

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Identifying and Overcoming Matrix Effects in Drug Discovery and Development

Terence G. Hall, Inese Smukste, Karen R. Bresciano,  
Yunxia Wang, David McKearn and Ronald E. Savage  
*ArQule Inc.*  
USA

## 1. Introduction

Liquid chromatography/tandem mass spectrometry (LC-MS/MS) with electrospray ionization is a highly specific and sensitive analytical technique that has become the industry standard for quantifying drugs, metabolites, and endogenous compounds in biological matrices (e.g. plasma). The technique is widely used because of its ability to accurately quantitate analytes of interest with minimal sample clean-up and rapid LC separation. Despite these advantages, LC-MS/MS methodology occasionally encounters problems, some of which are caused by matrix effects.

The “matrix” refers to all components in the sample other than the analyte(s) of interest. Some common matrices typically encountered by bioanalytical scientists include blood, plasma, urine, bile, feces, and tissue samples. Although these complex matrices have a number of common components, not all are known and levels may vary among individuals. For example, plasma samples obtained from different patients enrolled in clinical trial studies may contain different levels of endogenous components based on their genetics and/or disease state as well as different drugs used to manage their disease. Consequently, each patient’s plasma may have its own particular set of matrix components and therefore is viewed as being a unique sample. Matrix effects can arise from a number of matrix components including, but not limited to:

- Endogenous biological components such as phospholipids, carbohydrates, and endogenous metabolites (bilirubin)
- Residual formulation components from intraperitoneal (ip), intravenous (iv), or oral dosing (po) experiments; for example, polyethylene glycol (PEG), solutol, polysorbate (Tween 80), etc.
- An interaction between the analyte of interest and the matrix, such as covalent binding to plasma proteins or the enzymatic degradation of a prodrug
- Co-eluting drug metabolites
- Concomitant medications
- Mobile phase additives

A matrix effect is generally encountered when a component, for example a phospholipid (present in plasma) co-elutes with the analyte of interest and causes a decrease (suppression) or increase (enhancement) in ionization efficiency relative to the analyte eluting in the

absence of the matrix component. This may result in an erroneous reporting of sample concentrations. In the case of clinical trials, where dosing regimens are being optimized based on pharmacokinetic profiles and are dependant on accurate determination of drug plasma concentrations such miscalculations can lead to errors in determining optimum dosing regimens and in extreme cases failure of a drug in clinical trials.

The degree of enhancement or suppression of ionization of an analyte by a given matrix component can be dependant on the physicochemical properties of the analyte. For example, highly polar compounds generally appear to be affected to a greater degree than less polar molecules which may be due in part to their co-elution with other polar components causing matrix effects.

In most cases, matrix components which might cause ionization enhancement or suppression are removed during the sample clean-up process. The simplest form of sample clean-up is protein precipitation (PPT), in which an organic solvent, commonly acetonitrile (ACN), is combined with the sample of interest enabling most of the protein to be removed from the sample. Two additional methods of sample clean-up that will be discussed in greater detail later in this Chapter are liquid-liquid extraction (LLE) and solid phase extraction (SPE). These methods are more labor intensive, but generally result in better removal of matrix components. Along with sample clean-up, sample dilution is another simple technique effective in minimizing matrix effects.

The exact mechanisms by which matrix components cause ionization suppression (or enhancement) are not known. However, King et al. have postulated that matrix components interfere with the processes involved in the transfer of the charged analyte (ion) into the gas phase thereby increasing or decreasing the ionization efficiency (King et al., 2000).

Preparation of standards in the same matrix as the sample and use of internal standards (ISTDs) which have similar or nearly identical chemical and chromatographic properties to the analyte help in further minimizing matrix effects. For this reason, a stable-isotope-labeled analyte is typically the best choice for an internal standard and in many cases corrects for almost all matrix effects (Xu et al., 2007).

In many cases, it may not be readily apparent that ionization suppression is occurring in a given LC-MS/MS method. Hence, methodologies have been developed to remedy this. A commonly employed technique for detecting ionization suppression involves comparing the LC-MS/MS chromatographic profiles of injections of blank extracted matrix to neat blank matrix (water) which are obtained while continuously infusing diluted analyte solutions post column (prior to entering the MS detector).

Our understanding of matrix effects and how to handle them has continually progressed over the last decade. This Chapter will provide a short review of the current industry perspective on matrix effects.

## 2. Detection of matrix effects

There are several methodologies available to analytical scientists for detection and quantification of matrix effects. Selection of the particular methodology employed typically depends on the stage of the program (discovery or development). For example, compounds in early discovery generally receive a limited qualitative matrix effect evaluation, due to strict timelines for data generation, while compounds in development warrant more detailed quantitative evaluation.

### 2.1 Qualitative determination of matrix effects

Post-column infusion of an analyte, as diagrammed in Figure 1, is a fast and easy technique that can be used to qualitatively identify regions of ion suppression or enhancement in a particular matrix extract. In this technique, an extracted matrix sample is injected onto the HPLC column using the LC-MS/MS method for the analyte, while a steady flow of that analyte is infused into the effluent flow between the column and the mass spectrometer source. Additionally, a blank solution such as water, buffer, or the initial mobile phase mixture must also be injected to determine the baseline for the analysis. The regions of suppression or enhancement can be visualized in the resulting chromatograms by comparing the baseline obtained from the blank with each of the matrices tested (Figure 2). It is important to note that the degree of the effect will depend on the concentration of the analyte being infused. If the concentration of analyte being infused is too high, matrix effects could be masked. Any regions of enhancement or suppression must be compared with the retention time of the analyte. Investigation of matrix effects using this methodology with an internal standard is also highly recommended.

