentation pathways, making structural identification of product ions difficult;[224] and d) limited CID fragmentation yields at low collisional potentials.[225]

The possibility of adduct ion formation should be considered in relation to the following features: a) structural characteristics of the target compounds;[226–228] b) trace ionic impurities existing in the samples or in the solvents used as mobile phase components;[229] c) the basic principles controlling ionization proceses in the atmospheric pressure MS ion sources; and d) design of the MS equipment (including ion extraction and ion focusing technical solutions and mass analysis characteristics).

Under positive ionization conditions, the structures of the adducts frequently cited in literature are: $[M+Na]^+$; $[M+K]^+$; $[M+NH_4]^+$; $[M+H_2O+Na]^+$; $[M+OS+Na]^+$; $[M+2OS+Na]^+$, where M is the molecule of the analyte and OS represents the organic solvent used in the mobile phase.[230] Negative ionization is less favorable to adduct formation. However, formation of the following structures was observed in many cases:[226] $[2M\text{-}H]^-$; $[3M\text{-}H]^-$; $[2M\text{-}2H+Na]^-$.

The ability of adducts to be fragmented under collisional induced dissociation conditions strongly depends on the structural characteristics of the target compound. If considering only the nature of the ion producing cationization and the ability of dissociation of the resulting adducts, the following hierarchy was reported: $[M+H]^+ \sim [M+NH_4]^+ > [M+Li]^+ > [M\text{-}H+2Li]^+ > [M+Na]^+ >> [M+Cs]^+$, which appears logical as the ability to dissociate is reversely related to the electropositive character of the cation.[227] When using transitional metal ions to support ionization within the MS source and when coordinative interactions are the basis of adduct formation, collisional induced dissociation may produce stable molecular fragments containing the metal ion. In such cases, some usual fragmentation pathways may be hampered through the charge neutralization of the metal ion and formation of neutral organo-metallic complexes.[217]

The control upon adduct formation phenomena in MS sources is oriented in two directions: (1) if adduct formation is wanted, the introduction of the ion responsible for cationization in the mobile phase or its post-column addition are needed to shift equilibrium versus adduct formation;[219,220,225,227,228] and (2) if adduct formation needs to be suppressed, additivation of the mobile phase[222,223,231,232] is generally needed (addition of trifluoroacetic acid or alkyl-ammonium salts as formiates or acetates is frequently cited).

Based on the available literature data, and without claiming to advance an axiomatic rule, the order ESI > APCI > APPI ~ SSI is generally accepted for the ability of forming ion adducts.[232]

Carryover Effects

The carryover effect has instrumental origins and is strictly related to the LC system. Carryover basically involves a systematic error resulting from an amount of analyte from a previous sample transported to the following injection, generally by means of the autosampler constructive parts coming in direct contact with the sample.[233] Eliminating carryover from bioanalytical methods can be a time and resource consuming process. While it is necessary to investigate root causes of the carryover and reduce problem areas, complete elimination of carryover may not be practical or even possible.[234] Carryover becomes especially critical in LC/MS applications for bioanalysis[235] because of the following aspects: a) the intrinsic extreme sensitivity of the MS detector; b) the very low concentration thresholds usually targeted in bioanalysis together with strict limits of accuracy and precision being imposed; and c) the complex chemical profile of bioanalytical samples loaded to column, increasing the possibility that target compounds adhere to the active surfaces of the injector parts.

Constructive parts of the autosampler often involved in carryover processes are the needle (internal and external surfaces), needle seat, rotor and rotor seals from the injection valve, loop, and tubings. Different constructive designs of the instrumentation may be more or less prone to carryover effects. Structural characteristics of the analytes (i.e., apolar character) may also facilitate adherence on the active parts of an injection system. Residual matrix in the bio-sample may enhance the adherence ability. Outworn constructive active parts of the injector may seriously increase risks for carryover occurrence. Carryover effects are more often controlled through the rinsing programs (needle wash, needle seat wash) applied to the injection process and by intercalation of blank runs between samples. The first approach requires optimization in terms of solvent being used and duration (or solvent volume needed to eliminate the problem). Special applications in proteomics may involve special solvents, washing profiles, or constructive solutions.[236,237] The second approach is a time-consuming experimental solution and needs a precise evaluation of the number of blanks to be intercalated within samples.

Carryover effects have increased impact on samples having a concentration level of the target analyte close to the low level of quantitation (LLOQ) of the analytical method. In such respects, its influence impacts the dynamic range of the instrumental method.[238]

The structural information carried by the MS detector response is useful to discriminate between the carryover effect and interferences brought by the matrix. A carryover effect should only be considered if the mass spectra (or the intensity ratio between two signals attributed to product ions) of the residual peak appearing at the same retention time as the target compound is similar to the one produced by the analyte itself.

A carryover effect also appears in cases of accidental contamination of the mobile phase with the target compound(s). This may be specifically critical when a gradient elution profile is applied to the analytical column. In such circumstances, the amount of the analyte transferred between successive runs is proportional to the duration spent between consecutive injections (meaning that the carryover is variable).

Carryover should always be considered in accordance with the targeted LLOQ of the method.[239,240] If the LLOQ of the method produces an instrumental response at least 3.3 times more intense than the mean carryover response, no special measures have to be taken for elimination of such an effect.

The carryover effects are subject to a serious attention from the regulation bodies and, according to actual guidances in the field and the accepted rules for best practices should be attentively evaluated not only during analytical method validation but also during analysis of the incurred samples.[241–243]

CONCLUSIONS

The fate of bioanalysis without the benefits brought by the LC/MS hyphenation would be difficult to consider. In such circumstances the recent advances in various scientific fields (i.e., pharmaceuticals, medicine, bio-synthesis) would probably never exist. However, the extensive use of LC/MS technique is far from providing a delightful paradise. LC/MS practitioners know, from their day to day experiences, about the difficulties encountered and the imperious need to continuously observe and understand each detail, to encompass the frequent amazing and unpredictable behaviors of their equipment. For most of us, MS equipment is too complex an instrumentation for a punctual and in-depth understating of the functional features, and, consequently, a holistic approach is somehow needed. More often, the difficulties and shortcomings related to the achievement of the practical experiments are not transparently revealed in the published works, which is why it may be possible to create the false sensation to beginners that LC/MS is an absolute and infallible solution. Or, it is not the case.

Instrumentation for LC/MS applications is a complex, extremely variable (starting from basic functioning principles), and expensive. A perfect

agreement between the analysis particularities, declared goals, and the available instrumentation characteristics is not always possible. The major drawbacks to be considered in LC/MS applications are related to the inherent difficulties in structural identification and confirmation, the relative poor precision induced by residual matrix effects on ionization, the frequent cases of non-linear instrumental responses, the possibility of adduct formation within the atmospheric pressure ion sources, and the carryover effects often difficult to control if considering the extreme sensitivity of the detection device. Excepting the latter shortcoming, all the other addressed topics are intimately related to the phenomena arising during ionization. Undoubtedly, the ion source is by far the most delicate and unpredictable component of a mass spectrometer device. Consequently, special attention should be paid and specific procedures have to be adopted for a better knowledge on phenomena arising within ion sources, in close relation with the structural characteristics, and the solution/gas phase chemistry of the analyzed compounds. Practical solutions to existing problems always exist. It depends only on our awareness and ability to observe trends and to promptly identify the experimental problems.

