AN OVERVIEW OF MATRIX EFFECTS IN LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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Matrix-dependent signal suppression or enhancement represents a major drawback in quantitative analysis with liquid chromatography coupled to atmospheric pressure ionization mass spectrometry (LC–API-MS). Because matrix effects (ME) might exert a detrimental impact on important method parameters (limit of detection, limit of quantification, linearity, accuracy, and precision), they have to be tested and evaluated during validation procedure. This review gives a detailed description on when these phenomena might be expected, and how they can be evaluated. The major sources of ME are discussed and illustrated with examples from bioanalytical, pharmaceutical, environmental, and food analysis. Because there is no universal solution for ME, the main strategies to overcome these phenomena are described in detail. Special emphasis is devoted to the sample-preparation procedures as well as to the recent improvements on chromatographic and mass spectrometric conditions. An overview of the main calibration techniques to compensate for ME is also presented. All these solutions can be used alone or in combination to retrieve the performance of the LC–MS for a particular matrix–analyte combination. © 2010 Wiley Periodicals, Inc., Mass Spec Rev 30:491–509, 2011

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I. INTRODUCTION

Liquid chromatography in combination with atmospheric pressure ionization mass spectrometry (LC–API-MS) is a well-established analytical tool in many fields of application. Life-science progresses have benefited enormously from this technique. Also, new applications of environmental, food safety, and homeland security interest have seen an increased use of API science progresses have benefited enormously from this established analytical tool in many fields of application. Life-pressure ionization mass spectrometry (LC–API-MS) is a well

II. MECHANISTIC STUDIES

The processes that lead to ME in LC–MS are not fully understood, but they are probably due to the influence of co-eluting compounds on the analyte ionization. Whereas there are many hypotheses to explain the processes that lead to ion
suppression, information regarding the signal enhancement are still lacking. Tang and Kebarle (1993) were the first authors who demonstrated how the analyte signal in ESI-MS can be strongly influenced by the concomitant presence of other electrolytes in the electrosprayed solution. Many different mechanisms of ion suppression have been proposed; most are specific to the ionization technique used. In fact, although this phenomenon can have potential deleterious effects on ESI and APCI, evidence indicates that the ESI interface is more likely to be affected (Bruins et al., 1999; King et al., 2000; Matuszewsky, Constanzer, & Chavez-Eng, 2003). As widely reviewed in literature (Cole, 2000; Kebarle, 2000; Cech & Enke, 2001), the ESI process that leads to the formation of GP ions can be subdivided into various stages: (a) addition of a charge to the analyte in the liquid phase (LP); (b) production of the charged droplets at the ESI capillary tip through the Taylor cone formation; (c) evolution of the charged droplets due to solvent evaporation and droplet fission; and (d) formation of the GP ions by the charge residue model (CRM) (Dole et al., 1968) or the ion-evaporation model (IEM) (Iribarne & Thompson, 1976). Any event that might decrease the production rate of the droplets, and ultimately the formation of the GP ions, could participate in signal suppression. For this reason, mechanisms that lead to a decreased ionization efficiency can easily be subdivided into two different groups: suppression processes that occur in the LP and in the GP.

With the use of a dual ESI–APCI source and a dual-sprayer ESI source, King and co-workers demonstrated that the major contribute to signal suppression is due to the LP processes (King et al., 2000). Co-eluting compounds can interfere with the analyte signal in the LP by following four different mechanisms, which are deeply dependent on their physico-chemical properties:

1. A first mechanism responsible for ME in the LP is based on a competition for the available charges and the on access to the droplet surface between the analyte and the matrix components. Literature results demonstrate that, at high analyte concentration (>10^{-3} M), the linearity of the ESI response is often lost (Ikonomou, Blades, & Kebarle, 1990). This loss can be due to a limited amount of excess charge available on the ESI droplets, or to saturation of the ESI droplets with analyte at their surfaces, thus to inhibit ejection of an ion trapped inside the droplets. Some physico-chemical properties such as surface activity and polarity are essential to establish if an interfering compound would compete with the target analyte for the limited charge or space on the surface droplet (Cech & Enke, 2000; Zhou & Cook, 2001). In biological matrices, where large amounts of endogenous compounds with very high basicities and surface activities are present, the limit concentration of 10^{-3} M is reached quickly and ion suppression occurs (Bruins et al., 1999).

2. The presence of interfering compounds at high concentration can increase the viscosity and the surface tension of the droplets to change the efficiency of their formation and evaporation, which, in turn, affects the amount of charged ions in the GP that ultimately reaches the mass analyzer (King et al., 2000; Mallet, Lum, & Mazzeo, 2004).

3. To compare the performances of ESI-MS and APCI-MS, King and co-workers also demonstrated that non-volatile additives, such as ammonium sulfate, can be responsible for signal suppression through the formation of solid analyte-inclusion particles (King et al., 2000).

4. A last possible mechanism occurs when matrix components or mobile-phase additives act as ion-pairing reagents with the pre-formed analyte ions (Eshraghi & Chowdhury, 1993; Apffel et al., 1995; Gustavsson et al., 2001; Zhou & Cook, 2001; Holčapek et al., 2004).

The mechanisms responsible for ion suppression are various in the GP. The analyte can be transferred into the GP as an ion, or as a part of a charged solvent cluster. Once in the GP the charge can be lost through neutralization reactions or charge transfer due to the presence of interfering compounds or solvents with high GP basicity (Tang & Kebarle, 1993; Amad et al., 2000; Cole, 2000; King et al., 2000).

It has been suggested that APCI will not suffer from the same ion-suppression problems as those encountered with ESI (Barnes et al., 1995; Matuszewsky, Constanzer, & Chavez-Eng, 1998, 2000; King et al., 2000; Schuhmacher et al., 2003; Vanderford et al., 2003; Ching, Zhang, & Karna, 2004). However, some studies on bioanalytical and environmental samples have demonstrated that APCI is not completely free from ME (Li et al., 2003; Mei et al., 2003; Sangster et al., 2004; Zhao & Metcalfe, 2008). The main reason why APCI is sometimes less prone to ME than ESI can be found in the way each technique produces the charged analyte. In ESI, an analyte is ionized in the LP and transferred into the GP as a charged molecule. In APCI, the analyte is transferred into the GP as a neutral molecule, and ionization occurs in a second step by chemical ionization of the GP analyte (Sunner, Nicol, & Kebarle, 1988). As a result, most of the mechanisms that lead to a suppression in the ESI interface are not present in the APCI interface. The suppression phenomena related to APCI are not fully understood, but two main hypotheses can be formulated. A first mechanism can be found in the formation of solid precipitates due to the presence of non-volatile sample components (King et al., 2000). Sangster and co-workers suggest that differences in electron affinity between compounds in the GP can modify the efficiency of charge transfer from the corona-discharge needle (Sangster et al., 2004).

As it can be deducted from this literature overview, although there are many hypotheses to explain the processes that lead to ion suppression, information regarding the signal enhancement are still lacking. According to the equilibrium-partitioning model, it might depend on analyte’s relative affinity for the droplet surface (Cech & Enke, 2000). It is also a good practice to check the proper selection of the ions for the selected reaction monitoring (SRM) program. In fact, depending on fragmentation, ions at specific m/z values can overlap to cause signal enhancement. Overlapping can be easily detected with a simple blank run and eliminated by choosing a different combination of m/z values in the SRM program.

Understanding the mechanisms responsible for ME in ESI and APCI represents a fundamental step in the identification of the matrix constituents that can be a potential source of ME during the development of an LC–API-MS method. A wide range of molecules can lead to ME, especially if they are present at high concentration in the extract. In a recent publication, Antignac et al. (2005) suggested to subdivide the interference substances into two groups. A first group is represented by the so-called “endogenous suppressors,” which are constituted by the substances originally present in the matrix and retrieved in the
final extract. This group includes salts, highly polar compounds, surfactants, and various organic molecules such as carbohydrates, amines, lipids, peptides, or metabolites with a chemical structure close to the target analyte.

The “exogenous suppressors” represent the second source of ME: they are interfering compounds not originally present in the sample matrix, but coming from various external sources during the method development (sample preparation and chromatography). These groups of suppressors can include plastic and polymer residues, phthalates, ion pairing reagents, organic acids, buffers, calibration products, or materials released by the solid-phase extraction (SPE) cartridge (Eshraghi & Chowdhury, 1993; Gustavsson et al., 2001; Mei et al., 2003; Mallet, Lu, & Mazzeo, 2004; Antignac et al., 2005).

It is important to point out that the chemical nature of the individual compounds is a key factor to make an analyte a candidate or a source of ion suppression, or to make one compound a source of ion suppression for another. Bonfiglio et al. (1999) investigated ME in the analysis of four drugs with different polarities and demonstrated that the most-polar compounds were more affected by ion suppression. This result was confirmed in more recent publications (Müller et al., 2002; Liang et al., 2003; Naidong, 2003; Souverain, Rudaz, & Veuthey, 2004). It has also been shown that small molecular weight compounds are more prone to ME (Kloepfer, Quintana, & Reemtsma, 2005). It has been demonstrated that hydrophilic analytes are more easily suppressed when surface-active contaminants are present in the matrix. An exhaustive explanation of this phenomenon is given by the equilibrium partition model developed by Enke and co-workers that uses relative affinities for droplet surfaces to describe differences in response (Enke, 1997; Cech & Enke, 2000). This model postulates that two separate phases exist in the ESI droplet. Excess charge produced in the ESI process resides on the surface of the droplet. The phase of the droplet interior is neutral and consists of solvent molecules, electrolytes, and of the more polar analytes which are neutralized by counterions. Because the excess charge exists on the droplet surface, the ESI response is deeply related to the surface activity of the target analytes. As a consequence, compounds that can be most successfully analyzed by ESI-MS have both polar and non-polar portions. The polar regions are subjected to charge formation, either through protonation or adduct formation, whereas the non-polar portions are responsible for increasing the fraction of the analyte molecules that reside on the droplet surface. The application of the partitioning model for the analysis of a mixture of peptides with different polarities demonstrates that the more polar peptides remain in the droplet interior, where they are neutralized by counterions. Peptides with non-polar chains are contained on the droplet surface where these chains can be desolvated. Because the excess charge resides on the droplet surface, these peptides have a higher response and suppress the signal of the more polar peptides in the droplet interior (Cech & Enke, 2000).