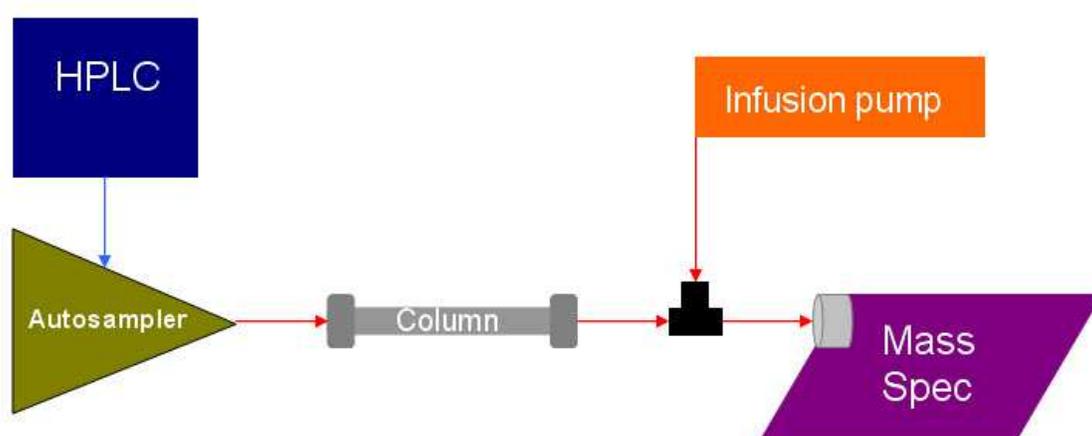


Fig. 1. Post-column infusion schematic.

### 2.2 Quantitative determination of matrix effects

Post-extraction analyte spiking provides a more quantitative measure of matrix effects. One technique involves extracting two sets of samples; one set contains the analyte added to an extracted matrix (post-extraction sample), and the other contains the analyte in mobile phase, solvent, or buffer (external solution). Both sets of samples are prepared with equivalent concentrations of the analyte and then are processed identically. One can quantitate the degree of enhancement or suppression caused by the matrix effect(s) by use of the equations shown below (Matuszewski et al., 2003):

- **Matrix effect (%)** =  $B / A * 100$  (can also be calculated using  $(B-A/A) * 100$ )
- **Recovery (%)** =  $C / B * 100$
- **Extraction Efficiency (%)** =  $C / A * 100$

**A** = external solution peak area, **B** = post-extraction sample peak area, **C** = extracted matrix peak area

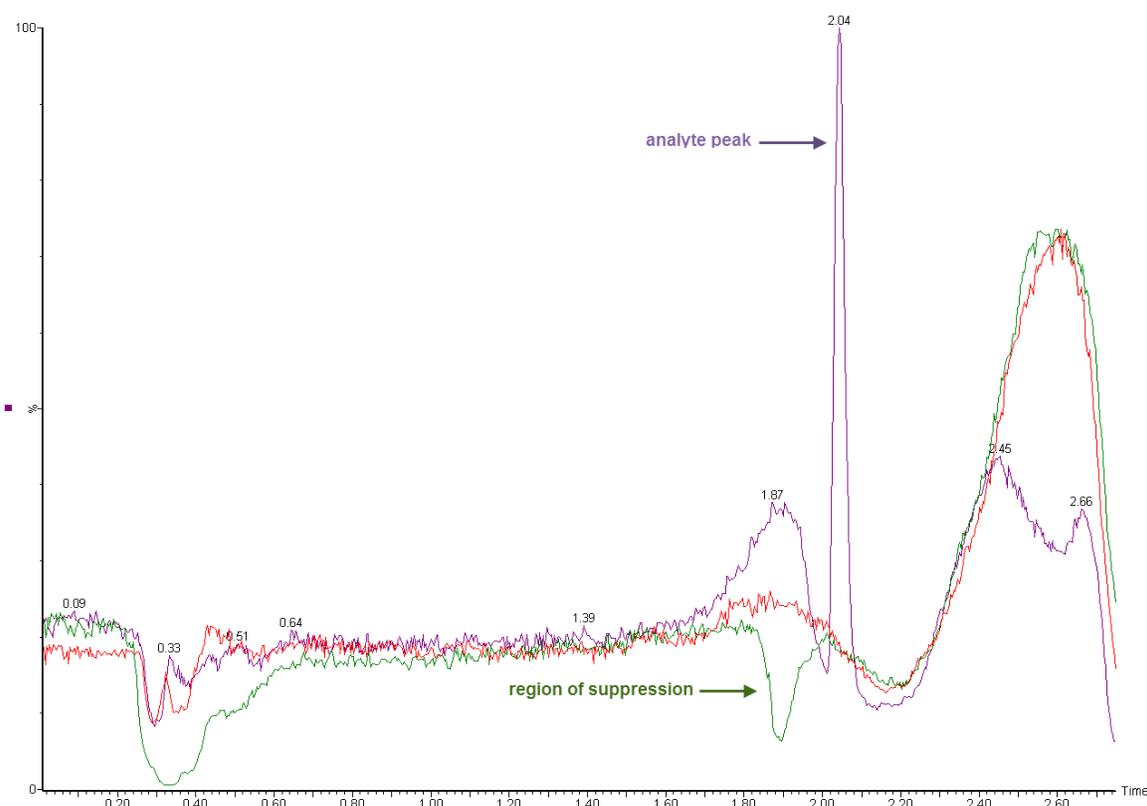


Fig. 2. Infusion test: the red trace is the baseline chromatogram for a solution of 50% acetonitrile in water (reagent blank), the green trace is the extracted matrix blank, and the purple trace is an analyte sample in reagent blank. In this example, the region of suppression does not coincide with the analyte peak. Therefore, this LC-MS/MS method could be used for the analysis of this single analyte, but if metabolite analysis was needed, this method could prove to be inadequate. These experiments would need to be repeated as metabolite standards became available.