REFERENCES

1. Hill, H. Development of bioanalysis: A short story. Bioanalysis **2009**, *1* (1), 3–7.
2. Hill, H. Developing trends in bioanalysis. Bioanalysis **2009**, *1* (8), 1359–1364.
3. Hill, H. The changing face of bioanalysis. Chromatogr. Today **2009**, *6*, 44–47.
4. Ardrey, R.E. *Liquid Chromatography-Mass Spectrometry: An Introduction*, Chapter 1, John Wiley & Sons Ltd.: Chichester, England, 2003; 1–5.
5. Niessen, W.M.A.; Tjaden, U.R.; van der Greef, J. Strategies for developing interfaces for coupling LC-MS. J. Chromatogr. **1991**, *554*, 3–26.
6. Ardrey, R.E. *Liquid Chromatography-Mass Spectrometry: an Introduction*, John Wiley & Sons Ltd.: West Sussex, England, 2003; 276.
7. Niessen, W.M.A. *Liquid Chromatography-Mass Spectrometry*, 3rd ed. CRC Press, Taylor & Francis Group: Boca Raton, Florida, USA, 2006; 30.
8. Blakley, C.R.; Vestal, M.L. Thermospray interface for liquid chromatography/mass spectrometry. Anal. Chem. **1983**, *55*, 750–754.
9. Arpino, P. Combined liquid chromatography mass spectrometry, ii: techniques and mechanisms of thermospray. Mass Spectrom. Rev. **1990**, *9*, 631–669.
10. Dass, C. *Principles and Practice of Biological Mass Spectrometry*, John Wiley & Sons, Inc.: New York, USA, 2001; 416.
11. Vestal, M.L. Methods for ion generation. Chem. Rev. **2001**, *101*, 361–375.
12. Singh, G.; Gutierrez, A.; Xu, K.; Blair, I.A. Liquid chromatography/electron capture atmospheric pressure chemical ionization/mass spectrometry: Analysis of pentafluorobenzyl derivatives of biomolecules and drugs in the attomole range. Anal. Chem. **2000**, *72*, 3007–3013.
13. Wang, K.; Davis, P.P.; Crews, T.; Gabriel, L.; Edom, R.W. An electron-capture dienophile derivatization agent for increasing sensitivity: Determination of a vitamin D analog (RO 24-2090) in plasma samples with liquid chromatography/mass spectrometry. Anal. Biochem. **1996**, *243*, 28–40.
14. Cappiello, A.; Famiglini, G.; Lombardozzi, A.; Massari, A.; Vadala, G.G. Electron capture ionization of explosives with a microflow rate particle beam interface. J. Am. Soc. Mass Spectrom. **1996**, *7*, 753–758.

15. Evans, C.S.; Sleeman, R.; Luke, J.; Keely, B. A rapid and efficient mass spectrometric method for the analysis of explosives. Rapid Commun. Mass Spectrom. **2002**, *16*, 1883–1891.
16. Hayen, H.; Jachmann, N.; Vogel, M.; Karst, U. LC-electron capture APCI-MS for the determination of nitroaromatic compounds. Analyst **2002**, *127*, 1027–1030.
17. Higashi, T.; Takido, N.; Shimada K. Detection and characterization of 20-Oxosteroids in rat brains using LC-electron capture APCI-MS after derivatization with 2-Nitro-4-trifluoromethylphenylhydrazine. Analyst **2003**, *128*, 130–133.
18. Robb, D.B.; Covey, T.R.; Bruins, A.P. Atmospheric pressure photoionization: An ionization method for liquid chromatography–mass spectrometry. Anal. Chem. **2000**, *72*, 3653–3659.
19. Raffaelli, A.; Saba, A. Atmospheric pressure photoionization. Mass Spectrom. Rev. **2003**, *22*, 318–331.
20. Syage, J.A. Mechanism of $[M+H]+$ formation in photoionization mass spectrometry. J. Am. Soc. Mass Spectrom. **2004**, *15*, 1521–1533.
21. Purcell, J.M.; Hendrickson, C.L.; Rogers, R.P.; Marshall, A.G. Atmospheric pressure photoionization fourier-transform ion cyclotron resonance mass spectrometry for complex mixture analysis. Anal. Chem. **2006**, *78*, 5906–5912.
22. Kauppila, T.; Kotiaho, T.; Kostiainen, R.; Bruins, A. P. Negative ion-atmospheric pressure photoionization mass spectrometry. J. Am. Soc. Mass Spectrom. **2004**, *15*, 203–211.
23. Fenn, J.B.; Mann, M.; Meng, C.K.; Wong, S.F.; Whitehouse, C.M. Electrospray ionization: Principles and practice. Mass Spectrom. Rev. **1990**, *9*, 37–70.
24. Amad, M.H.; Cech, N.B.; Jackson, G.S.; Enke, C.G. Importance of gas-phase proton affinities in determining the electrospray ionization response. J. Mass Spectrom. **2000**, *35*, 784–789.
25. Gamero-Castano, M.; de la Mora, J.F. Kinetics of small ion evaporation from the charge and mass distribution of multiply charged clusters in electrosprays. J. Mass Spectrom. **2000**, *35*, 790–803.
26. Kebarle, P. A brief overview of the present status of the mechanisms involved in electrospray mass spectrometry. J. Mass Spectrom. **2000**, *35*, 804–817.
27. de la Mora, J.F.; Van Berkel, G.J.; Enke, C.G.; Martinez-Sanchez, M.; Fenn, J.B. Electromechanical processes in electrospray ionization mass spectrometry. J. Mass Spectrom. **2000**, *35*, 939–952.
28. Wilm, M.; Mann, M. Analytical properties of the nanoelectrospray ion source. Anal. Chem. **1996**, *68*, 1–8.
29. Takats, Z.; Wiseman, J.M.; Gologan, B.; Cooks, R.G. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. Science **2004**, *306*, 471–473.
30. Takats, Z.; Wiseman, J.M.; Cooks, R.G. Ambient mass spectrometry using desorption electrospray ionization (DESI): Instrumentation, mechanisms, and applications in forensics, chemistry, and biology. J. Mass Spectrom. **2005**, *40*, 1261–1275.
31. Kertezs, V.; Ford, M.J.; Van Berkel, G.J. Automation of a surface sampling probe/electrospray mass spectrometry system. Anal. Chem. **2005**, *77*, 7183–7189.
32. Li, H.; Siu, K.W.M.; Guevremont, R.; Le Blanc, J.C.Y. Complexes of Silver(I) with peptides and proteins as produced in electrospray mass spectrometry. J. Am. Soc. Mass Spectrom. **1997**, *8*, 781–792.
33. Roussis, S.G.; Prouix, R. Molecular weight distributions of heavy aromatic petroleum fractions by Ag^+ electrospray ionization mass spectrometry. Anal. Chem. **2002**, *74*, 1408–1414.
34. Sandra, P.; Medvedovici, A.; Zhao, Y.; David, F. Characterization of triglycerides in vegetable oils by silver-ion packed-column supercritical fluid chromatography coupled to mass spectroscopy with atmospheric pressure chemical ionization and coordination ion spray. J. Chromatogr. A. **2002**, *974*, 231–241.
35. Cody, R.B.; Larame, J.A.; Durst, H.D. Versatile new ion source for the analysis of materials in open air under ambient conditions. Anal. Chem. **2005**, *73*, 2297–2302.
36. Dass, C. The role of a liquid matrix in controlling FAB-induced fragmentation. J. Mass Spectrom. **1996**, *31*, 77–82.
37. Dass, C. Fast atom bombardment combined with mass spectrometry for characterization of polycyclic aromatic hydrocarbons. J. Am. Soc. Mass Spectrom. **1990**, *1*, 405–412.
38. Caprioli, R. M. Continuous-flow fast atom bombardment mass spectrometry. Anal. Chem. **1990**, *62*, 477A–485A.
39. Demirev, P.A.; Feldman, A.B., Kongkasuriyachai, D.; Scholl, P.; Sullivan, D.; Kumar, N. Detection of malaria parasites in blood by laser desorption mass spectrometry. Anal. Chem. **2002**, *60*, 3262–3266.
40. Knochenmuss, R. A quantitative model of ultraviolet matrix – assisted laser desorption/ionization including analyte ion generation. Anal. Chem. **2003**, *75*, 2199–2207.