III. EVALUATION OF ME

The first attempts to evaluate ME and to estimate their influence in method performance can be found in the pioneering research of the groups of Buhrman (Buhrman, Price, & Rudewicz, 1996), King (Bonfiglio et al., 1999), and Matuszewsky (Fu, Woolf, & Matuszewsky, 1998; Matuszewsky, Constanzer, & Chavez-Eng, 1998). In these publications, two main strategies were proposed to estimate the degree of ME on a LC–MS method: post-extraction addition and post-column infusion.

In post-extraction addition, the signal of the analyte in a standard solution is compared to that of a post-extraction spiked sample at the same concentration (matrix-matched standard) (Fig. 1a). Any difference in response indicates either ion suppression or enhancement. Buhrman et al. were the first to use the post-extraction addition for the comparison of two different sample pre-treatment methods [SPE and hexane liquid–liquid extraction (LLE)] in the analysis of the platelet-activating factor receptor antagonist SR 27417 in human plasma (Buhrman, Price, & Rudewicz, 1996). In this study, ion suppression was estimated using the following formula:

\[
\text{ME(%) = } 100 - \frac{B}{A} \times 100
\]

where \(A\) represents the average peak area of the standard solution \((n = 5)\) and \(B\) represents the average peak area of a plasma extract spiked at the same concentration of the standard \((n = 5)\). With this approach, the authors estimated a significant signal suppression of the SR 27417 with SPE and hexane liquid–liquid extraction (LLE). In this key publication, the importance of carefully estimating the contribution of the “extraction efficiency” and “ion suppression” in the overall “process efficiency (PE)” during validation was first emphasized. As reported by these authors, “process efficiency” represents the combination between the ME and the recovery of the sample extraction method. Low PE can be deleterious for method precision and sensitivity. Understanding to what extent the PE is affected by ME or by low extraction efficiency represents a key step to develop strategies to improve the performances of the analytical method.

The findings of Buhrman were extensively investigated by the group of Matuszewsky in the analysis of several drugs in human plasma samples (Fu, Woolf, & Matuszewsky, 1998; Matuszewsky, Constanzer, & Chavez-Eng, 1998, 2003; Matuszewsky, 2006). These authors proposed detailed protocols to assess ME, recovery (RE), and overall PE, and introduced for the first time the terms “absolute” and “relative” ME. The “absolute ME” represents the difference in response between the solvent sample and a post-extraction spiked sample. The “relative ME” indicates the difference in response between various lots of post-extraction spiked samples. The absolute ME will affect the accuracy of the method, whereas the relative ME will affect precision and accuracy.

The “absolute ME,” together with method RE and PE, can be estimated by acquiring calibration plots relative to three different sample sets (Matuszewsky, Constanzer, & Chavez-Eng, 2003). In the first set (set 1), neat standard solutions of the analyte are analyzed at seven different concentrations, and analyses are repeated five times at each concentration. Set 1 provides a good insight into the overall LC–MS system reproducibility and accuracy. In the second set (set 2), the calibration plot is constructed as in set 1, but with matrix-matched standard solutions. Any variability in the peak areas with respect to set 1 would indicate an effect of sample matrix, because analytes at the same concentrations and in the same solvent were spiked into the dried extract. In the third set (set 3), calibration solutions are obtained from pre-extraction spiked matrix samples. The variability between sets 3 and 1 would reflect a combined effect of sample matrix and method recovery. From the peak areas
acquired from these calibration plots, the ME percentage, RE, and PE can be calculated as follows (Fig. 1a):

\[
\text{ME} \,(\%) = \frac{B}{A} \times 100 \quad (2)
\]

\[
\text{RE} \,(\%) = \frac{C}{B} \times 100 \quad (3)
\]

\[
\text{PE} \,(\%) = \frac{C}{A} \times 100 = \frac{\text{ME} \times \text{RE}}{100} \quad (4)
\]

The terms \( A \) and \( B \) are the same of Equation (1), whereas \( C \) corresponds to the peak area for standard spiked before extraction. Equation (2) is a modified version of Equation (1) from Buhrman, Price, and Rudewicz (1996), where the potential for signal enhancement was not considered. Obviously, a value of ME \((\%) > 100\%\) indicates ionization enhancement, whereas a value \(< 100\%\) indicates ionization suppression. It is important to point out that the RE \((\%)\) in Equation (3) represents a “true” recovery that is not affected by the matrix. The evaluation of the absolute ME has a limited relevance because the most important issue during method validation should be addressed to the estimation of the relative ME. The assessment of the presence of the relative ME can be performed through a comparison of the %RSD in repetitive injections \((n = 5)\) of standards and post-extraction spiked extracts that derive from different lots of the matrix. Alternatively, the variability of standard line slopes in at least five different lots of the investigated matrix can be used as a good indicator of the relative ME. A value of the slopes %RSD \(< 3–4\%\) has been suggested as a guide for method applicability (Matuszewsky, 2006). The presence of an absolute or even a relative ME for a given analyte does not necessarily indicate that the method cannot be accepted. If an IS is used, assuming that it exhibits the same ME profile, the analyte-to-IS ratio should not be affected and the method can be fully validated.

The post-column infusion method provides a qualitative assessment of ME, to identify the retention time zones in a chromatographic plot most likely to experience phenomena of ion suppression or enhancement. The assessment is carried out by monitoring the instrument response of a constantly infused...
analyte, after injecting an extract from a sample into the LC–MS system. The typical experimental system used to evaluate ME with a post-column infusion experiment is described in Figure 1b. A constant flow of a solution that contains the target analyte is delivered into the LC eluent at a point after the chromatographic column and before the mass spectrometer (Bonfiglio et al., 1999). A blank sample matrix is injected under the required chromatographic conditions, and the response from the infused analyte is recorded. Any endogenous matrix component that elutes from the column and induces ME can be seen as a suppression or enhancement of the infused analyte signal in specific regions of the chromatogram. A typical example of how signal suppression can be estimated with the post-column infusion method can be found in the experiments carried out by Antignac and co-workers (2005). These authors demonstrated that ME can severely compromise the quantitative determination of the beta-agonist isoproterenol in bovine meat (Fig. 2). A sample of bovine meat free of the beta-agonist underwent a sample-treatment procedure that involved an LLE and an enzymatic hydrolysis followed by two SPE. The extract was injected onto a post-column infusion ESI-MS system with isoproterenol as the infused analyte. During this experiment, an increase of the recorded total ion current (TIC) was observed when injecting the meat extract, caused by the organic material arriving in the ESI source (Fig. 2a). The spectrum recorded in this area showed that the intensity of the isoproterenol diagnostic ions (Fig. 2b) was decreased, with subsequent increment of the noise and detection of ions related to interfering compounds (Fig. 2d,c). When the main part of the extract was eluted, the TIC returned to its initial intensity, and returned to efficient ionization (Fig. 2a). Figure 2d presents the extracted ion chromatogram (EIC) of the m/z 194, to illustrate the ion-suppression area where the signal intensity of the infused isoproterenol was decreased. In this example, an estimation of the signal loss between the non-disturbed situation and the maximum of suppression indicates that only 5% of the real expected signal was detected.

Although post-extraction addition provides an estimation of ME at the point of elution of the analyte, the post-column infusion method identifies chromatographic regions where an analyte would be most susceptible to suppression or enhancement. Due to this feature, the post-column infusion is widely used during method development to evaluate the influence of different sample-preparation techniques, the appropriate chromatographic conditions, the role of mobile-phase additives on the analyte response, and to clarify the mechanistic aspects of ME (Müller et al., 2002; Dams et al., 2003; Mallet, Lu, & Mazzeo, 2004; Souverain, Rudaz, & Veuthey, 2004). However, this approach can be quite time-consuming and requires a significant effort, particularly if quantitation of multiple analytes in a single analysis is required (Marín et al., 2009). If several compounds need to be determined in one method, then all the analytes should be infused separately to investigate any possible ME for every analyte. Moreover, analytes are frequently infused at concentrations greater than the limit of quantitation (LOQ); thus, the ME evaluation for low concentrated samples is prevented. In contrast, with the post-extraction addition method, the ME of all analytes can be measured each time a change is made to the analytical method, even if the target compounds are present at low levels.

IV. STRATEGIES TO OVERCOME ME

Several operational strategies have been suggested to minimize the interferences of co-eluting matrix compounds. First of all, one can improve the sample-preparation procedure to reduce the
presence of interfering components in the final extract. However, the results might be compromised with very complex matrices, where a variety of interferences with different chemical properties are present. Another way is to modify the chromatographic conditions to shift the retention time of the target analytes farther from the area of the chromatogram affected by ME. Alternatively, an IS can be employed to compensate for the signal alteration. Finally, if sensitivity is not an issue, then one can use an alternative ionization source less sensitive to ME. All these strategies will be discussed in detail in the next paragraphs. However, it is important to point out that there is no universal strategy, and in many cases several approaches must be combined to achieve adequate quantitative results.

A. Sample Preparation

There are a variety of sample-preparation techniques available for LC–MS, which differ for their ability to remove interferences and in their ease of use. Such techniques are often specific to the sample matrix, because different matrices contain different components that need to be removed. The widespread pre-treatment methods employed in biological, food, and environmental fields involve SPE and LLE.