Nominal Concentration (ng/mL)	Analyte		Internal Standard	
	External solution peak area	Post-extraction peak area	External solution peak area	Post-extraction peak area
250	5483.6	5674.5	21700.3	21312.5
	5491.5	5478.7	22144.9	21916.0
	5554.8	5278.1	21034.5	19840.3
		5619.4		21238.6
		5171.7		20322.3
		5061.1		20447.8
<i>n</i>	3	6	3	6
Mean	5510.0	5380.6	21626.6	20846.3
SD	39.0	248.7	558.9	769.5
RSD (%)				
Matrix Effect* (%)	-2.3		-3.6	

\*Positive value indicates percent enhancement, negative value indicates percent suppression

Table 1. Example of matrix effect evaluation.

Nominal Concentration (ng/mL)	Analyte		Internal Standard	
	Extracted matrix peak area	Post-extraction peak area	Extracted matrix peak area	Post-extraction peak area
15	12155.0	14473.2	74739.9	88933.0
	12212.1	14139.8	74226.6	90050.2
	11467.6	14473.4	71490.1	92964.1
	9920.8		64027.5	
	12196.4		79932.9	
	13774.6		84727.1	
	<i>n</i>	6	3	6
<b>Mean</b>	11954.4	14362.1	74857.4	90649.1
<b>SD</b>	1253.1	192.5	7102.2	2081.2
<b>RSD (%)</b>	10.5	1.3	9.5	2.3
<b>Recovery (%)</b>		83.2		82.6
90	76391.8	80913.1	70177.3	87189.2
	74081.7	84259.3	74564.5	89830.9
	77464.8	82794.6	77328.6	91620.0
	80999.7		78844.2	
	79771.5		79697.3	
	79202.9		81323.1	
	<i>n</i>	6	3	6
<b>Mean</b>	77985.4	82655.7	76989.2	89546.7
<b>SD</b>	2522.2	1677.4	4048.6	2229.0
<b>RSD (%)</b>	3.2	2.0	5.3	2.5
<b>Recovery (%)</b>		94.3		86.0
750	649097.6	743563.2	75758.1	92358.5
	650131.4	755794.7	78693.2	92230.1
	632140.7	764104.4	76311.1	90620.6
	654594.0		81492.2	
	664467.5		77712.3	
	614953.9		72628.6	
	<i>n</i>	6	3	6
<b>Mean</b>	644230.9	754487.4	77099.3	91736.4
<b>SD</b>	17769.2	10332.8	2987.3	968.4
<b>RSD (%)</b>	2.8	1.4	3.9	1.1
<b>Recovery (%)</b>		85.4		84.0
<b>Overall Recovery (%)</b>		<b>87.6</b>		<b>84.2</b>

Table 2. Recovery for an analyte, at various concentrations, and internal standard (ISTD). The “extracted matrix” samples were spiked with analyte or ISTD prior to extraction, and the “post-extraction” samples were spiked with analyte or ISTD after extraction. The recovery of the analyte does not appear to be affected by concentration.

Matrix effect, as previously stated, is a measure of percent change in signal caused by matrix components. Table 1 shows results from an experiment designed to determine matrix effects. The calculated *matrix effect (%)* shows that the analyte and the internal standard responses in this experiment are not affected by matrix effects. Extraction efficiency is a measure of the percent of total response in an extracted sample when compared to the external solution. Sample recovery describes the percent of analyte that was recovered during the extraction process. Table 2 shows an evaluation of recovery for an analyte and internal standard for three concentrations.

The most rigorous technique for determining matrix effects is typically not performed until a compound advances into regulated animal studies where a Good Laboratory Practice (GLP) method validation is required. It involves analysis of three sets of samples for each matrix being evaluated. Each set is prepared as a series of calibration standards with internal standard; one set is prepared in mobile phase, solvent, or buffer (external solution), the second is prepared by addition of analyte to extracted matrix (post-extraction sample), and the third is prepared by addition of analyte directly into the study matrix (Matuszewski et al., 2003).

### 3. Mechanisms of matrix effects

#### 3.1 Mechanisms of matrix effects

Matrix effects arise at the interface between the LC system and the MS system (King et al., 2000). This interface is referred to as the 'ion source', and it is here that analytes are desolvated and charged. The principals of MS detection dictate that only charged (positive or negative) gas phase ions are detectable. Anything that interferes with either the charging or the desolvation of the analyte will produce a matrix effect.