41. Knochenmuss, R.; Zenobi, R. MALDI ionization: The role of in-plume processes. Chem. Rev. **2003**, *103*, 441–452.
42. Knochenmuss, R. Photoionization pathways and free electrons in UV-MALDI. Anal. Chem. **2004**, *76*, 3179–3184.
43. Armstrong, D.W.; Zhang, L.-K.; He, L.; Gross, M.L. Ionic liquids as matrices for matrix – assisted laser desorption/ionization mass spectrometry. Anal. Chem. **2001**, *73*, 3679–3686.
44. Carda-Broch, S.; Berthod, A.; Armstrong, D.W. Ionic matrices for matrix – assisted laser desorption/ionization time-of-flight detection of DNA oligomers. Rapid Commun. Mass Spectrom. **2003**, *17*, 553–560.
45. Xu, S.; Li, Y.; Zou, H.; Qiu, J.; Guo, Z.; Guo, B. Carbon nanotubes as assisted matrix for laser desorption/ionization time-of-flight mass spectrometry. Anal. Chem. **2003**, *75*, 6191–6195.
46. Hoang, T.T.; Chen, Y.; May, S.W.; Browner, R.F. Analysis of organoselenium compounds using active carbon and chemically modified silica sol-gel by surface assisted laser desorption/ionization time-of-flight mass spectrometry. Anal. Chem. **2004**, *76*, 2062–2070.
47. Ren, S.F.; Zhang, L.; Cheng, Z.H.; Guo, Y.L. Immobilized carbon nanotubes for MALDI–TOF-MS analysis: applications to neutral small carbohydrates. J. Am. Soc. Mass Spectrom. **2005**, *16*, 333–339.
48. Trauger, S.A.; Go, E.P. Sensitivity and analyte capture with desorption/ionization on silylated porous silicon. Anal. Chem. **2004**, *76*, 4484–4489.
49. Laiko, V.V.; Baldwin, M.A.; Burlingame, A.L. Atmospheric pressure matrix assisted laser desorption/ionization time-of-flight mass spectrometry. Anal. Chem. **2000**, *72*, 652–657.
50. Moyer, S.C.; Cotter, R.J. Atmospheric MALDI. Anal. Chem. **2002**, *74*, 468A–476A.
51. Issaq, H.J.; Conrads, T.P.; Prieto, D.A.; Tirumalai, R.; Veenstra, T.D. SELDI-TOF for diagnostic proteomics. Anal. Chem. **2003**, *75*, 149A–155A.
52. Feuerstein, I.; Najam-ul-Haq, M.; Rainer, M.; Trojer, L.; Bakry, R.; Aprilita, N.H.; Stecher, G.; Huck, C.W.; Bonn, G.K.; Klocker, H.; Bartsch, G.; Guttman, A. Material-enhanced laser desorption/ionization (MELDI)—a new protein profiling tool utilizing specific carrier: materials for time-of-flight mass spectrometric analysis. J. Am. Soc. Mass Spectrom. **2006**, *17*, 1203–1208.
53. Volmer, D.A. Analyzing thermally unstable compounds by LC/SSI/MS. LC-GC Eur. **2000**, *15*, 838–844.
54. Hiraoka, K.; Saito, S.; Katsuragawa, J.; Kudaka, I. A new LC-MS interface: Laser spray. Rapid. Commun. Mass Spectrom. **1998**, *12*, 1170–1174.
55. Dass, C. *Fundamental of Contemporary Mass Spectrometry*, John Wiley & Sons, Inc.: Hoboken, New Jersey, USA, 2007; 585.
56. Hoffman, E. de; Stroobant, V. *Mass Spectrometry: Principles and Applications*, John Wiley & Sons Ltd.: West Sussex, England, 2007; 489.
57. Mattauch, J.; Herzog, R. Double-focusing mass spectrograph and the masses of N15 and O18. Phys. Rev. **1936**, *44*, 617.
58. Johnson, E.G.; Nier, A.O. Angular aberrations in sector shaped electromagnetic lenses for focusing beams of charged particles. Phys. Rev. **1953**, *84*, 10.
59. Birkinshaw, K., Ed. Special issue: detectors and the measurement of mass spectra. Int. J. Mass Spectrom. **2002**, *215* (1–3), 1–213.
60. Wollnik, H. Time-of-flight mass analyzers. Mass Spectrom. Rev. **1993**, *12*, 89–114.
61. Guilhaus, M. Principles and instrumentation in time-of-flight mass spectrometry: Physical and instrumental concepts. J. Mass Spectrom. **1995**, *30*, 1519–1532.
62. Guilhaus, M.; Mlynski, V.; Selby, D. Perfect timing: Time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. **1997**, *11*, 951–962.
63. Cotter, R.J. The new time-of-flight mass spectrometry. Anal. Chem. **1999**, *71*, 445A–451A.
64. Dawson, P.H. Quadrupole mass analyzers: Performance, design, and some recent applications. Mass Spectrom. Rev. **1986**, *5*, 1–37.
65. Campana, J.E. Elementary theory of quadrupole mass spectrometry. Int. J. Mass Spectrom. Ion Proc. **1980**, *33*, 101–117.
66. Miller, P.E.; Bonner Denton, M. The quadrupole mass filter: basic operating concepts. J. Chem. Educ. **1986**, *63*, 617–622.
67. Todd, J.F.J. Ion trap mass spectrometer: Past, present, and future(?). Mass Spectrom. Rev. **1991**, *10*, 3–52.

68. March, R.E. Introduction to quadrupole ion trap mass spectrometry. J. Mass Spectrom. **1997**, *32*, 351–369.
69. Jonscher, K.R.; Yates, J.R. The quadrupole ion trap mass spectrometer: A small solution to a big challenge. Anal. Biochem. **1997**, *244*, 1–15.
70. March, R.E.; Todd, J.F.J. *Quadrupole Ion Trap Mass Spectrometry*, 2nd Ed. Wiley-Interscience: Hoboken, NJ, USA, 2005; 346.
71. Schwartz, J.C.; Senko, M.W.; Syka, J.E.P. A two dimensional quadrupole ion trap mass spectrometer. J. Am. Soc. Mass Spectrom. **2002**, *13*, 659–669.
72. Hager, J.W. A new linear ion trap mass spectrometer. Rapid Commun. Mass Spectrom. **2002**, *16*, 512–526.
73. Hofgartner, G.; Varesio, E.; Tschappat, V.; Grivet, C.; Bourgogne, E.; Leuthold, L.A. Triple quadrupole linear ion trap mass spectrometer for the analysis of small molecules and macromolecules. J. Mass Spectrom. **2004**, *39*, 845–855.
74. Hashimoto, Y.; Hasegawa, H.; Baba, T.; Waki, I. Mass selective ejection by axial resonant excitation from a linear ion trap. J. Am. Soc. Mass Spectrom. **2006**, *17*, 685–690.
75. Ouyang, Z.; Wu, G.; Song, Y.; Li, H.; Plass, W.R.; Cooks, R.G. Rectilinear ion trap: Concepts, calculations, and analytical performance of a new mass analyzer. Anal. Chem. **2004**, *76*, 4595–4605.
76. Douglas, D.J.; Frank, A.J.; Mao, D. Linear ion traps in mass spectrometry. Mass Spectrom. Rev. **2005**, *24*, 1–29.
77. Hager, J.W. A new linear ion trap mass spectrometer. Rapid Commun. Mass Spectrom. **2002**, *16*, 512–26.
78. Makarov, A. Electrostatic axially harmonic orbital trapping: High-performance technique of mass analysis. Anal. Chem. **2000**, *72*, 1156–1162.
79. Hardman, M.; Makarov, A. Interfacing the orbitrap mass analyzer to an electrostatic ion source. Anal. Chem. **2003**, *75*, 1699–1701.
80. Hu, Q.; Noll, R.J.; Li, H.; Makarov, A.; Hardman, M.; Cooks, R.G. The orbitrap: a new mass spectrometer. J. Mass Spectrom. **2005**, *40*, 430–443.
81. Makarov, A.; Hardman, M.E.; Schwartz, J.C.; Senko, M. US Patent. 2004108450, 2004.
82. Amster, I.J. Fourier transform mass spectrometry. J. Mass Spectrom. **1996**, *31*, 1325–1337.
83. Holliman, C.L.; Rempel, D.L.; Gross, M.L. Detection of high mass-to-charge ions by fourier transform mass spectrometry. Mass Spectrom. Rev. **1994**, *13*, 105–132.
84. Dienes, T.; Pastor, S.J.; Schurch, S.; Scott, J.R.; Yao, J.; Cui, S.; Wilkins, C.A. Fourier transform mass spectrometry-advancing years (1992–mid. 1996). Mass Spectrom. Rev. **1996**, *15*, 163–211.
85. Albu, F.; Georgita, C.; David, V.; Medvedovici, A. Liquid chromatography-electrospray tandem mass spectrometry method for determination of indapamide in serum for single/multiple dose bioequivalence studies of sustained release formulations. J. Chromatogr. B **2005**, *816*, 35–40.
86. Georgita, C.; Albu, F.; David, V.; Medvedovici, A. Simultaneous assay of metformin and glibenclamide in human plasma based on extraction-less sample preparation procedure and LC/(APCI)MS. J. Chromatogr. B **2007**, *854*, 211–218.
87. Giancotti, V.; Medana, C.; Aigotti, R.; Pazzi, M.; Baiocchi, C. LC–high-resolution multiple stage spectrometric analysis of diuretic compounds: Unusual mass fragmentation pathways. J. Pharm. Biomed. Anal. **2008**, *48*, 462–466.
88. Medvedovici, A.; Sora, D.I.; Ionescu, S.; Hillebrand, M.; David, V. Characterization of a new norfloxacin metabolite monitored during a bioequivalence study by means of mass-spectrometry and quantum computation. Biomed. Chromatogr. **2008**, *22*, 1100–1107.
89. Scholz, K.; Dekant, W.; Volkel, W.; Pahler, A. Rapid detection and identification of N-Acetyl-L-Cysteine thioethers using constant neutral loss and theoretical multiple reaction monitoring combined with enhanced product-ion scans on a linear ion trap mass spectrometer. J. Amer. Soc. Mass Spectrom. **2005**, *16*, 1976–1984.
90. Mazzarino, M.; Turi, S.; Botre, F. A screening method for the detection of synthetic gluco-corticosteroids in human urine by liquid chromatography–mass spectrometry based on class-characteristic fragmentation pathways. Anal. Bioanal. Chem. **2008**, *390*, 1389–1402.
91. Burinsky, D.J.; Wang, F. Mass spectral characterization. Sep. Sci. Technol. **2004**, *5*, 249–299.
92. McLuckey, S.A.; Huang, T.-Y. Ion/ion reactions: New chemistry for analytical MS. Anal. Chem. **2009**, *81*, 8669–8676.