As previously stated, although LLE often creates rather clean extracts, but this procedure is cumbersome and has many pitfalls with LC–ESI-MS (Jessome & Volmer, 2006). To transfer an ionizable analyte into the organic extraction solvent, it first must be converted into a non-ionic form in the aqueous medium, with an appropriate pH. Next, a suitable solvent that efficiently and preferentially extracts the analyte needs to be found. However, even with an appropriate solvent, a single step rarely extracts the analyte quantitatively, and multiple extraction steps are needed. Often, a final re-suspension in water at the original pH is needed, with an additional risk of significant analyte losses. Another issue with LLE is SPE is the requirement of a rather large solvent volume.

Compared to LLE, SPE can be scaled down to sample volumes below 50 µL, and can be easily automated (Hopfgartner & Bourgogne, 2003). Furthermore, it can also be realized on-line with the LC separation, with switching valves to direct the flow either to waste or to the LC column (Hopfgartner & Bourgogne, 2003; Xu et al., 2007). As widely reported in the literature, SPE represents the most efficient way to overcome ME. With respect to the other sample-pre-treatment procedures, SPE has the advantage that a large number of specific protocols can be generated to selectively purify the sample from the interferences. These operational strategies are based on two key steps: the first one is based on choosing the sorbents that better match the properties of the analytes of interest (van Hout et al., 2000; Koeber et al., 2001; Souverain, Rudaz, & Veuthey, 2004; Van De Steene, Mortier, & Lambert, 2006; Tachon et al., 2008), whereas the second one focuses on a proper selection of the washing and elution solvents (Benijts et al., 2004; Mallet, Lu, & Mazzeo, 2004). Alternatively, a two-step extraction procedure might be employed, in which the first extraction removes the matrix interferences from the sample, and the second step extracts the target analytes (Hogenboom et al., 2000; Niessen, Manini, & Andreoli, 2006). Recently, mixed-mode SPE has been proposed as an effective tool to reduce ME (Müller et al., 2002; Mallet, Lu, & Mazzeo, 2004; Chambers et al., 2007). Mixed-mode SPE uses a dual-retention mechanism based on reverse-phase coupled to ion exchange, to extract the analytes from the complex matrix with the same SPE cartridge. This approach makes use of a rigorous interference-elution procedure, to selectively remove matrix compounds from the SPE column, prior to elution of the analytes of interest. In a recent study, two different mixed-mode sorbents were evaluated for their ability to produce high recoveries of acidic and basic drugs from plasma, with minimal ion suppression (Mallet, Lu, & Mazzeo, 2004). Data were compared to those obtained with a single SPE extraction based on reverse phase. The results demonstrated that a clean-up of plasma based on mixed-mode SPE leads to minimal ion suppression enhancement for the selected compounds. Several types of mixed-mode and ion-exchange sorbents were evaluated to determine their abilities to remove phospholipids (Shen et al., 2005). Although mixed-mode phases can fulfill the requirements to retain the investigated analytes, this study suggests that reverse-phase retention mechanisms can be detrimental to eliminate ion suppression caused by late-eluting phospholipids. Indeed, if an analyte and its metabolites can be retained with an ion-exchange mechanism alone, then mixed-mode extraction phases should be avoided. A new sample clean-up technique called HybridSPE-PPT (Sigma–Aldrich, St. Louis, MO) has been recently proposed as a powerful tool to minimize ME due to the concomitant presence of phospholipids and proteins (Pucci et al., 2009). Biological samples are first subjected to protein precipitation (PPT) via the addition and mixing of acidified acetonitrile. The resulting supernatant is loaded onto a HybridSPE-PPT 96-well plate, which acts as a chemical filter that specifically targets the removal of endogenous sample phospholipids. The phospholipids-retention mechanism is based on a highly selective Lewis acid–base interaction between zirconia ions, functionally bonded to the HybridSPE-PPT stationary phase, and the phosphonate moiety consistent with all phospholipids.

Recent breakthroughs in sample clean-up have been achieved to exploit on-line sample-preparation procedures such as on-line SPE and turbulent-flow chromatography (TFC) with valve-switching devices. Diverting matrix components to waste before and after compound elution reduces the amount of undesirable and non-volatile components. Due to the availability of a wide range of extraction supports [e.g., restricted access materials (RAM), monolithic material, disposable cartridges], these approaches represent a valid tool to limit ME and, with respect to the off-line procedures, they offer speed, good recoveries, and low extraction costs. A detailed overview of validated LC–MS methods that use the strategy of on-line sample clean-up can be found elsewhere (Koeber et al., 2001; Hopfgartner & Bourgogne, 2003; Stoob et al., 2005; Xu et al., 2007; Du & White, 2008; Kuster, López de Alda, & Barceló, 2009).

In addition to LLE and SPE, several other sample-preparation techniques can be employed in LC–MS, such as ultrafiltration and microdialysis. Ultrafiltration based on the use of RAM with a mass cut-off of 15 kDa reduces ME related to high molecular weight humic substances from groundwater or sediment extracts (Petrovic, Tavazzi, & Barceló, 2002). However, a recent article by Kloepfer, Quintana, and Reentsma (2005) demonstrated that this approach would hardly be suitable to reduce suppression from wastewater samples, where matrix components with a MW below 1,000 Da account for more than 70% of the dissolved organic carbon (DOC). Microdialysis is an
in vivo sampling technique used to monitor changes in the composition of the extracellular fluid of different tissues, and is based on the principle of dialysis through a semi-permeable membrane. Microdialysis enables the collection of small, hydrophilic endogenous compounds, such as neurotransmitters and peptides, and exogenous compounds, such as drugs. Although microdialysates are protein-free aqueous solutions, they contain a large amount of salts (>150 mM) and other small molecules. These non-volatile compounds can cause ion suppression of the analytes of interest. It was shown that column switching is a pre-requisite to minimize salt effects from dialysates (Lanckmans et al., 2008).

Two alternative approaches could be sample dilution or a reduction of the injected volume (Schuhmacher et al., 2003); however, these solutions clearly appear inappropriate for trace analysis. Indeed, a dilution factor of 2–5 might be critical for the analyte present at trace level and without any effect on an interfering substance present at high concentration. As a consequence, the improvement of sample purification is usually preferable.

B. Chromatography

An improved chromatography represents a widely used strategy to overcome ME. With this approach, the elution conditions are modified to shift the retention time of the analyte far away from the area affected by signal suppression or enhancement. Usually the chromatographic areas that are most affected by interferences are the solvent front, where highly polar and unretained compounds are eluted, and the end of the elution gradient, where the strongly retained compounds are eluted (Bonfiglio et al., 1999; van Hout et al., 2000; Müller et al., 2002; Shou & Weng, 2003; Souverain, Rudaz, & Veuthey, 2004; Chambers et al., 2007). Consequently, it is recommended to adjust the chromatographic conditions to elute the target analytes between these two regions. An easy and effective way to do this adjustment is to modify the mobile-phase strength or gradient conditions (Bogialli et al., 2004; Taylor, 2005; Weaver & Riley, 2006; Chambers et al., 2007). However, this modification generally involves an increase in the time of analysis, which can be deleterious in those applications where the development of fast and cost-effective analytical methods is mandatory (e.g., pharmacokinetic studies). If high throughput is required, then an effective sample pretreatment becomes critical, because fast-gradient LC is not able to overcome ME due to its reduced resolution. Jemal, 2000; Chambers et al., 2007; Xu et al., 2007). An alternative approach can be the application of a rapid gradient or “ballistic gradient” to separate the analytes from the solvent front, while maintaining high throughput (Hopfgartner & Bourgogne, 2003).

As reported in Section II, the influence of mobile-phase composition on ionization efficiency is a well-described phenomenon in LC–MS (Law & Temesi, 2000; King et al., 2000; Choi, Hercules, & Gusev, 2001a; Giorgianni et al., 2004; Mallet, Lu, & Mazzeo, 2004). Therefore, a proper selection of the mobile-phase additives represents an important step to develop a LC–MS method.

Formic and acetic acids were added to a group of 35 endocrine-disrupting compounds (ECDs) in water at two concentration levels of 0.01% and 0.1%, v/v, and the MSMS response expressed as the ratio between standard and spiked extracts was used to calculate the ME (Benijts et al., 2004). The signal of the analytes was significantly affected by ME regardless of the concentration of the additives. The addition of buffers such as ammonium acetate and ammonium formate to the mobile phase at a concentration of 1 and 5 mM, respectively, was also attempted, but resulted in a strong suppression at the highest concentration. The latter effect was probably due to an increased number of ions (matrix interferences and additives) in the spray. This increase could reduce the access of the target analyte to the droplet surface, to eventually lead to complete droplet saturation and ionization suppression of target analyte. ME was compensated for with an extended sample clean-up and the use of stable isotope-labeled internal standards (SIL-Iss).

Volatile ion-pairing reagents are widely used in LC–UV because they improve peak shape and retention time. Unfortunately, due to their combined effect of ion-pairing and surface-tension modification, these additives can induce a severe signal suppression with ESI-MS. The signal intensities of eight amine analytes in the presence of three fluorinated carboxylic acids (trifluoroacetic acid, heptfluorobutanoic acid, and perfluorohexanoic acid) were measured (Gustavsson et al., 2001). The results from these experiments were compared with the signal intensity with an ion-pair free formic acid–ammonium formate buffer. It was shown that the ESI signal from most of the analytes decreased approximately 30–80% when the fluorinated carboxylic acids were added to the mobile phase at useful concentrations. Ionization suppression from organic modifiers such as TFA can be minimized or corrected for through the use of weaker acids (e.g., acetic acid or formic acid). If TFA must be used as an ion-pairing agent to achieve acceptable chromatography and peak shape, then it might decrease the TFA concentration and still obtain adequate separation. The use of surface tension-lowering modifiers in the ESI source was successful when TFA is used as a mobile-phase modifier (Eshraghi & Chowdhry, 1993). The post-column addition of acids and solvent carriers to displace TFA from compounds and to aid ionization has also successfully been used (Apffel et al., 1995).