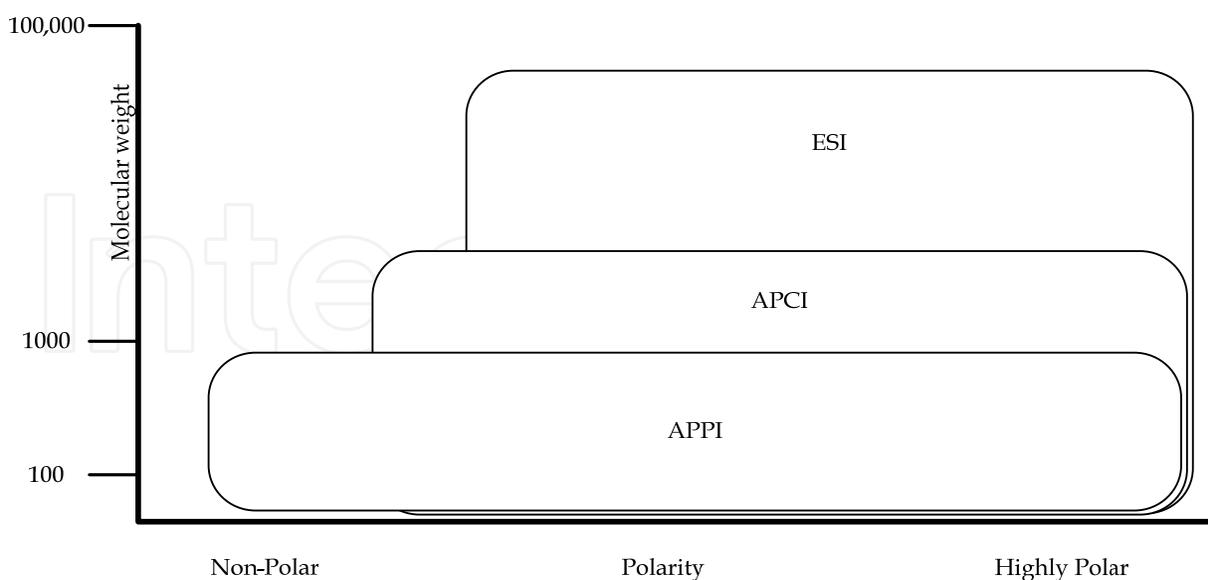


Fig. 3. Applicability of various ion sources depending on analyte polarity and molecular weight.

A complete overview of MS interface design is beyond the scope of the present work; however, some understanding of the different operating principles of the major ion sources is useful for examining the root causes of matrix effects. In modern LC-MS there are a variety of ion sources available from which the analyst can choose. This choice often depends on a number of factors including source availability, type of analyte, and sensitivity of the instrument. Figure 3 shows the three most commonly used ion sources, and their applicability for use based on analyte polarity and molecular weight. Electrospray ionization (ESI) is the most widely used ion source. It has broad applicability to a wide spectrum of analytes of varying molecular weight and polarity, and it is capable of ionizing larger macromolecules such as peptides and proteins. Atmospheric pressure chemical ionization (APCI) is the next most widely used ionization method. Additionally, there are a number of alternative source designs such as atmospheric pressure photo ionization (APPI), nano-spray (low volume ESI), and inductively coupled plasma mass spectrometry (ICPMS). Regardless of the type of source, all share a single goal of transitioning uncharged analytes in solution to detectable gas phase ions.

### 3.2 Electrospray Ionization

In the ESI source, analytes must acquire a charge in solution and then successfully transition to gas phase while maintaining their charge. Figure 4 depicts a generic ESI source. The acquisition of charge in the solution phase and successful transitioning to the gas phase makes the ESI source the most vulnerable to matrix effects when compared to either APCI or APPI (Jessome and Volmer, 2006; King et al., 2000; Truffelli et al., 2011).

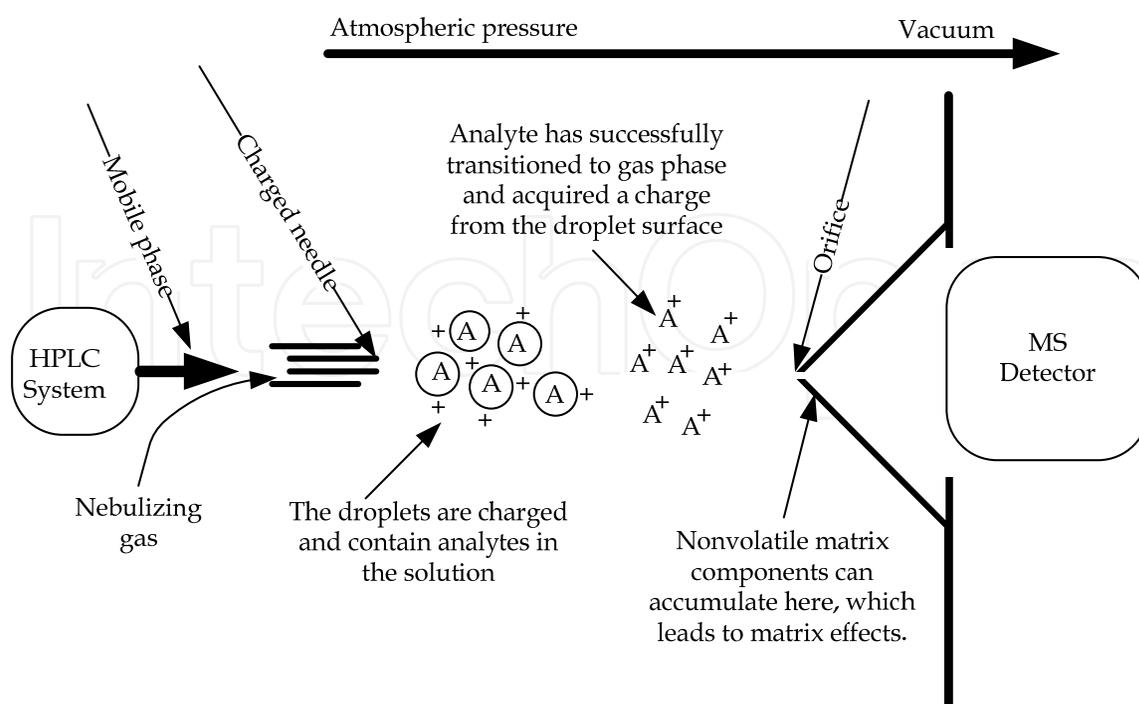


Fig. 4. ESI source.

The mobile phase containing the analyte along with appropriate mobile phase additives such as formic acid or ammonium hydroxide are passed through a charged needle, which transfers this charge to the exterior surface of the solution. At the tip of this charged needle, the solution takes the form of a narrow cone of charged liquid called a Taylor cone. Through the use of nebulising gasses and heat, the droplets are aerosolized. As these charged droplets transverse the ESI source they are progressively reduced in size through evaporation until they become completely desolvated gas-phase ions. These ions are then directed into the orifice of the mass spectrometer. Figure 5 depicts the desolvation of the charged droplets (containing the uncharged analyte) to the charged gas phase ions that are detected by the mass spectrometer.