93. Bogusz, M.J.; Maier, R.D., Krüger, K.D.; Webb, K.S.; Romeril, J.; and Miller, M.L. Poor reproducibility of in-source collisional atmospheric pressure ionization mass spectra of toxicologically relevant drugs. J. Chromatogr. A **1999**, *844*, 409–418.
94. Halket, J.M.; Waterman, D.; Przyborowska, A.M.; Patel, R.K.P.; Fraser, P.D.; Bramley, P.M. Chemical derivatization and mass spectral libraries in metabolic profiling by GC/MS and LC/MS/MS. J. Experim. Botany **2005**, *56*, 219–243.
95. Chong, X.-M.; Hu, C.-Q. Compilation of an ESI-MS library of β-lactam antibiotics for rapid identification of drugs. Chromatographia **2008**, *68*, 759–766.
96. Lips, A.G.A.M.; Lameijer, W.; Fokkens, R.H.; Nibbering, N.M.M. Methodology for the development of a drug library based upon collision-induced fragmentation for the identification of toxicologically relevant drugs in plasma samples. J. Chromatogr. B **2001**, *759*, 191–207.
97. Weinmann, W.; Stoertzel, M.; Vogt, S.; Wendt, J. Tune compounds for electrospray ionisation/in-source collision-induced dissociation with mass spectral library searching. J. Chromatogr. A **2001**, *926*, 199–209.
98. Shin, Y.G.; van Breemen, R.B. Analysis and screening of combinatorial libraries using mass spectrometry. Biopharm. Drug Dispos. **2001**, *22*, 353–372.
99. Muller, C.; Schafer, P.; Stortzel, M.; Vogt, S.; Weinmann, W. Ion suppression effects in liquid chromatography–electrospray-ionisation transport-region collision induced dissociation mass spectrometry with different serum extraction methods for systematic toxicological analysis with mass spectra libraries. J. Chromatogr. B **2002**, *773*, 47–52.
100. Maurer, H.H.; Kratzsch, C.; Kraemer, T.; Peters, F.T.; Weber, A.A. Screening, library-assisted identification and validated quantification of oral antidiabetics of the sulfonylurea-type in plasma by atmospheric pressure chemical ionization liquid chromatography–mass spectrometry. J. Chromatogr. B **2002**, *773*, 63–73.
101. Lee, H.; Shen, S.; Grinberg, N. Identification and control of impurities for drug substance development using LC/MS and GC/MS. J. Liq. Chromatogr. Rel. Technol. **2008**, *31*, 2235–2252.
102. Norwood, D.L.; Jenke, D.; Manolescu, C.; Pennino, S.; Grinberg, N. HPLC and LC/MS Analysis of pharmaceutical container closure system leachables and extractables. J. Liq. Chromatogr. Rel. Technol. **2009**, *32*, 1768–1827.
103. Lee, H. Pharmaceutical applications of liquid chromatography coupled with mass spectrometry (LC/MS). J. Liq. Chromatogr. Rel. Technol. **2005**, *28*, 1161–1202.
104. Liu, H.-C.; Liu, R.H.; Ho, H.-O.; Lin, D.-L. Development of an information-rich LC-MS/MS database for the analysis of drugs in postmortem specimens. Anal. Chem. **2009**, *81*, 9002–9011.
105. Schuhmacher, J.; Zimmer, D.; Tesche, F.; Pickard, V. Matrix effects during analysis of plasma samples by electrospray and atmospheric pressure chemical ionization mass spectrometry: Practical approaches to their elimination. Rapid Commun. Mass Spectrom. **2003**, *17*, 1950–1957.
106. Jemal, M.; Xia, Y.-Q. The need for adequate chromatographic separation in the quantitative determination of drugs in biological samples by high performance liquid chromatography with tandem mass spectrometry. Rapid Commun. Mass Spectrom. **1999**, *13*, 97–106.
107. Hsieh, Y.; Chintala, M.; Mei, H.; Agans, J.; Brisson, J.-M.; Ng, K.; Korfmacher, W.A. Quantitative screening and matrix effect studies of drug discovery compounds in monkey plasma using fast-gradient liquid chromatography/tandem mass Spectrometry. Rapid Commun. Mass Spectrom. **2001**, *15*, 2481–2487.
108. Tiller, P.R.; Romanyshyn, L.A. Implications of matrix effects in ultra-fast gradient or fast isocratic liquid chromatography with mass spectrometry in drug discovery. Rapid Commun. Mass Spectrom. **2002**, *16*, 92–98.
109. Avery, M.J. Quantitative characterization of differential ion suppression on liquid chromatography/atmospheric pressure ionization mass spectrometric bioanalytical methods. Rapid Commun. Mass Spectrom. **2003**, *17*, 197–201.
110. van Hout, M.W.J.; Niederländer, H.A.G.; de Zeeuw, R.A.; de Jong, G.J. Ion suppression in the determination of clenbuterol in urine by solid-phase extraction atmospheric pressure chemical ionization ion-trap mass spectrometry. Rapid Commun. Mass Spectrom. **2003**, *17*, 245–250.