Hydrophilic-interaction liquid chromatography (HILIC) on a silica column with a low aqueous/high organic mobile-phase content is a valuable alternative to the reverse-phase LC–MS for the analysis of highly polar compounds (Naidong, 2003; Xu et al., 2007). With HILIC columns, a sufficient retention can be achieved also to move the more polar analytes away from the solvent front (Naidong, 2003; Xu et al., 2007; Ji et al., 2008). In addition, the highly volatile organic mobile phases used in HILIC provide an increased sensitivity ESI-MS (Naidong, 2003).

Recent technology advances have made available reverse-phase chromatography media with sub-2 μm particle size along with liquid-handling systems that can operate such columns at much higher pressures. This technology, termed ultra-high-performance liquid chromatography (UHPLC), offers significant theoretical advantages in resolution, speed, and sensitivity for analytical applications, particularly when coupled with mass spectrometers capable of high-speed acquisitions. The improved resolution provides a benefit with respect to ME, through improved separation from endogenous components (Chambers et al., 2007; Marin et al., 2009).

Due to its increased resolution, on-line orthogonal two-dimensional liquid chromatography (2D-LC) provides another efficient way to compensate for signal suppression and to improve qualitative and quantitative data for the analysis of a wide variety of compounds in complex matrices (Choi, Hercules, & Gusev, 2001a,b; Dijkman et al., 2001; Pascoe, Foley, & Gusev,
2001; Hernández, Sancho, & Pozo, 2004; Rogatsky et al., 2006). The selection of the proper chromatographic system for 2D-LC is critical for a successful analysis. It might be advantageous to select columns of various types that further enhance chromatographic selectivity. As an example, if two C18 columns are used in series, then the same separation mechanism would be involved in both columns. Therefore, matrix components with similar retention to the target analyte might induce continued signal suppression due to matrix–analyte co-elution. If the column combination is varied, then the difference in selectivity between columns could further separate matrix components from analytes. Different column combinations were tested to investigate their capabilities to overcome ME in the analysis of complex matrices such as wheat forage, straw, pecan nuts, and mouse plasma (Pascoe, Foley, & Gusev, 2001). The results demonstrated that a reduction in ME was obtained when sufficient orthogonality of the phase systems was achieved. High-flow on-line reverse-phase extraction was coupled with normal phase on silica columns with aqueous-organic mobile-phase LC–ESI-MSMS to quantify drug candidates in biological fluids (Deng et al., 2005). The orthogonal separation effect obtained from this configuration considerably reduced ME and increased sensitivity for highly polar compounds. This approach also significantly improved the robustness and LOD of the assay.

Recent studies have proven that miniaturized ESI methods that employ nanoLC columns are more tolerant toward contaminations in the analyte solution (Van Eckhaut et al., 2009). It has been shown that reducing the ESI flow rate to the nL/min range leads to increased desolvation, ionization, and ion-transfer efficiency over ESI conducted at higher flow rates (Wilm & Mann, 1996; Enke, 1997; Abian, Oosterkamp, & Gelpí, 1999). The higher surface-to-volume ratios of the smaller nano-ESI droplets result not only in improved concentration sensitivity but also in resistance to ionization suppression effects. The droplets generated by nanospray are already small to produce GP ions directly, without relying on fissioning (Karas, Bahr, & Dulčiks, 2000). Thus, analytes with poor surface activity are not lost in the fissioning process, but become charged in the initial charged droplets, which have very high surface-to-volume ratio. The effect of flow rate on ion signals was deeply investigated by Schmidt, Karas, and Dulčiks (2003) using mixtures of compounds with different physico-chemical properties. For these model systems, the functional dependence of the analyte signals upon the flow rate has been correlated to changes in analyte partition during droplet fission, prior to ion release. In this study, analyte suppression was significantly reduced at nL/min flow rates. Similar results were obtained by Gangl et al. (2001) who investigated how the ionization and ion-transfer efficiency were affected by drastically reducing the flow rate. The target analyte for their study was carvedilol and the suppressing agents were dimethyl sulfoxide, indinavir, and taurocholic acid. Metabolites generated from an in vitro incubation of indinavir with rat liver microsomes were also examined. A significant improvement in concentration and mass sensitivity, as well as a reduction in signal suppression, were observed when the flow rate was decreased from 200 to 0.1 µL/min. A high-throughput assay for SCH 211803, a M2 muscarinic receptor antagonist, in human plasma using nano-ESI infusion MSMS has been proposed by Chen and co-workers (2004). This method exhibited a fourfold reduction in matrix suppression when compared to an ESI source operating at conventional flow rate.

### C. Calibration Methods

If ME cannot be eliminated by the above-described methods, then an appropriate calibration technique to compensate for signal alterations must be used. The standard-addition method probably represents the most-effective way to compensate for the adverse influence of the matrix on method performances (Stüber & Reemtsma, 2004). However, this approach is laborious and time-consuming because spiked samples must be analyzed for each sample. Calibration with external matrix-matched standards is often proposed in literature (Kang, Hick, & Price, 2007). Unfortunately, the method is time-consuming and offers an appreciable compensation only with diluted samples in a uniform matrix. Furthermore, the availability of appropriate blanks (i.e., material free of residues of the target analyte) might not always be accomplished.

The most widely used calibration approach to compensate for ME uses IS (structural or SIL-IS). The use of an adequate IS allows one to balance the variation of the analyte signal with an equivalent disturbance on the IS. For this reason, the IS and the target analyte must have similar ionization properties and elute at the same retention time. It was shown that the precision of a method in which an analog IS is used can be significantly improved by modifying the mobile-phase conditions in such a way that analyte and IS co-elute (Kitamura et al., 2001; Weaver & Riley, 2006; Leverence et al., 2007). It appears to be a general belief that SIL-ISs yield better assay performance because they show almost identical behavior to the analyte in sample pretreatment, chromatography, as well as ionization (Freitas et al., 2004; Ismaiel et al., 2008; Zhao & Metcalfe, 2008). However, their use is rather expensive and for many compounds SIL-ISs are not commercially available. In addition, although it is generally believed that the use of an SIL-IS corrects for any ME, many data reported in literature indicate that this issue needs proper attention during method development and validation. The investigation of ME for nine drugs and their corresponding SIL-IS with ESI- and APCI-MS was reported (Liang et al., 2003). The results showed that all of the investigated target drugs and their co-eluting SIL-ISs suppress each other’s ionization response in ESI. The factors that affect the extent of suppression in ESI were investigated, including flow rate, structure, and concentrations of drugs. The authors demonstrated that the mutual ionization can be minimized by selecting an appropriate concentration for each SIL-IS. During method development for the analysis of mevalonic acid in urine, an unexpected variability of the analyte/SIL-IS ratio that depended on the urine batches and sample volume used for the extraction was observed (Jemal, Schuster, & Whigan, 2003). For this reason, the authors suggested that one performs calibration with different batches of biological matrix. In a study of the quantitation of the antimalarial piperazine in plasma, it was found that triethylamine residues that remain after SPE suppressed the signal of piperazine and its deuterated IS differently (Lindegardh et al., 2008). This phenomenon induced an underestimation of 50% of the true concentration. Stokvis, Rosing, and Beijnen (2005) compared the use of structural analogs and SIL-IS for several anticancer agents with ESI-MSMS. These authors found that 13C-, 15N-, or 17O-labeled compounds might be more appropriate than deuterium-labeled compounds. Similar results were obtained by Wang, Cyronak, and Yang (2007). The replacement of the carbon-bound hydrogen with deuterium slightly alters the
lipophilicity of the analyte, and hence the retention times during the chromatographic separation. This phenomenon is commonly known as a deuterium-isotope effect. The $^{13}$C, $^{15}$N, or $^{17}$O-labeled IS might be more ideal than the $^2$H-labeled ones, because deuterium and hydrogen have greater differences in their physical properties than, for example, $^{12}$C and $^{13}$C.

In multicomponent methods such as the multiresidue analysis of pesticides, generally no ISs are applied because of the scarcity of appropriate SIL-IS. Furthermore, the use of an IS for every analyte would seriously limit the sensitivity of the method, because it doubles the number of SRM transitions that have to be monitored. Kang and co-workers proposed different calibration methods to assess ME in the quantitative analysis of several phytoestrogens in aqueous environmental samples from sewage-treatment plants (Kang, Hick, & Price, 2007). The aim of the authors was to find a more-effective approach for multiresidue analysis to compensate for ME even after extensive sample clean-up and a careful method set-up. The authors compared three different calibration methods: (1) solvent standard calibration with one IS; (2) external matrix-matched standard calibration; (3) matrix-matched standard calibration with one IS. The results were compared to those obtained with standard addition and the ratio [concentration (analyte)$_{by}$ calibration/concentration (analyte)$_{by$ standard addition}] was calculated. The concentrations estimated using the matrix-matched standard calibration with one IS were consistently similar to those obtained by standard addition, with the concentration ratios close to 100%. These results demonstrated that the matrix-matched standard calibration with one IS could be a practical alternative to compensate for ME in multicomponent analysis: it is not time-consuming, such as standard addition, and it makes unnecessary the requirement to find an IS for every target analyte.