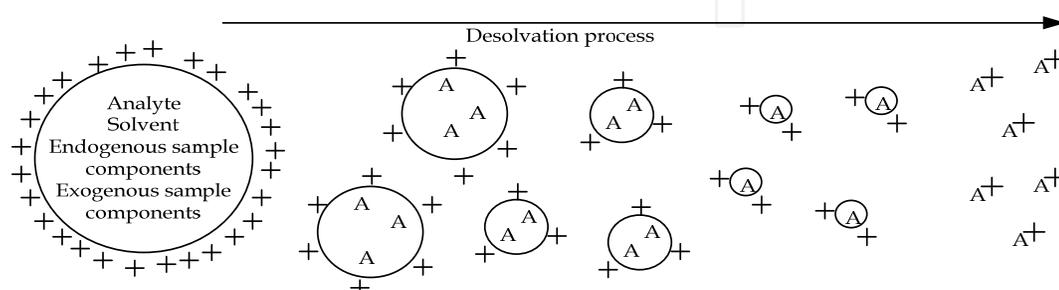


Fig. 5. ESI desolvation process.

Within the ESI source, there are a number of factors which can affect the ability of the analyte to be consistently ionized. Figure 6 depicts some of the causes of matrix effects that may occur while the analyte is still in the liquid phase in an ESI source. Endogenous and exogenous co-eluting sample components can cause suboptimal droplet formation. Oversized droplets, or droplets with non-volatile components, are difficult to completely desolvate and can result in solution phase analyte collecting around the orifice or inside the quadrupole chamber, which can cause signal suppression (Bonfiglio et al., 1999; King et al., 2000). Certain sample components such as the surfactants Tween 80 or PEG 400 have a high affinity for the air-liquid interface of a droplet. As the charge is also localized at the surface of the droplet, this affinity for the surface of the droplet can limit an analyte with less surface affinity from gaining access to the charge (Xu et al., 2005). In addition to surfactants, phospholipids can also interfere with an analyte's access to the surface of the droplet, and thus hinder access to the charges located on the droplet's surface (Bennett and Liang, 2004; Chambers et al., 2007). Ion-pairing reagents, typically used to improve chromatography, can interfere with an analyte's ability to accept a charge. In ESI, the charge is localized on the surface of the droplet; therefore any co-eluting sample component can interfere with an analyte's migration to the surface of the droplet. This interference occurs via competition with the analyte for the charge or repulsion of the analyte away from the surface thereby preventing ionization of the analyte. In charge competition scenarios, the charge will go to analyte or matrix component with the greatest ionization potential at the pH of the mobile phase.

Non-volatile materials, such as phosphate (a common HPLC mobile phase buffer that is generally avoided for LC-MS/MS work), can accumulate inside the MS source housing, on the orifice of the detector, or on the front face of the mass spectrometer's quadrupoles. Samples may also contain components (for example endogenous lipids) that can precipitate inside an ion source. The accumulation of non-volatile materials can increase electrical

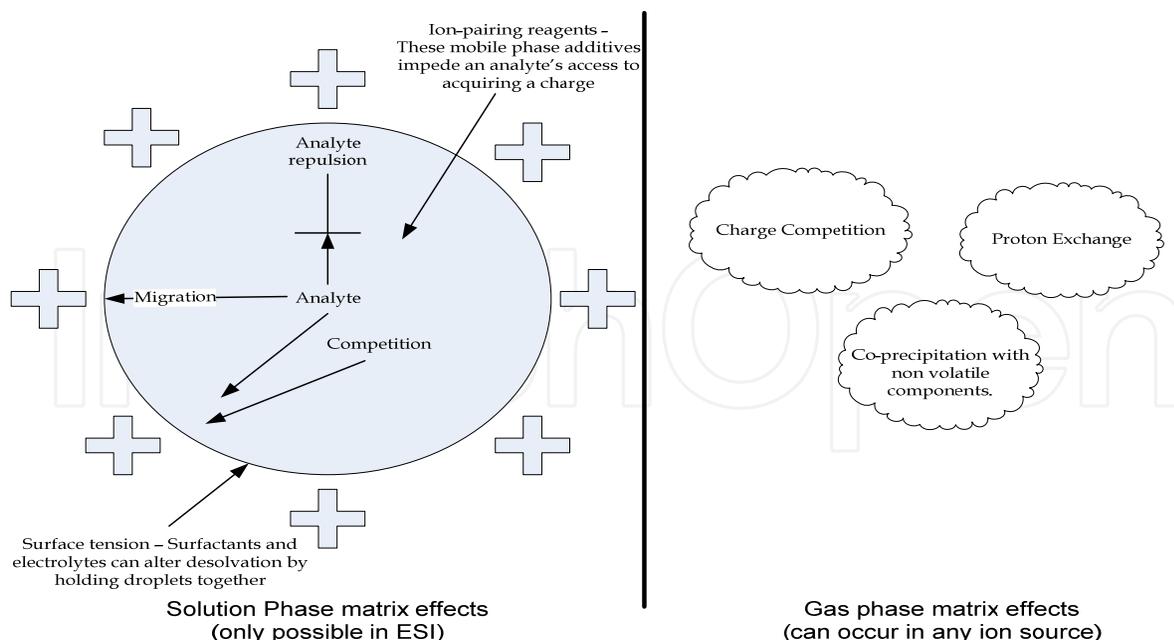


Fig. 6. Mechanisms of ion suppression in LC-MS.

resistance, thereby preventing the ions from following the electromagnetic gradient into the detector, resulting in signal loss (Mei, 2005). These deposits can also block the orifice, physically preventing entrance into the MS detector.