111. Müller, C.; Schäfer, P.; Störtzel, M.; Vogt, S.; Weinmann, W. Ion suppression effects in liquid chromatography–electrospray-ionization transport-region collision induced dissociation mass spectrometry with different serum extraction methods for systematic toxicological analysis with mass spectra libraries. J. Chromatogr. B **2002**, *773*, 47–52.
112. Matuszewski, B.K.; Constanzer, M.L.; Chavez-Eng, C.M. Matrix effect in quantitative LC/MS/MS analyses of biological fluids: A method for determination of finasteride in human plasma at picogram per milliliter concentrations. Anal. Chem. **1998**, *70*, 882–889.
113. Matuszewski, B.K.; Constanzer, M.L.; Chavez-Eng, C.M. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC−MS/MS. Anal. Chem. **2003**, *75*, 3019–3030.
114. Sojo, L.E.; Lum, G.; Chee, P. Internal standard signal suppression by co-eluting analyte in isotope dilution LC-ESI-MS. Analyst **2003**, *128*, 51–55.
115. Tong, X.S.; Wang, J.; Zheng, S.; Pivnichny, J.V.; Griffin, P.R.; Shen, X.; Donnelly, M.; Vakerich, K.; Nunes, C.; Fenyk-Melody, J. Effect of signal interference from dosing excipients on pharmacokinetic screening of drug candidates by liquid chromatography/mass spectrometry. Anal. Chem. **2002**, *74*, 6305–6313.
116. King, R.; Bonfiglio, R.; Fernandez-Metzler, C.; Miller-Stein, C.; Olah, T. mechanistic investigation of ionization suppression in electrospray ionization. J. Am. Soc. Mass Spectrom. **2000**, *11*, 942–950.
117. Sangster, T.; Spence, M.; Sinclair, P.; Payne, R.; Smith, C. Unexpected observation of ion suppression in a liquid chromatography/atmospheric pressure chemical ionization mass spectrometric bioanalytical method. Rapid Commun. Mass Spectrom. **2004**, *18*, 1361–1364.
118. Srinivas, N.R. Dodging matrix effects in liquid chromatography tandem mass spectrometric assays-compilation of key learnings and perspectives. Biomed. Chromatogr. **2009**, *23*, 451–454.
119. Mislanova, C.; Hutta, M. Role of biological matrices during the analysis of chiral drugs by liquid chromatography. J. Chromatogr. B **2003**, *797*, 91–109.
120. Skelly, J.P.; Swann, P.G.; Weiner, R. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharm. Res. **2007**, *24*, 1962–1973.
121. *Guidance for Industry, Bioanalytical Method Validation.* U.S. Department of Health and Human Services, FDA, CDER, CVM (2001).
122. Wieling, J. LC-MS-MS experiences with internal standards. Chromatographia **2002**, *55*, S107–S113.
123. Stokvis, E.; Rosing, H.; Beijnen, J.H. Stable isotopically labeled internal standards in quantitative bioanalysis using liquid chromatography/mass spectrometry: Necessity or not? Rapid Commun. Mass Spectrom. **2005**, *19*, 401–407.
124. Nilsson, L.B.; Eklund, G. Direct quantification in bioanalytical LC-MS/MS using internal calibration via analyte/stable isotope ratio. J. Pharm. Biomed. Anal. **2007**, *43*, 1094–1099.
125. Delahunty, T.; Bushman, L.; Robbins, B.; Fletcher, C.V. The simultaneous assay of tenofovir and emtricitabine in plasma using LC/MS/MS and isotopically labeled internal standards. J. Chromatogr. B **2009**, *877*, 1907–1914.
126. Bruce, S.J.; Tavazzi, I.; Parisod, V.; Rezzi, S.; Kochhar, S.; Guy, P.A. Investigation of human blood plasma sample preparation for performing metabolomics using ultrahigh performance liquid chromatography/mass spectrometry. Anal. Chem. **2009**, *81*, 3285–3296.
127. Wang, S.; Cyronak, M.; Yang, E. Does a stable isotopically labeled internal standard always correct analyte response? A matrix effect study on a lc/ms/ms method for the determination of carvedilol enantiomers in human plasma. J. Pharm. Biomed. Anal. **2007**, *43*, 701–707.
128. Hsieh, S.; Selinger, K. High-throughput bioanalytical method using automated sample preparation and liquid chromatography-atmospheric pressure ionspray mass spectrometry for quantitative determination of glybenclamide in human serum. J. Chromatogr. B **2002**, *772*, 347–356.
129. Lindegardh, N.; Annerberg, A.; White, N.J.; Day, N.P.J. Development and validation of a liquid chromatographic-tandem mass spectrometric method for determination of piperaquine in plasma: Stable isotope labeled internal standard does not always compensate for matrix effects. J. Chromatogr. B **2008**, *862*, 227–236.
130. Fierens, C.; Thienpont, L.M.R.; Stöckl, D.; Willekens, E.; De Leenheer, A.P. Quantitative analysis of urinary c-peptide by liquid chromatography–tandem mass spectrometry with a stable isotopically labelled internal standard. J. Chromatogr. A **2000**, *896*, 275–278.

131. Zhang, G.; Wujcik, C.E. Overcoming ionization effects through chromatography: A case study for the ESI-LC–MS/MS quantitation of a hydrophobic therapeutic agent in human serum using a stable-label internal standard. J. Chromatogr. B **2009**, *877*, 2003–2010.
132. Stokvis, E.; Rosing, H.; López-Lázaro, L.; Schellens, J.H.M.; Beijnen, J.H. Switching from an analogous to a stable isotopically labeled internal standard for the LC-MS/MS quantitation of the novel anticancer drug kahalalide f significantly improves assay performance. Biomed. Chromatogr. **2004**, *18*, 400–402.
133. Bergeron, A.; Furtado, M.; Garofolo, F. Importance of using highly pure internal standards for successful liquid chromatography/tandem mass spectrometric bioanalytical assays. Rapid Commun. Mass Spectrom. **2009**, *23*, 1987–1297.
134. Doyle, E.; McDowall, R.D.; Murkitt, G.S.; Picot, V.S.; Rogers, S.J. Two systems for the automated analysis of drugs in biological fluids using high-performance liquid chromatography. J. Chromatogr. B **1990**, *527*, 67–77.
135. Clavijo, C.; Bendrick-Peart, J.; Zhang, Y.L.; Johnson, G.; Gasparic, A.; Christians, U. An automated, highly sensitive LC-MS/MS assay for the quantification of the opiate antagonist naltrexone and its major metabolite 6β-Naltrexol in dog and human plasma. J. Chromatogr. B **2008**, *874*, 33–41.
136. Wells, D. *High Throughput Bioanalytical Sample Preparation: Methods and Automation Strategies, in Progress in Pharmaceutical and Biomedical Analysis*, Elsevier Science: Amsterdam, The Netherlands, 2003; 640.
137. Agasoster, T. Rasmussen, K.E. Fully automated high-performance liquid chromatographic analysis of whole blood and plasma samples using on-line dialysis as sample preparation: Determination of oxytetracycline in bovine and salmon whole blood and plasma. J. Chromatogr. B **1991**, *570*, 99–107.
138. Ba, B.B.; Nso, B.B.B.; Quentin, C.; Saux, M.-C. Determination of linezolid in growth media by high-performance liquid chromatography with on-line extraction. J. Chromatogr. B **2007**, *854*, 104–108.
139. Robinson, S.; Mcdonnell, S. An on-line extraction LC-MS/MS screen for the quantitative analysis of multiple classes of illicit drugs. Toxicol. Lett. **2008**, *180*, S162–S163.
140. Sadagopan, N.; Pabst, B.; Cohen, L. Evaluation of on-line extraction/mass spectrometry for in vivo cassette analysis. J. Chromatogr. B **2005**, *820*, 59–67.
141. Zeng, H.; Wu, J.-T.; Unger, S.E. The investigation and the use of high flow column-switching LC/MS/MS as a high-throughput approach for direct plasma sample analysis of single and multiple components in pharmacokinetic studies. J. Pharm. Biomed. Anal. **2002**, *27*, 967–982.
142. Rogatsky, E.; Braaten, K.; Cruikshank, Jayatillake, G.H.; Zheng, B.N.; Stein, D.T. Flow inconsistency: The evil twin of column switching—hardware aspects. J. Chromatogr. A **2009**, *1216*, 7721–7727.
143. Heinig, K., Bucheli, F. Application of column-switching liquid chromatography-tandem mass spectrometry for the determination of pharmaceutical compounds in tissue samples. J. Chromatogr. B **2002**, *769*, 9–26.
144. Choi, S.O.; Um, S.Y.; Jung, S.H.; Jung, S.J.; Kim, J.I.; Lee, H.J.; Chung, S.Y. Column-switching high-performance liquid chromatographic method for the determination of zaltoprofen in rat plasma. J. Chromatogr. B **2006**, *830*, 301–305.
145. Medvedovici, A.; Albu, F.; Georgita, C.; Sora, D.I.; Galaon, T.; Udrescu, S.; David, V. Achiral–chiral LC/LC–FLD coupling for determination of carvedilol in plasma samples for bioequivalence purposes. J. Chromatogr. B **2007**, *850*, 327–335.
146. Herman, J.L. Generic method for on-line extraction of drug substances in the presence of biological matrices using turbulent flow chromatography. Rapid Commun. Mass Spectrom. **2002**, *16*, 421–426.
147. Grant, R.P.; Cameron, C.; Mackenzie-McMurter, S. Generic serial and parallel on-line direct-injection using turbulent flow liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom. **2002**, *16*, 1785–1792.
148. Zimmer, D.; Pickard, V.; Czembor, W.; Müller, C. Comparison of turbulent-flow chromatography with automated solid-phase extraction in 96-well plates and liquid–liquid extraction used as plasma sample preparation techniques for liquid chromatography–tandem mass spectrometry. J. Chromatogr. A **1999**, *854*, 23–35.