A new and interesting alternative to the IS concept is the echo-peak technique (Alder et al., 2004). This technique simulates the use of IS, without the demand for a SIL-IS of the target analyte. It consists of two injections, the unknown sample and a standard solution, within a short time period. As a result, the compound in the unknown sample and its standard elute in close proximity are, therefore, expected to be affected by the matrix interferences in the same way. In a set of experiments to determine eight pesticides in apple, Zrostlíková et al. (2002) compared the method performances of the echo-peak technique with those of the external standard calibration and external matrix-matched standard calibration. According to expectation, with external standard solvent calibration, the results were underestimated due to suppression or enhancement of analyte signal by matrix components. On the other hand, with the use of matrix-matched calibration, accurate data were obtained. With echo-peak technique accurate results comparable to those obtained with matrix calibration were obtained for six out of eight pesticides.

D. Mass Spectrometry

Another action level to overcome ME is to modify the mass spectrometric conditions. MS modification can be an advantageous solution because it does not require any change in the rest of the analytical procedure (sample preparation and chromatography). In fact, the occurrence of ion suppression might differ between ionization modes (positive or negative), source designs, and ionization techniques. A change in ionization mode, especially with ESI-MS, was a successful strategy to overcome ME. The negative-ion mode was more specific, and consequently less prone to ME. Source geometries also can influence the amount of ME (Mei et al., 2003; Antignac et al., 2005). Holčapek et al. investigated the extent of ion suppression using five different mass analyzers and instrument geometries. A higher signal suppression was observed with linear geometry instruments in comparison to orthogonal or even Z-spray geometry mass spectrometers (Holčapek et al., 2004).

As widely described in Section II, several studies have demonstrated that APCI is less prone to ME. Therefore, a shift from ESI to APCI is an excellent way to reduce ME (Barnes et al., 1995; Matuszewsky, Constanzer, & Chavez-Eng, 1998, 2003; King et al., 2000; Schuhmacher et al., 2003; Vanderford et al., 2003; Ching, Zhang, & Karnes, 2004). However, as previously reported, the occurrence of a signal suppression phenomenon has also been shown with APCI (Liang et al., 2003; Mei et al., 2003; Sangster et al., 2004; Zhao & Metcalfe, 2008).

Atmospheric-pressure photoionization (APPI) is a more recent technique and consequently less-investigated versus ME. The APPI response to conditions that typically lead to ion suppression and chemical noise with APCI and ESI was tested. The results indicate that APPI is less susceptible to ion suppression and salt-buffer effects than APCI and ESI (Hanold et al., 2004).

In a recent study, LC coupled to direct-electron ionization mass spectrometry (direct-EI-MS) has been proposed as a valid alternative to the API techniques to overcome ME (Cappiello et al., 2008) for small molecules. This instrumentation allows a straight connection between a nanoLC system and a mass spectrometer equipped with an EI source, to form a reliable LC–MS system that resembles the immediacy and straightforwardness of GC-MS (Cappiello et al., 2007a). Different from API, hard-ionization techniques such as EI operate in a high-vacuum, high-temperature environment and making use a GP, physical mechanism that is much less dependent by adverse reaction induced by the matrix. In fact, co-eluted compounds are simultaneously vaporized and subsequently ionized by a multitude of independent, single molecule–electron interactions. In this condition the total ion signals are dependent only on the concentration of each component without any mutual adverse interaction. Experiments carried out on biological and environmental matrices (human plasma and river water) well demonstrated this assumption. Pesticides that belong to the class of triazines and carbamates, and anti-inflammatory drugs, were used as target compounds (Cappiello et al., 2008). ME were assessed with post-column infusion and post-extraction addition experiments. The results from the investigated analytes demonstrated that direct EI-MS allowed the analysis of the target molecules regardless of the presence of co-eluted interferences, whereas ME was always observed with LC–ESI-MS with a combination of enhancement and suppression. A strong signal suppression affected the ESI-MSMS response, whereas no signal alteration was observed with direct EI-MS. The interface capability to overcome ME was further demonstrated in other applications on the analysis of diethylene glycol in seawater (Cappiello et al., 2007b), and the determination of organochlorines and phenoxy acidic pesticides in river water (Famiglini et al., 2008, 2009).
V. ME IN DIFFERENT APPLICATIONS

A. Bioanalytical and Pharmaceutical Analysis

The application of LC–API-MS has expanded rapidly in the field of bioanalytical and pharmaceutical research (Xu et al., 2007), and it has now become the method of choice in a wide variety of applications such as the detection of biomarkers in biological fluids (Taylor, 2005), the therapeutic monitoring of drugs and their metabolites (Hopfgartner & Bourgogne, 2003; Prakash, Shaffer, & Nedderman, 2007), the screening of metabolic disorders (Chace, 2001), and toxicological and forensic investigation (Wood et al., 2006). However, recent articles highlighted the importance of evaluating ME in method development, validation, and routine use of LC–MS for bioanalytical purposes (Annesley, 2003; Matuszewsky, 2006; Van Eeckhaut et al., 2009). Although LC–API-MS is the most powerful tool for quantitative bioanalysis, this technique is not a “turn key” solution to analytical problems, because ME can be its “Achille’s heel” (Taylor, 2005). Recent studies on the investigation of drugs and metabolites demonstrated the occurrence of ion suppression or enhancement in many different biological samples, such as plasma, urine, oral fluids, and tissues (Antignac et al., 2005; Wood et al., 2005; Van Eeckhaut et al., 2009). The LC–ESI-MSMS behavior of indinavir, an HIV protease inhibitor, in human urine was presented as one of the first examples of ME in a biological application (Fu, Woolf, & Matuszewsky, 1998). Results from two different sample-preparation procedures (direct dilution of urine vs. urine extraction) and two chromatographic systems were compared. Sample clean-up with LLE coupled to an improved chromatography increases the instrument response. Additionally, the use of a SIL-IS improved the precision of the method. These results demonstrated, for the first time, that an evaluation of the potential effects of co-eluting species from the sample matrix must be adequately assessed before the large-scale employment of an assay in clinical studies with biological fluids.

The necessity to improve sample preparation and chromatography to minimize ME in bioanalytical LC–MS was highlighted in many other recent studies (Souverain, Rudaz, & Veuthey, 2004; Jessome & Volmer, 2006; Chambers et al., 2007; Van Eeckhaut et al., 2009). Biological fluids such as plasma or urine, the most widely used strategies for sample clean-up, are represented by PPT, LLE, and SPE. The increasing focus on high-throughput analysis has led to the common practice to prepare biological samples with the simplest and fastest method possible, in most cases PPT. However, this method does not result in clean extracts because it fails to sufficiently remove those endogenous components (salts, amines, fatty acids, phospholipids, triglycerides, etc.) known to induce variability in analyte signal (King et al., 2000; Mei et al., 2003; Schuhmacher et al., 2003; Jessome & Volmer, 2006). LLE is viewed as a cleaner option than PPT (Avery, 2003). However, highly polar and ionic compounds can be difficult to extract with this technique, and multiple extraction steps are commonly needed (Jessome & Volmer, 2006). Thanks to the wide variety of stationary phases, much more specific protocols can be generated with SPE to selectively clean the samples from the interferences (Avery, 2003; Mallet, Lu, & Mazzeo, 2004). However, especially when dealing with complex matrices such as plasma, interfering components might not be efficiently separated from the analyte—even when an SPE procedure is employed (van Hout et al., 2000; Dans et al., 2003; Souverain, Rudaz, & Veuthey, 2004). For post-column infusion experiments with morphine, Dans et al. (2003) have evaluated the synergistic effect of ionization type, sample-preparation technique, and biofluid on ME in the quantitative analysis of illicit drugs and opioid therapeutic medications. Three biofluids (urine, oral fluid, and plasma) were pre-treated with four sample-preparation procedures (direct injection, dilution, PPT, SPE) and the preparations were analyzed by both LC–ESI-MS and LC–APCI-MS. The results demonstrated that: (1) large differences in ME were observed among the investigated sample-preparation techniques. A signal suppression was also observed even with SPE, which is often proposed as a solution for ME. In fact, the SPE pre-concentration step increased the concentration of the non-removed interfering substances together with the concentration of the target analyte. Much less suppression in LC–ESI-MS was observed using direct injection and dilution. However, the drawback to omit a pre-concentration step from the sample-preparation procedure induced a loss of sensitivity. (2) ME were observed with both ionization types, but it was more prevalent with ESI than APCI. (3) Matrix components, characteristic of each biofluid, interfered at different retention times and to varying extent throughout the analysis. The major interferences in urine were hydrophilic residual components, most likely inorganic salts. The endogenous interferences in oral fluids were represented by residual matrix components of hydrophilic and hydrophobic nature, including protein, amino acids, phospholipids, and especially mucin. Plasma has more interferences than urine and oral fluids and represents the most complex matrix due to the presence of components with a wide polarity range.

Recent studies clearly demonstrated that a major contribution to ME in biofluids can be ascribed to glycerophosphocholine (GPCho) lipids because of their surfactant-like properties (Shen et al., 2005; Little, Wempe, & Buchanan, 2006; Lihong & White, 2008; Wu, Schoener, & Jemal, 2008). Therefore, the removal of phospholipids as principle agents of ion suppression is extremely important in any extraction process. With the post-extraction addition method, Chambers and co-workers evaluated several clean-up methods to detect basic and neutral drugs in rat plasma samples, including PPT, LLE, and SPE with respect to ME and recovery (Chambers et al., 2007). The authors monitored the levels of various GPChos to compare the relative cleanliness of final extracts. The results obtained in this study demonstrated that PPT failed in efficiently removing the endogenous phospholipids. Reversed-phase and cation-exchange SPE both resulted in cleaner extracts and reduced ME compared to PPT. LLE also provides clean final extracts. However, the recovery, particularly for polar analytes, was low. Phospholipids that belong to the class of GPChos and 2-lyso-glycerophosphocholines (2-lyso-GPChos) were used in a recent study as markers for endogenous matrix components, and to compare between ESI and APCI in the determination of chlorpheniramine and diphenhydramine in human plasma samples extracted with LLE (Ismail et al., 2008). Post-column infusion experiments with different chromatographic conditions demonstrated that ME coincided with the elution profile of the phospholipids. It was also found that GPChos and 2-lyso-GPChos demonstrated a very low ionization efficiency in the APCI mode, that made APCI the ionization method of choice in this specific application, even though the absolute response for the target analytes was lower.


than in ESI mode. These results demonstrated that the monitoring of phospholipids represents a key step in method development to select the appropriate stationary phase, mobile-phase composition, and ionization source.