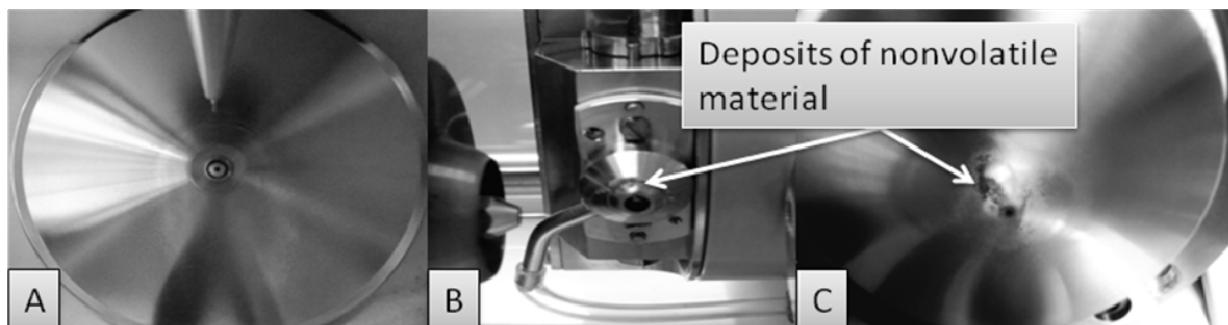


Fig. 7. Accumulation of non-volatile components in ESI sources: A: shows a recently cleaned source with no accumulations; B: shows a crystalline ring of deposits resulting from polar matrix components and endogenous electrolytes; C: shows a slightly greasy smear around the orifice that may be the result of insufficient sample clean-up of endogenous lipids.

In modern mass spectrometers, the LC inlet is orthogonal to the MS orifice. This configuration allows ions to follow the electrical charge gradient to the orifice and into the MS. Previous generations of LC-MS/MS instruments were designed with the LC inlet and the MS orifice aligned in a linear placement. This linear source design tended to have more issues with matrix effects and non-volatile accumulation in the source and the front of the MS quadrupoles, which often resulted in degradation of MS performance more rapidly. Figure 7 shows the orifices of several LC-MS/MS systems including clean and with build up of non-volatile components.

Nano-scale ESI, a variant of conventional ESI sources that relies on ultra low amounts of solvent, has been reported to reduce matrix effects (Chiu et al., 2010). The reduced volumes

used in nano-scale ESI sources diminish the potential impact of matrix effects on the droplet formation and desolvation.

### 3.3 Atmospheric pressure chemical ionization

Atmospheric pressure chemical ionization (APCI) is an alternate method of analyte ionization to ESI. Although less susceptible to matrix effects, APCI can have significantly lower ionization efficiencies for some analytes. In such cases, the loss of analyte signal sensitivity must be weighed against the reduction of matrix effects (Trufelli et al., 2011). APCI, unlike ESI, does not rely on solution phase analyte charging, therefore many of the matrix effects due to droplet formation and phase transformation are eliminated. Instead, desolvation of the analyte occurs almost instantly in a heated ceramic vaporizing collar at the inlet from the LC. However, the high temperature required for desolvation makes APCI unsuitable for the quantitative analysis of thermally labile molecules. Figure 8 depicts a typical APCI ion source.

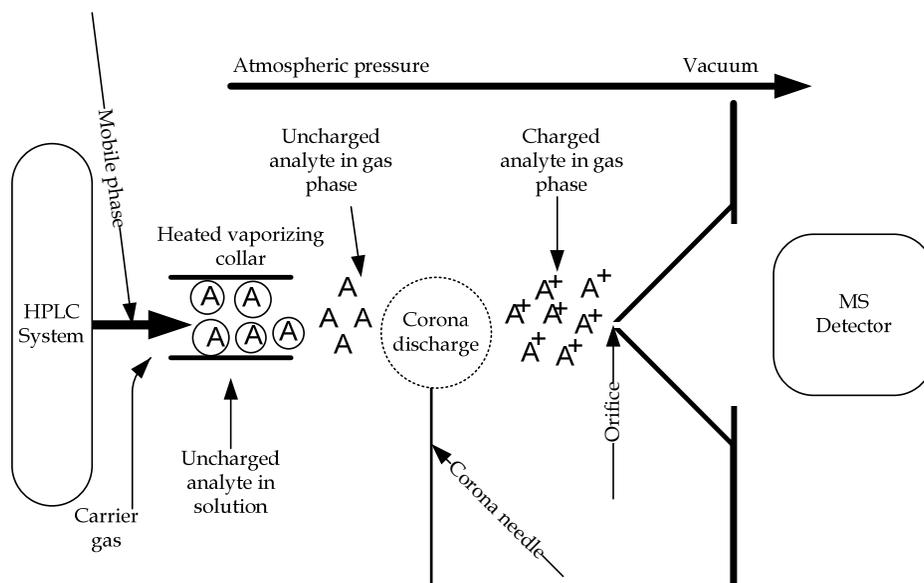


Fig. 8. APCI source.

APCI is not immune to matrix effect issues of its own (Gosetti et al., 2010). In APCI, ion creation occurs as the desolvated neutral analyte and solvent molecules pass through the gas phase solvent ions (plasma) produced by the corona needle. The corona needle discharges electrons that initially charge the gas phase solvent molecules, and this charge is then transferred to the analyte. The ionization process must occur with a limited amount of transferrable charge in a very short amount of time. Thus, co-eluting species passing simultaneously through the corona often compete with the analyte for available charge. In a positive ionization mode, any component with a higher pKa can affect analyte signal intensity (van Hout et al., 2003). For example, Tween 80, a common excipient in drug formulations, competes with the analyte for charge thereby lowering the ionization efficiency of the analyte (Xu et al., 2005). Co-precipitation of analyte with non-volatile matrix components can also play a role in APCI signal suppression (van Hout et al., 2003).