149. Zhou, J.-L.; An, J.-J.; Li, P.; Li, H.-J.; Jiang, Y.; Cheng, J.-F. Two-dimensional turbulent flow chromatography coupled on-line to liquid chromatography–mass spectrometry for solution-based ligand screening against multiple proteins. J. Chromatogr. A **2009**, *1216*, 2394–2403.
150. Turnpenny, P.; Fraier, D.; Chassaing, C.; Duckworth, J. Development of a μ-turbulent flow chromatography focus mode method for drug quantitation in discovery bioanalysis. J. Chromatogr. B **2007**, *856*, 131–140.
151. Edge, T. Turbulent flow chromatography in bioanalysis, Ch. 4, in *Handbook of Analytical Separations*; Wilson, I. D. Ed.; Elsevier: Amsterdam, The Netherlands, 2003; Vol. 6 (Bioanalytical Separations), 91–128.
152. Krebber, R.; Hoffend, F.-J.; Ruttmann, F. Simple and rapid determination of enrofloxacin and ciprofloxacin in edible tissues by turbulent flow chromatography/tandem mass spectrometry (TFC–MS/MS). Anal. Chim. Acta **2009**, *637*, 208–213.
153. Verdirame, M.; Veneziano, M.; Alfieri, A.; Di Marco, A.; Monteagudo, E.; Bonelli, F. Turbulent flow chromatography TFC-tandem mass spectrometry supporting in vitro/vivo studies of NCEs in high throughput fashion. J. Pharm. Biomed. Anal. **2010**, *51*, 834–841.
154. Ceglarek, U.; Lembcke, J.; Fiedler, G.M.; Werner, M.; Witzigmann, H.; Hauss, J.P.; Thiery, J. Rapid simultaneous quantification of immunosuppressants in transplant patients by turbulent flow chromatography combined with tandem mass spectrometry. Clin. Chim. Acta **2004**, *346*, 181–190.
155. Ynddal, L.; Hansen, S.H. On-line turbulent-flow chromatography–high-performance liquid chromatography–mass spectrometry for fast sample preparation and quantitation. J. Chromatogr. A **2003**, *1020*, 59–67.
156. Vintiloiu, A.; Mullett, W.M.; Papp, R.; Lubda, D.; Kwong, E. Combining restricted access material (RAM) and turbulent flow for the rapid on-line extraction of the Cyclooxygenase-2 inhibitor rofecoxib in plasma samples. J. Chromatogr. A **2005**, *1082*, 150–157.
157. Kousoulos, C.; Dotsikas, Y.; Loukas, Y.L. Turbulent flow and ternary column-switching on-line clean-up system for high-throughput quantification of risperidone and its main metabolite in plasma by LC–MS/MS: application to a bioequivalence study. Talanta **2007**, *72*, 360–367.
158. Smalley, J.; Marino, A.M.; Xin, B.; Olah, T.; Balimane, P.V. Development of a quantitative LC–MS/MS analytical method coupled with turbulent flow chromatography for Digoxin for the in vitro P-gp inhibition assay. J. Chromatogr. B **2007**, *854*, 206–267.
159. Hlushkou, D.; Tallarek, U. Transition from creeping via viscous-inertial to turbulent flow in fixed beds. J. Chromatogr. A **2006**, *1126*, 70–85.
160. Zeng, W.; Musson, D.G.; Fisher, A.L.; Chen, L.; Schwartz, M.S.; Woolf, E.J.; Wang, A.Q. Determination of sitagliptin in human urine and hemodialysate using turbulent flow online extraction and tandem mass spectrometry. J. Pharm. Biomed. Anal. **2008**, *46*, 534–542.
161. Xu, Y.; Willson, K.J.; Musson, D.G. Strategies on efficient method development of on-line extraction assays for determination of MK-0974 in human plasma and urine using turbulent-flow chromatography and tandem mass spectrometry. J. Chromatogr. B **2008**, *863*, 64–73.
162. Papp, R.; Mullett, W.M.; Kwong, E. A method for the direct analysis of drug compounds in plasma using a single restricted access material (RAM) column. J. Pharm. Biomed. Anal. **2004**, *36*, 457–464.
163. Souverain, S.; Rudaz, S.; Veuthey, J.-L. Restricted access materials and large particle supports for on-line sample preparation: An attractive approach for biological fluids analysis. J. Chromatogr. B **2004**, *801*, 141–156.
164. Chiap, P.; Piette, M.; Evrard, B.; Frankenne, F.; Christiaens, B.; Piel, G.; Cataldo, D.; Foidart, J.-M.; Delattre, L.; Crommen, J.; Hubert, Ph. Automated method for the determination of a new matrix metalloproteinase inhibitor in ovine plasma and serum by coupling of restricted access material for on-line sample clean-up to liquid chromatography. J. Chromatogr. B **2005**, *817*, 109–117.
165. Heinig, K.; Bucheli, F. Application of column-switching liquid chromatography-tandem mass spectrometry for the determination of pharmaceutical compounds in tissues samples. J. Chromatogr. B **2002**, *769*, 9–26.
166. Boss, K.-S.; Rudolphi, A.; Vielhauer, S.; Walfort, A.; Lubda, D.; Eisenbeiß, F. Alkyl-diol silica (ADS): restricted access precolumn packings for direct injection and coupled-column chromatography of biofluids. Fresenius J. Anal. Chem. **1995**, *352*, 684–690.

167. Chiap, P.; Rbeida, O.; Christiaens, B.; Hubert, P.; Lubda, D.; Boss, K.-S.; Crommen, J. Use of a novel cation-exchange restricted-access material for automated sample clean-up prior to the determination of basic drugs in plasma by liquid chromatography. J. Chromatogr. A **2002**, *975*, 145–155.
168. Yoshiaki, S.; Eiichi, Y.; Susumu, T.; Takashi, K.; Naok, A. Evaluation of a weak cation exchange restricted access material for on-line purification of basic compounds in plasma. Chromatography **2006**, *27*, 83–84.
169. Deng, L.Q.; Lun, Z.H.; Yan, C.; Gao, R.Y. Preparation and evaluation of molecularly imprinted monolithic column for felodipine in micro-liquid chromatography. Chinese Chem. Lett. **2005**, *16*, 955–958.
170. Boss, K.-S.; Fleischer, C.T. Multidimensional on-line solid-phase extraction (SPE) using restricted access materials (RAM) in combination with molecular imprinted polymers (MIP). Fresenius J. Anal. Chem. **2001**, *371*, 16–20.
171. Pinkerton, T.C. High-performance liquid chromatography packing materials for the analysis of small molecules in biological matrices by direct injection. J. Chromatogr. **1991**, *544*, 13–23.
172. Baeyens, W.R.G.; Van der Weken, G.; Haustraete, J.; Aboul-Enein, H.Y.; Corveleyn, S.; Remon, J.P.; Garca-Campana, A.M.; Deprez, P. Application of the restricted-access precolumn packing material Alkyl-Diol silica in a column-switching system for the determination of ketoprofen enantiomers in horse plasma. J. Chromatogr. A **2000**, *871*, 153–161.
173. Amini, N.; Crescenzi, C. Feasibility of an on-line restricted access material/liquid chromatography/tandem mass spectrometry method in the rapid and sensitive determination of organophosphorus triesters in human blood plasma. J. Chromatogr. B **2003**, *795*, 245–256.
174. Borlak, J.; Walles, M.; Levsen, K. Application of restricted access material (RAM) with precolumn-switching and matrix solid-phase dispersion (MSPD) to the study of the metabolism and pharmacokinetics of verapamil. Anal. Bioanal. Chem. **2002**, *374*, 1179–1186.
175. Sora, I.; Galaon, T.; Udrescu, S.; Negru, J.; David, V.; Medvedovici, A. Fast RPLC-UV method on short sub-two microns particles packed column for the assay of tenoxicam in plasma samples. J. Pharm. Biomed. Anal. **2007**, *43*, 1437–1443.
176. Albu, F.; Georgita, C.; Tache, F.; Mutihac, L.; Medvedovici, A.; David, V. Considerations on MS/MS detection of bromazepam after liquid chromatographic separation from plasma samples: Application to a bioequivalence study. J. Liq. Chromatogr. R. T. **2007**, *30*, 2699–2715.
177. Albu, F.; Georgita, C.; David, V.; Medvedovici, A. Determination of glibenclamide in human plasma by liquid chromatography and atmospheric pressure chemical ionization/ms-ms detection. J. Chromatogr. B **2007**, *846*, 222–229.
178. Lavagnini, I.; Magno, F. A Statistical overview on univariate calibration, inverse regression, and detection limits: Application to gas chromatography/mass spectrometry Technique. Mass Spectrom. Reviews **2007**, *26*, 1–18.
179. Bolton, S.; Bon, C. *Pharmaceutical Statistics: Practical and Clinical Applications*, 4th Ed.; Marcel Dekker: New York, U.S.A., 2004; 755.
180. Massart, D.L.; Vandeginste, B.G.M.; Morgan, S.N.; Michotte, Y.; Kaufman, L. *Chemometrics: A Textbook*, Elsevier: Amsterdam, The Netherlands, 1988; 500.
181. Brueggemann, L.; Morgenstern, P.; Wenrich, R. Comparison of regression techniques for linear calibration. Accred. Qual. Assur. **2005**, *10*, 344–351.
182. Garden, J.S., Mitchell, D.G.; Mills, W.N. Non-constant variance regression techniques for calibration-curve-based analysis. Anal. Chem. **1980**, *52*, 2310–2315.
183. Shapiro, S.S.; Wilk, M.B. An analysis of variance for normality (complete samples). Biometrika **1965**, *52*, 591–611.
184. Mocak, J.; Bond, A.M.; Mitchell, S.; Scollary, G. A statistical overview of standard (IUPAC and ACS) and new procedures for determining the limits of detection and quantification: Application to voltametric stripping techniques. Pure Appl. Chem. **1997**, *69*, 297–328.
185. Vial, J.; Jardy, A. Experimental comparison of the different approaches to estimate LOD and LOQ of an HPLC method. Anal. Chem. **1999**, *71*, 2672–2677.
186. Schwartz, L.M. Calibration curves with non-uniform variance. Anal. Chem. **1979**, *51*, 723–727.