Co-eluting endogenous components are not the only source of ME that need to be investigated in a bioanalytical assay. Recent studies highlighted the importance to prevent any alteration of the signal response induced by exogenous interfering compounds during method development (Souverain, Rudaz, & Veuthey, 2004; Larger et al., 2005; Weaver & Riley, 2006; Leverence et al., 2007). It has been demonstrated that polymers contained in different brands of plastic tubes and the anticoagulant Li-heparin can contribute to ME when analyzing plasma samples (Mei et al., 2003). These exogenous ME can be easily eliminated or avoided by a judicious selection of test tubes to process plasma samples, and by a proper choice of the anticoagulant. The same authors demonstrated that ME induced by Li-heparin anticoagulant can be ascribed to the formation of lithiated adducts, which enhance the ionization efficiency of the plasma GPChos.

Exogenous interference compounds can also be found in sampling devices used to collect biological fluids. An example is reported in a recent publication on the quantitative analysis of opiates and cannabinoids in preserved oral fluid with LC–ESI–MS (Wood et al., 2005). The authors demonstrated that the Intercept®, an FDA-approved sampling device, contains some ingredients (stabilizing salts, non-ionic surfactants, and anti-bacterial agents) that induce signal suppression without a suitable sample clean-up method.

In pharmacokinetic studies, a major contribution to ME can be ascribed to the excipients often used as dosing vehicles (Tong et al., 2002; Shou & Weng, 2003; Larger et al., 2005; Weaver et al., 2006). Tween-80 and PEG400 are commonly employed in dosing formulations, via either intravenous or oral route, during the early drug discovery. A series of study was performed to evaluate the potential ME, when these dosing vehicles are assayed in rat plasma by LC coupled to ESI and APCI (Xu et al., 2005). Due to their high surface activity, these formulations are easily eliminated or avoided by a judicious selection of test tubes to process plasma samples, and by a proper choice of the anticoagulant. The same authors demonstrated that ME induced by Li-heparin anticoagulant can be ascribed to the formation of lithiated adducts, which enhance the ionization efficiency of the plasma GPChos.

To overcome signal enhancement or suppression of concomitant medications, a comprehensive sample clean-up and LC method need to be developed with sufficient separation of co-administered drugs from the analyte and its IS. This literature overview demonstrated that the occurrence of ME in the bioanalytical field must be carefully evaluated as a part of method development. For this reason, the recent Food and Drug Administration (FDA, 2001) guidelines on bioanalytical analysis explicitly require the evaluation of ME. However, there is no agreement on how this evaluation should be performed during method validation. An overview of validated LC–MS methods for the analysis of drugs in biological fluids has been given by Van Eeckhaut et al. (2009).

B. Environmental and Food Analysis

A very important application area of LC–APE-MS is environmental and food analysis. Many active compounds that derive from human activities can be found in different compartments where, in many cases, they can persist for a long time and bioaccumulate. Their toxicity can be conveyed in a long range; therefore, they can be found even at a long distance from the site of release. The new generations of molecules that enter the environment show physico-chemical properties that make them particularly appropriate for LC–MSMS detection with ESI and APCI. Environmental specimens commonly exhibit a wide and complex composition also within the same kind of matrix (soil, water) that affects analytes quantification in unpredictable ways that limit method accuracy and precision, and impinge on compound characterization and confirmation.

Hexabromocyclododecane (HBCDs) is an environmentally active compound that is used in various industrial productions to reduce the risk of fire, with an estimated increase of use. From an environmental standpoint, it behaves like other persistent organic pollutants (POPs), in the sense that it tends to accumulate in soil and sediments. The commercially available product is a mixture of three predominant diastereoisomers: α-, β-, and γ-HBCD; the γ isomer is present at the highest concentration. The three diastereoisomers show dissimilar physico-chemical properties that can explain their different effects on the environment. Each diastereoisomer has several enantiomers: (+)α, (−)α, (+)β, (−)β, (+)γ, (−)γ, that share the same properties, but have different biological and toxicological properties (Guerra, Eljarat, & Barceló, 2008). Fu et al. investigated a clean-up procedure for soil and air samples to eliminate ME that involves the use of 13C-labeled HBCDs before injection (Yu et al., 2008). Four air and three surface-soil samples were extracted and prepared by following a procedure reported in the literature (Mai et al., 2005). To evaluate ME, they compared the amounts of three 13C-labeled standards (A) with those of air and soil-sample spiked after extraction (B). They calculated the ratio (B/A) to define the absolute ME. For all samples, they obtained values that ranged between 0.93 and 1. That meant that the extracted matrix components from air and soil did not interfere with the analysis. The authors claim that this result is also due to an improved chromatographic separation that allows baseline separation among all stereoisomers and potential interferences.

The use of SIL-IS is a suggested practice to compensate for ME, also in environmental and food samples. However, the compensation can be dependent on the retention time; therefore, several ISs might be necessary. Such compounds are not always available on the market and most of the time they are rather expensive; thus, as an alternative, standard enrichment is also...
used to compensate for the unavoidable signal modification induced by the matrix interferences. Extensive clean-up prior to the LC–MS analysis would be an effective way to clear the sample. A size-exclusion process was used in the analysis of a municipal wastewater treatment plant (influent and effluent) that used benzothiazoles and some pharmaceuticals as model compounds (Kloepffer, Quintana, & Reemtsma, 2005). This approach was combined with a flow-rate decrease during ESI-MS analysis. Reducing the ESI flow rate leads to an overall higher degree of sensitivity compared to high flow rates, but also reduces ME, and limits the competition between target analytes and matrix components during desolvation and ionization. However, the benefit of these advantages is that a nanoelectrospray source is required (Wilm & Mann, 1996; Gangl et al., 2001). The selected model compounds were analyzed with reverse-phase and ion-pair LC with positive and negative-ion mode ESI-MS detection. The evaluation of ME was performed with standard addition into SPE extracts and the calibration curves of these experiments were compared to those of pure aqueous standards at the same concentration levels. The clean-up of the samples was performed using membranes with cut-offs of 1.3 and 10 kDa, the permeates were extracted with SPE and aliquots of the extracts were spiked at different concentration levels. This procedure did not reduce ME from wastewater samples; in fact, the authors concluded that most of the signal suppression that they observed was due to components with a molecular weight lower than 1 kDa. This approach is more successful when humic substances or, in general, high molecular weight materials are present in the matrix. A reduced ESI flow rate of ca. 50 μL/min for pharmaceuticals and 20 μL/min for benzothiazoles was used to verify whether a flow reduction was beneficial in terms of ME and whether it was compatible with a conventional ESI interface. The reduction was achieved with a post-column T-piece to allow the use of a conventional system rather than a nanoelectrospray device. As expected, a higher sensitivity was obtained for most compounds, although the ideal flow rate was different and depended on the ion modes. This advantage was coupled to a considerable reduction of the ME for many analytes to the point that, for some of them, external calibration was sufficient for quantitation. Yet, for some compounds flow reduction did not provide any appreciable advantage.

In the determination of carbamazepine and its four metabolites (10,11-dihydro-10,11-dihydroxycarbamazepine, 2-hydroxy carbamazepine, 3-hydroxy carbamazepine, 10,11-dihydro-10-hydroxy carbamazepine) in aqueous samples with ESI-MSMS, after SPE with Oasis HLB cartridges (Waters Corporation, Milford, MA), a signal suppression strongly affects quantification. Dihydroxy carbamazepine was used as IS, due to the unavailability of the SIL-IS of carbamazepine and its metabolites (Miao & Metcalfe, 2003). The same approach, with IS analogs, was used also in another application in which antibiotic substances in hospital sewage water were determined with SPE–ESI-MSMS (Lindberg et al., 2004). ME can occur also when ion-pairing agents are necessary to improve the chromatographic separation, as described by Quintana and Reemtsma (2004) in the determination of acidic pharmaceutical drugs, their metabolites, and triclosan in water with ion-pair LC–ESI-MS in the negative-ion mode.

Extensive sample clean-up was successfully used to limit ME. In the determination of polar pesticides and their transformation products in estuarine, sea, and surface water, the performances of graphitized carbon black (GCB) extraction disks, styrene–divinylbenzene co-polymer extraction cartridges (LiChrolut EN, Merck KGaA, Darmstadt, Germany), and aminopropyl cartridges were compared. LiChrolut EN yielded better results in terms of recovery percentage and also showed a better capability to remove humic acids present in the final extract (Steen et al., 1999).

The same approach was adopted by Lindsey, Meyer, and Thurman in the analysis of sulfonamide (SA) and tetracycline antimicrobials (TCs) in groundwater and surface water with positive-ion ESI-LC–MS. Although SA did not show ME, TC quantitation was impossible due to a fluctuating signal behavior (Lindsey, Meyer, & Thurman, 2001). The APCI approach was attempted in positive- and negative-ion modes, to produce a signal enhancement in both modes. C18 clean-up removed the natural organic matter, and afterwards the signal enhancement was considerably reduced.