### 3.4 Atmospheric pressure photo ionization

Atmospheric pressure photo ionization (APPI) is a recently introduced ionization source that achieves ionization by channeling the uncharged gas phase sample molecules through a charged photon beam. APPI has been demonstrated to be less susceptible to matrix effects than ESI and APCI. APPI, like APCI, ionizes the analyte in the gas phase, eliminating potential issues that arise from solvent phase ionization. Additionally, APPI produces higher energy protons that can overcome potential charge competition between the analyte and solvent or extraneous materials, which sometimes occurs in APCI sources (Gosetti et al., 2010). Figure 9 shows an APPI source diagram.

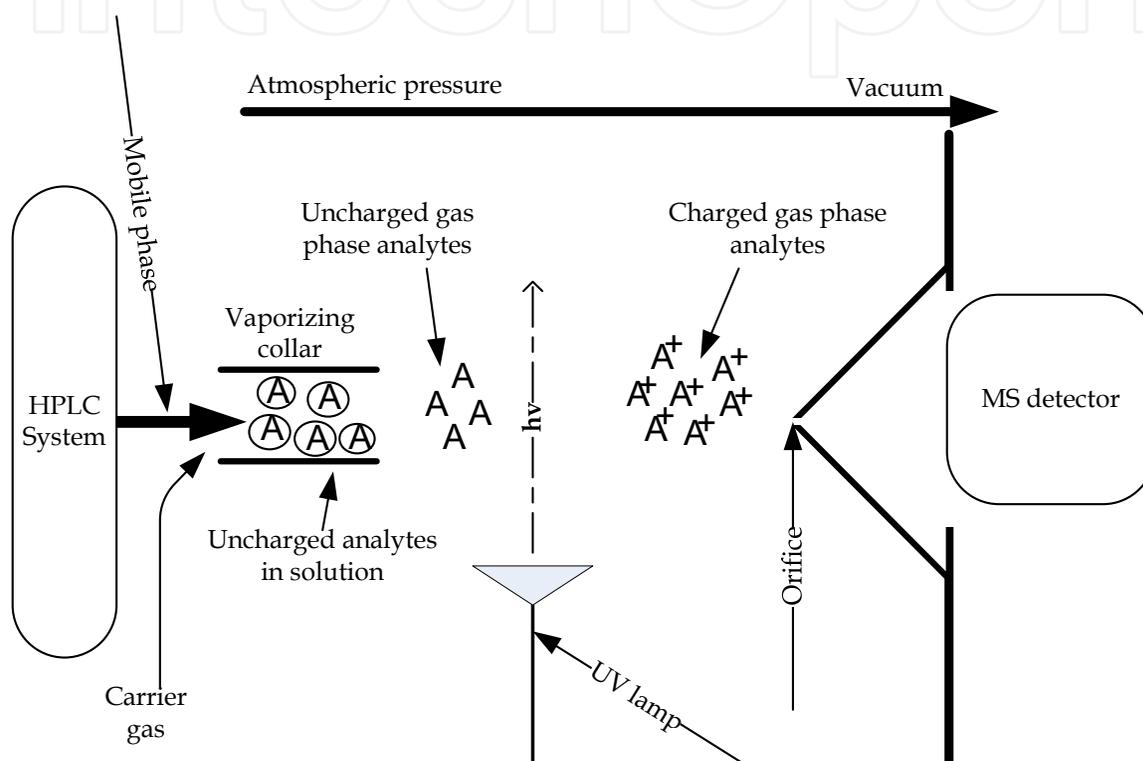


Fig. 9. APPI interface.

### 3.5 General Considerations

In experiments with multiple analytes, it is necessary to evaluate potential matrix effects for each analyte. Many factors, including pKa, solvation energy, solvent properties, etc., affect electrospray ion formation. Furthermore, each of these factors can impact various analytes within a mixture differently (King et al., 2000).

In general, the consensus is that APCI and APPI systems are less prone to suffer from matrix effects than ESI, however, each method has its advantages and disadvantages (King et al., 2000). In certain matrices and for certain analytes, no significant improvement over ESI sources was observed, emphasizing that matrix effects should be assessed on a compound-by-compound and matrix-by-matrix basis (Lien et al., 2009).

Although it may be possible to resolve an observed matrix effect by changing sources, this is not always an option. For example, analytes often exhibit different ionization efficiencies depending on the mode of ionization. Moreover, changing ion sources does not guarantee

elimination of matrix effects. A better option is to understand the specific cause of the matrix effect and then address it directly.

#### 4. Sources of matrix effects

Ionization suppression and/or enhancement due to sample matrices have become one of the most important causes for failures and errors in bioanalysis. Matrix effects are subdivided into two groups: 1) endogenous matrix effects caused by components naturally occurring in the biological or environmental sample and 2) exogenous matrix effects caused by components introduced prior to or during sample collection and analysis.

Commonly encountered sources of matrix effects are summarized below:

- Endogenous components of matrices such as lipids, phospholipids, proteins, and bile salts
- Exogenous components of matrices that are introduced during analysis such as formulation excipients, leachables from the labware, anticoagulants, analyte stabilizers used during sample collection, and reagents used in the preparation of bioanalytical samples
- Degradation products of the analyte created during sample preparation and analysis (e.g. degradation products of prodrugs and compounds sensitive to pH, temperature, or light)
- Impurities and salts contained in analytes and ISTDs
- Poor recovery of analytes due to binding to biological matrices or containers (e.g. non-specific binding of hydrophobic analytes to plastic)
- Solvents and additives used for LC
- Xenobiotics and their metabolites present in analytical samples (e.g. other drugs present in patient samples)

The sources of matrix effects are extremely diverse and are analyte-, LC-MS/MS method- and ion source-dependent. Therefore, great care should be taken during method development and validation to identify potential issues (Matuszewski, 2006; Taylor, 2005; Trufelli et al., 2011; Vogeser and Seger, 2010).