187. Saini, G.S.; Wani, T.A.; Gautam, A.; Varshney, B.; Ahmed, T.; Rajan, K.S.; Pillai, K.K.; Paliwal, J.K. Validation of the LC-MS/MS method for the quantification of mevalonic acid in human plasma and determination of the matrix effect. J. Lipid Research **2006**, *47*, 2340–2345.
188. Roy, S.M.N.; Mangaonkar, K.V.; Yetal, S.M.; Joshi, S.S. LC-MS-MS method for determination of metolazone in human plasma. E-J. Chem. **2008**, *5*, 634–640.
189. Cesari, N.; Fontana, S.; Montanari, D.; Braggio, S. Development and validation of a high-throughput method for the quantitative analysis of D-amphetamine in rat blood using liquid chromatography/MS^3 on a hybrid triple quadrupole-linear ion trap mass spectrometer and its application to a pharmacokinetic study. J. Chromatogr. B **2010**, *878*, 21–28.
190. Kousoulos, C.; Tsatsou, G.; Dotsikas, Y.; Apostolou, C.; Loukas, Y.L. Validation of a fully automated high throughput liquid chromatographic/tandem mass spectrometric method for roxithromycin quantification in human plasma: Application to a bioequivalence study. Biomed. Chromatogr. **2008**, *22*, 494–501.
191. Sreenivas, N.; Narasu, M.L.; Shankar, B.P.; Mullangi, R. Development and validation of a sensitive LC-MS/MS method with electrospray ionization for quantitation of doxofylline in human serum: application to a clinical pharmacokinetic study. Biomed. Chromatogr. **2008**, *22*, 654–661.
192. Jain, L.; Gardner, E.R.; Venitz, J.; Dahut, W.; Figg, W.D. Development of a rapid and sensitive LC-MS/MS assay for the determination of sorafenib in human plasma. J. Pharm. Biomed. Anal. **2008**, *46*, 362–367.
193. Chen, X.; Gardner, E.R.; Price, D.K.; Figg, W.D. Development and validation of an LC-MS assay for finasteride and its application to prostate cancer prevention trial sample analysis. J. Chromatogr. Sci. **2008**, *46*, 356–361.
194. Murphy, C.M.; Huestis, M.A. LC–ESI-MS/MS analysis for the quantification of morphine, codeine, morphine-3-*β*-D-glucuronide, morphine-6-*β*-D-glucuronide, and codeine-6-*β*-D-glucuronide in human urine. J. Mass Spectrom. **2005**, *40*, 1412–1416.
195. Yao, M.; Srinivas, N. Liquid chromatography/mass spectrometry for the quantitation of muraglitazar in monkey plasma. Biomed. Chromatogr. **2006**, *20*, 1017–1023.
196. Dams, R., Murphy, C.M.; Lambert, W.E.; Huestis, M.A. Urine drug testing for opioids, cocaine, and metabolites by direct injection liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom. **2003**, *17*, 1665–1670.
197. Sinnaeve, B.A.; Storme, M.L.; Van Bocxlae, J.F. Capillary liquid chromatography and tandem mass spectrometry for the quantification of enkephalins in cerebrospinal fluid. J. Sep. Sci. **2005**, *28*, 1779–1784.
198. Song, N.; Zhang, S.; Lib, Q.; Liu, C. Liquid chromatographic/mass spectrometry assay of bromotetrandrine in rat plasma and its application to pharmacokinetic study. Biomed. Chromatogr. **2009**, *23*, 623–629.
199. Patel, B.N., Sharma, N., Sanyal, M.; Shrivastav, P.S. Simultaneous determination of simvastatin and simvastatin acid in human plasma by LC-MS/MS without polarity switch: Application to bioequivalence study. J. Sep. Sci. **2008**, *31*, 301–313.
200. Morita, J.B.; Berton, D.; Boldin, R.; Bowos, F.A.; Meurer, E.C.; Amaranta, A.R.; Campos, D.R.; Calatatti, S.A.; Pereira, R.; Abib, E.; Pedrazolli, J. Determination of levocetirizine in human plasma by liquid chromatography–electrospray tandem mass spectrometry: Application to a bioequivalence study. J. Chromatogr. B **2008**, *862*, 132–139.
201. Xu, D.H.; Lou, H.G.; Yuan, H.; Jiang, B.; Zhou, Q.; Zhang, Z.M.; Ruan, Z.R. Quantitative determination of domperidone in human plasma by ultraperformance liquid chromatography with electrospray ionization tandem mass spectrometry. Biomed. Chromatogr. **2008**, *22*, 433–440.
202. Tarinas, A.; Tápanes, R.D.; Ferrer, G.; Pérez, J. Validation of high performance liquid chromatography methods for determination of zidovudine, stavudine, lamivudine and indinavir in human plasma. Farm. Hospital. **2007**, *31*, 243–247.
203. Mistri, H.N.; Jangid, A.G.; Shrivastav, P.S. Liquid chromatography tandem mass spectrometry method for simultaneous determination of antidiabetic drugs metformin and glyburide in human plasma. J. Pharm. Biomed. Anal. **2007**, *45*, 97–106.
204. Georgita, C.; Albu, F.; David, V.; Medvedovici, A. Nonlinear calibrations on the assay of dilitiazem and two of its metabolites from plasma samples by means of liquid chromatography and ESI/MS^2 detection: Application to a bioequivalence study. Biomed. Chromatogr. **2008**, *22*, 289–297.