In many cases, SPE stationary phases do not appear to have the required selectivity. In fact, retention of the analytes is based on non-selective, hydrophobic, or polar interactions that can allow the partial co-extraction of matrix compounds. Molecules that were determined by the method were retained by molecular imprinting (MIP) as synthetic sorbents that involve a mechanism of molecular recognition and represent a valid alternative when a higher degree of selectivity is needed (Pichon, 2007). In a recent article, Zorita and co-workers report the determination of acidic pharmaceuticals, such as non-steroidal anti-inflammatory drugs, NSAIDs, (ibuprofen, naproxen, and diclofenac sodium salt), and clofibric acid (metabolite of clofibrate), at low ng/L in wastewater with ESI-LC–MSMS in the negative multiple-reaction monitoring mode (MRM) (Zorita et al., 2008). Sample preparation was carried out from sewage water with molecularly imprinted solid-phase extraction (MISPE). This method allowed the complete removal of matrix components, and neither signal suppression nor enhancement was reported, compared to tap water. The same result was obtained with water from a pond that receives treated water from a sewage-treatment plant. Analogous results were obtained by Sun et al. (2008) in the analysis of diclofenac in wastewater. The same approach, MIP, was used to determine chloramphenicol (CAP) from milk (raw milk, skimmed milk, and milk powder) using isotope dilution LC–ESI-MSMS in the negative ionization acquisition mode (Mohamed et al., 2007). The method was compared with classic SPE and LLE procedures to produce better recovery and selectivity.

Atmospheric pressure chemical ionization (APCI)-MSMS was used in a recent application by Metcalfe et al. for the analysis of neutral pharmaceuticals in water (Zhao & Metcalfe, 2008). Sample preparation used Oasis HLB cartridges; however, this step was not sufficient to remove the interfering compounds from the final extract. Therefore, the significant signal enhancement (as high as 178%) was corrected with SIL-IS. Different SPE-packing materials were tested for their capability to remove humic substances from polar and acidic microcontaminants in river water, including various types of C2, C8, C18, CN, and polymeric stationary phases (Hogenboom et al., 2000). The method was evaluated with an on-line dual-SPE–LC–UV set-up, and diode array-UV (DAD-UV) and MSMS quantitative results were compared. The behavior of the different SPE phases, in different combinations, was assessed by keeping as a reference the retention of a 14-compound mixture on a dual pre-column, packed with PLRP-S (15–25 μm Polymer Laboratories, Church
In this set-up, the first pre-column retained the medium and non-polar compounds, and acidic and highly polar compounds were efficiently retained in the second pre-column. This approach can reduce ME when ESI-MSMS detection is required. A similar approach was attempted by Van De Steene, Mortier, and Lambert and co-workers to evaluate ME in the simultaneous determination of nine pharmaceutical compounds in water samples with ESI-MS in the positive-ion mode (Van De Steene, Mortier, & Lambert, 2006). A selection of stationary phases was tested: Oasis HLB, C8, Bond Elut Phenyl (Varian, Sint-Katelijne-Waver, Belgium), Strata X-polymer SCX/RP sorbent, and Strata X-polymer RP sorbent (Phenomenex, Bester, Amstelveen, the Netherlands). The phenyl one was the most effective to reduce of ME. A further reduction was achieved with an additional step of clean-up with an NH2 cartridge. A 1:5 reduction of the flow-rate intake, with the use of a post-column splitter, had the advantage to diminish ME that was assessed with structural analogues IS. Speedisk phenyl columns were used to decrease the time of analysis. In addition, a further reduction in ME was obtained.

A comprehensive study of matrix interferences in the analysis of acidic pesticides in water was made by Dijkman and co-workers, who explored three different LC modes: a single column, on-line SPE–LC, and coupled column LC–LC. MS detection consisted of either ESI and APCI interfaces in the positive- and negative-ion modes (Dijkman et al., 2001). The experiments were carried out on Milli-Q and tap water fortified with 12 mg/L DOC to simulate ME and salinity. Signal suppression caused by salinity could be overcome by SPE–LC and LC–LC. They also explored different LC–LC combinations to overcome ME. C18/Supleco ABZ+ (Supleco, Bellefonte, PA) was the most favorable one to achieve a reliable quantification of the analytes.

A group of 53 multiclass emerging organic pollutants (EOPs) that belong to different classes were determined in water samples (wastewater, surface, and drinking water) with SPE followed by LC–ESI-MSMS in the positive- and negative-ion modes. They include acidic herbicides, UV filters, insect repellents, organophosphorus flame retardants, a bactericide, pharmaceuticals, and metabolites (Rodil et al., 2009). The sample preparation was carried out on Oasis HLB 200 mg cartridges, and the ME compensation was performed with seven isotopically labeled standards.

The same approach was used in the determination of 51 EOPs in environmental waters with LC–ESI-MSMS, including pharmaceutically active, endocrine-disrupting, and perfluoroalkylated compounds (Hao et al., 2008). SPE was performed on Oasis HLB 200 mg cartridges, and ME was assessed for drinking water, wastewater, and surface water with SIL-IS.

The presence of humic substances can be even more central in the case of extraction of analytes from soil; therefore, the extraction and the sample-preparation steps become extremely critical. The determination of four tetracyclines chlorotetracycline (CTC), doxycycline (DXY), oxytetracycline (OTC), and tetracycline (TC) from soil has been reported by O’Connor, Locke, and Aga (2007). They investigated different extraction solvents and pressurized liquid-extraction conditions (PLE) and optimized the SPE procedure. The extraction of six different soils was performed on a Dionex ASE 200 (Dionex Corporation, Sunnyvale, CA) accelerated solvent extractor with several solvents to determine the best operating conditions. For sample clean-up, different SPE sorbents were taken into account, including the Sep-pak C18, Oasis HLB, Discovery strong-anion exchange (SAX), and StrataX polymeric-sorbert cartridges. LC–ESI-MS analyses were carried out with ion-trap and a single-quadrupole mass spectrometers for the experiments on ionization suppression; for quantitative analysis, the single quadrupole was the instrument of choice. In a previous attempt, tandem clean-up was set up with a SAX cartridge (Waters Corporation) connected to a reverse-phase cartridge (Blackwell et al., 2004). The SAX cartridge retained the anionic fraction of humic acids and allowed the cationic tetracyclines to pass through and be retained on the second reverse-phase cartridge. After the removal of the SAX, the analytes were eluted from the other one with fewer matrix interferences. In this approach, the SAX cartridge was placed after the reverse-phase one with the purpose to further purify the sample without the risk of eluting the matrix from the SAX back into the sample extract.

A comparison among three different sample-preparation methods was carried out by Kruve et al. to investigate the differences in ME in 15 fruits for 14 pesticides residues with LC–ESI-MSMS. In particular, Luke, QuEChERS (quick, easy, cheap, effective, rugged, and safe) and matrix solid-phase dispersion (MSPD) were taken into account (Luke, Froberg, & Masumoto, 1975; Kruve et al., 2008). The selected pesticides (aldicarb sulfoxide, aldicarb sulfone, demethion-S-methyl sulfoxide, carbandazim, methomyl, thiamidazole, methiocarb sulfoxide, methiocarb sulfone, aldicarb, amazalil, phorate sulfoxide, methiocarb) were prepared at three concentration levels (0.01, 0.10, and 1.00 mg/kg). ME was calculated according to Matuszewski, Constanzer, and Chavez-Eng (2003). To evaluate the most-effective sample-preparation method in limiting ME, apple extracts were analyzed in six replicates at a concentration of 0.10 mg/kg. Luke method gave the best results to limit signal alteration, although also the QuEChERS method gave satisfactory results. Thanks to the fact that it provided better recovery data and it is more economic, Luke was considered the method of choice. Reproducibility of ME over different fruits at different analytes concentrations and from variety to variety were also considered.

When multiresidue analysis is performed, the typical approaches (use of IS, standard addition) to reduce ME can be unsatisfactory due to the large number of compounds with a wide range of physico-chemical properties. A systematic study on ME in 20 plant matrices for 129 pesticides, that range from acidic to basic and from hydrophilic to hydrophobic, from surface active to inactive, allowed one to evaluate a large number of analyte/matrix combination in LC–ESI-MSMS in positive-ion mode (Stahinke, Reemtsma, & Alder, 2009). The assessment of ME was performed in two sets of experiments: the first one was carried out with post-column infusion, the second one by spiking the final sample extracts prior to injection into the system. ME was calculated for different analytes in the same matrix and in different matrices. After a total of some 2,388 analyses, the authors concluded that ME is more matrix-dependant than compound-dependant. They also claim that different batches of the same fruit or vegetable give different ME and that this consideration can be extended to other matrices (biomedical, environmental, metabolomics, etc.). These findings offer a new possibility to correct for ME. In fact, it could be possible to assess ME on only one analyte that is considered representative of the
entire set to, therefore, reduce the effort and the number of analyses in multiresidue determination.

In food analysis and in particular in food safety, it is mostly important to determine the presence of protein toxins such as those from Staphylococcus aureus, Campylobacter jejuni, Vibrio cholerae, and Escherichia coli O157:H7. An LC–ESI-MSMS method to determine staphyloccocal enterotoxin B (SEB) in apple juice as a model food matrix has been described in a recent article (Callahan et al., 2006). The protein of interest is separated from the low molecular weight components with ultrafiltration; it is digested with trypsin, and the tryptic peptides are characterized with QToF MSMS. The authors observed that the main limitation of the method is a signal suppression induced by the apple juice matrix. This drawback has been reduced by a selective fractionation of the sample to remove low molecular weight components; high molecular weight proteins were digested into smaller peptides separated from other heavy components. ME assessment was made with IS with a tryptic cleavage site, such as leu-enkephalin, rather than an expensive SIL-IS. This method was the most effective on low solubility protein-content matrices, and was unsuccessful with milk and its derivatives. This limitation is mainly due to the use of ESI as ionization technique, but it can be circumvented in the sample-preparation step with antibodies for SEB multidimensional cartridges in the extraction process.

In the determination of 13 SAs in raw meat and infant foods with LC–ESI-MSMS after accelerated solvent extraction (ASE), the evaluation of ME was based on instrumental calibration curves compared with matrix-matched calibration curves (Gentili et al., 2004). Different matrices gave different results; namely, the calibration curves from infant food (all meat) did not evidence ME, whereas a signal suppression of ca. 30% was observed for beef and pork meat, and ca. 10% for veal and poultry meat from supermarkets. The authors hypothesized that these results are caused by the fatty materials that form a film on the droplet surface that inhibits ion evaporation. Signal suppression is reported for the same class of compounds also in various honey samples by Verzegnassi, Savoy-Perroud, and Stadler (2002), who used the same approach to assess ME. SAs were extracted from honeys by acidic hydrolysis followed by LLE. LC–ESI-MSMS in the positive-ion mode was used for the characterization. The authors did not find any correlation between the suppression and the chemical composition of the samples, and claimed that a further purification step could eventually circumvent the problem.

A sample-preparation method based on a MSPD technique with heated water as the extracting medium was used to determine tetracycline antibiotics and three related epimers in bovine, swine, and poultry muscle tissues (Bogiali et al., 2006). Heated water (70°C) was used to extract the target analytes, and the aqueous extract was acidified and filtered before LC–ESI-MSMS analysis. Based on the same approach, Blasco, Di Corcia, and Pico (2009) used a commercial and automated system to determine the same analytes in the same matrices. An SPE-clean-up step was included in the method to improve the LOQs. Calibration curves were calculated in methanol and in extracts of the various matrices, and the differences in the slopes were used to assess ME. With the only exception of lamb that induced a signal suppression of ca. 10%, ME was negligible for all other matrices.

A careful selection of the LC–MS conditions can affect the extent of ME. The use of HILIC coupled with hybrid triple-quadrupole/linear ion has been used to determine biogenic amines (cadaverine, histamine, spermidine, spermine, tryptamine, tyramine, and putrescine) in several kinds of cheese (Gianotti et al., 2008). The LC–APCI-MSMS response was strongly affected by signal enhancement, whereas in a previous article by the same research group LC–ESI-MSMS in the positive-ion mode with a C18 LC column, the results were strongly affected by signal suppression (Gosetti et al., 2007). In both cases, ME was assessed with calibration curves based on a standard addition method.

The ionization technique can have a significant influence on limiting ME, as demonstrated by two articles in which the direct EI–LC–MS interface has been used to determine organochlorine (OCPs) and phenoxyacid pesticides in water samples (sea, river, and Milli-Q). After SPE extraction, the samples were analyzed with the LC–MS system described above. ME was evaluated in both articles by comparing the response of an analyte in a standard solution and in a post-extraction spiked sample. Due to the GP ionization, the method is not influenced by ME for the selected compounds (Famigliini et al., 2008, 2009).

VI. CONCLUSIONS

There is no doubt that ME represents a complex problem to address in LC–API-MS quantitative analysis. The fundamental mechanisms that lead to an undesired signal suppression or enhancement are still under investigation, though many steps forward have been recently accomplished toward a deeper understanding of this phenomenon. In general, it is commonly recognized that a careful evaluation of ME is an essential step in any validation process based on LC–MS. In this review article, we focused our attention on the most-recognized mechanisms, and on the strategies to limit and assess ME. Due to the enormous variety of matrices and to the unpredictable effect that they might have on the final results, it is impossible to propose a univocal protocol that could totally circumvent or eliminate ME. As a general rule, each step of the entire analytical process must be optimized to limit the effect of the interfering compounds from the matrix. This aspect becomes particularly relevant with samples at trace level and where the complexity of the matrix can strongly influence the quantification step. Although ME seem impossible to be eliminated completely, many authors report that a careful sample-preparation step, either with the use of new stationary phases or adopting more innovative solutions, is a key point in the whole process. A great attention has been devoted to extensive sample clean-up; however, it is important to keep in mind that, in the case of multiresidue analysis, additional complexity can take place because many compounds with different physico-chemical properties are present in the sample at the same time. Different stationary phases in one or more purification steps have been exploited, although they might be insufficient when high selectivity is required. In this case, MIP can represent a valid solution to isolate the compound of interest from the matrix. Liquid extraction has also been demonstrated to be an effective way to selectively separate the analytes of interest from the interfering compounds.

When the sample-preparation step cannot minimize ME, calibration approaches are needed to compensate for signal variation. The use of an IS is one of the most-used ways; however, because different analytes can show a different behavior in the same matrix, ideally each one needs its own IS. This goal is, of
course, impossible to realize; in particular when SIL-ISs are needed. The matrix-matched calibration approach with a single, or with an analog, IS represents in many cases a convenient alternative.

As an alternative, calibration curves compared with those obtained with blank extracts spiked with analytes (external matrix-matched calibration) are also used to compensate for ME.

All authors agree that an improvement in LC separation is also welcome to reduce ME; as a consequence much effort has been addressed to find the right combination of mobile and stationary phases for the different applications.

When possible, a careful selection of the MS interface can be the right solution; in fact, an APCI interface is less prone to ME than ESI, even if a certain degree of signal variation is reported as well. Alternative techniques, in particular the EI-based direct EI–LC–MS, thanks to a GP ionization process, have demonstrated to be ME-free in matrices of different nature and with a wide variety of analytes.

VII. ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ACE</td>
<td>accelerated solvent extraction</td>
</tr>
<tr>
<td>APCI</td>
<td>atmospheric pressure chemical ionization</td>
</tr>
<tr>
<td>API</td>
<td>atmospheric pressure ionization</td>
</tr>
<tr>
<td>CAP</td>
<td>chloramphenicol</td>
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<tr>
<td>CRM</td>
<td>charge residue model</td>
</tr>
<tr>
<td>CYC</td>
<td>chlortetracycline</td>
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<tr>
<td>DOC</td>
<td>dissolved organic carbon</td>
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<tr>
<td>DXC</td>
<td>doxycycline</td>
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<tr>
<td>EDC</td>
<td>endocrine disrupting compound</td>
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<tr>
<td>EI</td>
<td>electron ionization</td>
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<td>EIC</td>
<td>extracted ion chromatogram</td>
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<tr>
<td>ESI</td>
<td>electrospray ionization</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>GCB</td>
<td>graphitized carbon black</td>
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<tr>
<td>GP</td>
<td>gas phase</td>
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<tr>
<td>GPCho</td>
<td>glycerophosphocholine</td>
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<tr>
<td>HBDC</td>
<td>hexabromocyclododecane</td>
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<tr>
<td>HILC</td>
<td>hydrophilic interaction liquid chromatography</td>
</tr>
<tr>
<td>HPCB</td>
<td>hydroxypropyl-β-cyclodextran</td>
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<tr>
<td>IEM</td>
<td>ion evaporation model</td>
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<tr>
<td>IS</td>
<td>internal standard</td>
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<tr>
<td>LC–API-MS</td>
<td>liquid chromatography–atmospheric pressure ionization mass spectrometry</td>
</tr>
<tr>
<td>LC–MS</td>
<td>liquid chromatography–mass spectrometry</td>
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<tr>
<td>LC–MSMS</td>
<td>liquid chromatography–tandem mass spectrometry</td>
</tr>
<tr>
<td>LC–UV</td>
<td>liquid chromatography–ultraviolet detection</td>
</tr>
<tr>
<td>LLE</td>
<td>liquid–liquid extraction</td>
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<tr>
<td>LOD</td>
<td>limit of detection</td>
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<tr>
<td>LOQ</td>
<td>limit of quantitation</td>
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<tr>
<td>LP</td>
<td>liquid phase</td>
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<tr>
<td>MC</td>
<td>methyl cellulose</td>
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<tr>
<td>ME</td>
<td>matrix effects</td>
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<tr>
<td>MISPE</td>
<td>molecular imprinted solid-phase extraction</td>
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<tr>
<td>MSPD</td>
<td>matrix solid-phase dispersion</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>non-steroidal anti-inflammatory drugs</td>
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<tr>
<td>OTC</td>
<td>oxytetracycline</td>
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<tr>
<td>PE</td>
<td>process efficiency</td>
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<tr>
<td>PPT</td>
<td>protein precipitation</td>
</tr>
<tr>
<td>QuEChERS</td>
<td>quick, easy, cheap, effective, rugged, and safe</td>
</tr>
<tr>
<td>RAM</td>
<td>restricted access materials</td>
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<tr>
<td>RE</td>
<td>recovery</td>
</tr>
<tr>
<td>SA</td>
<td>sulfonamide</td>
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<tr>
<td>SAX</td>
<td>strong-anion exchange</td>
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<tr>
<td>SEB</td>
<td>staphylococcal enterotoxin B</td>
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<tr>
<td>SIL-IS</td>
<td>stable isotope-labeled internal standard</td>
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<tr>
<td>SPE</td>
<td>solid-phase extraction</td>
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<tr>
<td>SRM</td>
<td>selected reaction monitoring</td>
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<tr>
<td>TC</td>
<td>tetracycline</td>
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<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
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<tr>
<td>TFC</td>
<td>turbulent-flow chromatography</td>
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<tr>
<td>TIC</td>
<td>total ion current</td>
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<tr>
<td>UHPLC</td>
<td>ultra-high-performance liquid chromatography</td>
</tr>
<tr>
<td>2D-LC</td>
<td>two-dimensional liquid chromatography</td>
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REFERENCES


Hao C, Zhao X, Tabe S, Yang P. 2008. Optimization of a multiresidual method for the determination of waterborne emerging organic pollutants using...