205. Singtoroj, T.; Tarning, J.; Annerberg, A.; Ashton, M.; Bergqvist, Y.; White, N.J.; Lindergardh, N.; Day, N.P. A new approach to evaluate regression models during validation of bioanalytical assays. J. Pharm. Biomed. Anal. **2006**, *41*, 219–227.
206. Tarning, J.; Singtoroj, T.; Annerberg, A.; Ashton, M.; Bergqvist, Y.; White, N.Y.; Day, N.P.; Lindegardh, N. Development and validation of an automated solid phase extraction and liquid chromatographic method for the determination of piperaquine in urine. J. Pharm. Biomed. Anal. **2006**, *41*, 213–218.
207. Szabo, G.K.; Browne, H.K.; Ajami, A.; Josephs, E.G. Alternatives to least square regression analysis for computation of standard curves for quantitation by high performace liquid chromatography: Application to clinical pharmacology. J. Clin. Pharmacol. **1994**, *34*, 242–249.
208. Karnes, H.T.; March, C. Calibration and validation of linearity in chromatographic biomedical analysis. J. Pharm. Biomed. Anal. **1991**, *9*, 911–918.
209. Srinivas, N.R. Sensitivity enhancement in tandem liquid chromatographic mass spectrometric assays by summation of two transition ion pairs – perspectives. J. Sep. Sci. **2009**, *32*, 483–486.
210. Srinivas, N.R. Applicability of nonlinear calibration regression for quantitative determination of parent and metabolite(s) in bioequivalence assessment. Biomed. Chromatogr. **2008**, *22*, 1315–1317.
211. Yang, L.; Wu, N.; Rudewicz, P.J. Applications of new liquid chromatography-tandem mass spectrometry technologies for drug development support. J. Chromatogr. A **2001**, *926*, 43–55.
212. Carter, P. Preparation of ligand-free human serum for radioimmunoassay by adsorption on activated charcoal. Clin. Chem. **1978**, *24*, 362–364.
213. Li, W.; Cohen, L.H. Quantitation of endogenous analytes in biofluids without a true blank matrix. Anal. Chem. **2003**, *75*, 5854–5859.
214. Jemal, M. High-throughput quantitative bioanalysis by LC/MS/MS. Biomed. Chromatogr. **2000**, *14*, 422–429.
215. Jemal, M.; Xia, Y.-Q. LC-MS development strategies for quantitative bioanalysis. Curr. Drug Metabolism **2006**, *7*, 491–502.
216. Xu, F.; Zou, L.; Lin, Q.; Ong, C.N. Use of liquid chromatography/tandem mass spectrometry and online databases for identification of phosphocholines and lysophosphatidylcholines in human red blood cells. Rapid Commun. Mass Spectrom. **2009**, *23*, 3243–3254.
217. Medvedovici, A.; Lazou, K.; d'Oosterlinck, A.; Zhao, Y.; Sandra, P. Analysis of jojoba oil by LC-coordination ion spray-MS (LC-CIS-MS). J. Sep. Sci. **2002**, *25*, 173–178.
218. Sandra, P.; Medvedovici, A.; David, F. Comprehensive pSFC × pSFC-MS for the characterization of triglycerides in vegetable oils. LC-GC Europe **2003**, *16*, 32–34.
219. Wujcik, C.E.; Kadar, E.P. Reduction of in-source collision-induced dissociation and thermolysis of sulopenem prodrugs for quantitative liquid chromatography/electrospray ionization mass spectrometric analysis by promoting sodium adduct formation. Rapid Commun. Mass Spectrom. **2008**, *22*, 3195–3206.
220. Garofolo, F.; Bradley, T.; Taillon, M.P.; Latour, S.; Michon, J.; Côté, C.S. Use of ion adducts to increase selectivity and sensitivity in LC-MS/MS. The AAPS Journal. **2008**, *10* (S2).
221. Van Aerden, C.; Debrauwer, L.; Paris, A.; Tabet, J.C. Analysis of nucleoside-estrogen adducts by LC-ESI-MS-MS. Analyst **1998**, *123*, 2677–2680.
222. Mortier, K.A.; Zhang, G.-F.; Van Peteghem, C.H.; Lambert, W.E. Adduct formation in quantitative bioanalysis: Effect of ionization conditions on paclitaxel. J. Am. Soc. Mass Spectrom. **2004**, *15*, 585–592.
223. Zhao, J.J.; Yang, A.Y.; Douglas Rogers, J. Effects of liquid chromatography mobile phase buffer contents on the ionization and fragmentation of analytes in liquid chromatographic/ionspray tandem mass spectrometric determination. J. Mass Spectrom. **2002**, *37*, 421–433.
224. Giancotti, V.; Medana, C.; Aigotti, R.; Pazzi, M.; Baiocchi, C. LC-high-resolution multiple stage spectrometric analysis of diuretic compounds: Unusual mass fragmentation pathways. J. Pharm. Biomed. Anal. **2008**, *48*, 462–466.
225. Schug, K.; McNair, H.M. Adduct formation in electrospray ionization. Part 1: Common acidic pharmaceuticals. J. Sep. Sci. **2002**, *25*, 759–766.
226. Eichhorn, P.; Knepper, T.P. Electrospray ionization mass spectrometric studies on the amphoteric surfactant cocamidopropylbetaine. J. Mass Spectrom. **2001**, *36*, 677–684.

227. Volmer, D.A.; Lock, C.M. Electrospray ionization and collision-induced dissociation of antibiotic polyether ionophores. Rapid Commun. Mass Spectrom. **1998**, *12*, 157–164.
228. Poquette, M.A.; Lensmeyer, G.L.; Doran, T. C. Effective use of liquid chromatography-mass spectrometry (LC/MS) in the routine clinical laboratory for monitoring sirolimus, tacrolimus, and cyclosporine. Ther. Drug Monitor. **2005**, *27*, 144–150.
229. Annesley, T.M. Methanol-associated matrix effects in electrospray ionization tandem mass spectrometry. Clin. Chem. **2007**, *53*, 1827–1834.
230. Warrack, B.M., DiDonato, G.C. Ion spray liquid chromatographic/mass spectrometric characterization of bile acids. Biol. Mass Spectrom. **1993**, *22*, 101–111.
231. Yamaguchi, J.; Matsuno, Y.; Hachiuma, K.; Ogawa, N.; Higuchi, S. A Strategy for quantitative bioanalysis of non-polar neutral compounds by liquid chromatography/electrospray ionization tandem mass spectrometry: Determination of TS-962, a novel acyl-coa:Cholesterol acyltransferase inhibitor, in rabbit aorta and liver tissues. Rapid Commun. Mass Spectrom. **2001**, *15*, 629–636.
232. Cai, S.S.; Syage, J.A. Comparison of atmospheric pressure photoionization, atmospheric pressure chemical ionization, and electrospray ionization mass spectrometry for analysis of lipids. Anal. Chem. **2006**, *78*, 1191–1199.
233. Hughes, N.C.; Wong, E.Y.K.; Fan, J.; Bajaj, N. Determination of carryover and contamination for mass spectrometry – based chromatographic assays. The AAPS Journal **2007**, *9*, Article 42.
234. Clouser-Roche, A.; Johnson, K.; Fast, D.; Tang, D. Beyond pass/fail: A Procedure for evaluating the effect of carryover in bioanalytical LC/MS/MS methods. J. Pharm. Biomed. Anal. **2008**, *47*, 146–155.
235. Wei, Z.; Musson, D.G.; Fisher A.L.; Qiu W.A. A new approach for evaluating carryover and its influence on quantitation in high-performance liquid chromatography and tandem mass spectrometry assay. Rapid Commun. Mass Spectrom. **2006**, *20*, 635–640.
236. Wang, H.; Hanash, S.M. Increased throughput and reduced carryover of mass spectrometry-based proteomics using a high-efficiency nonsplit nanoflow parallel dual-column capillary HPLC system. J. Proteome Res. **2008**, *7*, 2743–2755.
237. Mitulović, G.; Stingl, C.; Steinmacher, I.; Hudecz, O.; Hutchins, J. R.; Peters, J.M.; Mechtler, K. Preventing carryover of peptides and proteins in nano LC-MS separations. Anal. Chem. **2009**, *81*, 5955–5960.
238. Hodgin, J.C.; Thurmond, M. Improved LC/MS dynamic range through carryover elimination. LC GC North America **2003**, *2*, 53–54.
239. Vallano, P.T.; Shugarts, S.B.; Woolf, E.J.; Matuszewski, B.K. Elimination of autosampler carryover in a bioanalytical HPLC-MS/MS method: a case study. J. Pharm. Biomed. Anal. **2005**, *36*, 1073–1078.
240. Yoshiki, A.; Chinami, O.; Katsunobu, O.; Shoji, K.; Naoki, A. Reduction of carry-over in column-switching HPLC/MS system with automated system washing procedure for highly sensitive direct analysis of Donepezil in dog plasma. Chromatography **2006**, *27*, 85–88.
241. Weng, N.; Halls, T.D.J. Systematic troubleshooting for LC/MS/MS. Pharm. Technol. **2002**, *3*, 102–120.
242. Briscoe, C.J.; Stiles, M.R.; Hage, D.S. System suitability in bioanalytical LC/MS/MS. J. Pharm. and Biomed. Anal. **2007**, *44*, 484–491.
243. Jones, A.B. Bioanalytical quality assurance: Concepts and concerns. Qual. Assur. J. **2006**, *10*, 101–106.