During the bioanalytical method development and validation for GLP studies, many of the exogenous matrix effects are addressed by careful evaluation of the reagents and supplies used for sample collection, preparation, and analysis. However, some matrix effects are difficult to identify and prevent during early stage drug discovery when a large number of diverse compounds are evaluated in a high-throughput manner and general LC-MS/MS methods are used. The problem is exacerbated by matrix effects resulting from impurities present in investigational compounds, such as inorganic and organic salts, and degradation products. Some exogenous and endogenous matrix effects are well known and have been described in literature, but many remain undetermined and are rarely explored in detail. A limited number of extensive and systematic studies have been performed to assess the broad nature of matrix effects providing much needed information regarding mechanisms of matrix effects in general and methods for their elimination (Chambers et al., 2007; Ismaiel et al., 2010; Little et al., 2006; Mallet et al., 2004; Marchi et al., 2010; Muller et al., 2002; Tong et al., 2002; Xu et al., 2005). In contrast, most of the current knowledge is based on studies focused on specific matrix effects. In every day practice, ion suppression is often overcome using empirical methods such as modification of LC-MS/MS methods, substitution of

HPLC columns, more rigorous sample clean-up, and optimization of labware and reagents without identifying or fully exploring the underlying causes.

#### **4.1 Exogenous components leading to matrix effects**

##### **4.1.1 Eluents and additives**

The composition of the mobile phase profoundly influences the ionization efficiency of all sample components due to the mechanisms discussed previously (Gao et al., 2005; Kostianen and Kauppila, 2009). Multi-fold increases in signal of the analyte can be achieved by modifying composition of the mobile phase during the LC-MS/MS analysis. For example, an increase in the organic solvent portion of the mobile phase usually leads to increased ionization efficiency (Dams et al., 2002). Such modifications can also lead to changes in retention times of analytes and matrix components thereby altering elution patterns and potentially introducing matrix effects.

Acidic, basic, ion-pairing or buffer salt additives in the mobile phase may have signal suppression or enhancement effects. Mallet and colleagues performed a systematic analysis of formic acid (FA), acetic acid (AA), trifluoroacetic acid (TFA), ammonium hydroxide, ammonium formate, ammonium biphosphate, ammonium bicarbonate, and nonafluoropentadecanoic acid as mobile phase additives and studied their effect on the MS signal intensity of a diverse set of compounds (Mallet et al., 2004). Ionization enhancement or suppression was highly dependent upon each additive, analyte and ionization mode. Consistent with numerous other reports, TFA strongly suppressed ionization of all tested compounds in both positive and negative ionization modes. The signal suppressing effects of TFA and other fluorinated acids are due to the ion-pairing and surface tension effect (as described previously), which disrupts the ionization of the analyte (Gustavsson et al., 2001). The post-column addition of a propionic acid and 2-propanol mixture may counteract the deleterious effects of TFA by facilitating TFA evaporation during ionization (Apffel et al., 1995). Benijts and colleagues reported that addition of acids resulted in significant signal suppression in the analysis of environmental water samples for 35 endocrine disrupting chemicals in negative and positive ESI, while 1 mM ammonium formate reduced the matrix effect. Further improvements were achieved by sample clean-up and use of SIL-ISTDs (Benijts et al., 2004).

Additionally, reagents used during sample collection and preparation that are retained in the analytical sample may lead to matrix effects. Mei et al. reported that commonly used anticoagulant Li-heparin enhanced ionization efficiency for some of the analytes (Mei et al., 2003). Tris(hydroxymethyl)aminomethane (TRIS) buffer and nicotinamide adenine dinucleotide phosphate (NADPH) led to significant matrix effects during the analysis of 27 highly diversified pharmaceutical compounds in microsomal samples when PPT was utilized for sample clean-up (Zheng et al., 2002). SPE based sample clean-up was shown to significantly reduce these matrix effects. Phosphate buffer and non-volatile ion-pairing reagents such as sodium dodecyl sulfate (SDS) lead to severe ion suppression due to accumulation in the source as discussed in Section 3.2.

##### **4.1.2 Leachables and impurities**

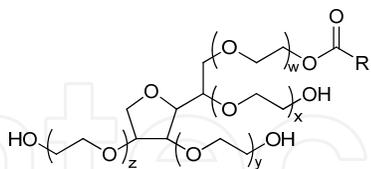
Matrix effects caused by polymers and plasticizers leaching from common labware are often overlooked (Guo et al., 2006; Mei et al., 2003; van Hout et al., 2003). Importantly, ion source contamination with these polymers may lead to long-lasting interference with analysis.

Particular care should be exercised when organic solvents such as ethyl acetate, dichloromethane, or ethers are used with polymer-based containers, caps, or solid phase materials, as they may solubilize polymers and plasticizers. Minor solvent impurities and additives may also affect the accuracy of analyses (Annesley, 2007).

#### 4.1.3 Formulation agents

Some formulation agents may also lead to matrix effects. In particular, excipients used in early drug discovery for solution or suspension formulations are known to interfere with analyses. These excipients are present in plasma at high concentrations (>1 mg/mL) in early PK sampling time points. In studies conducted by Xu and colleagues, 20% hydroxypropyl- $\beta$ -cyclodextrin (HP $\beta$ CD) or 0.4% methyl cellulose vehicles did not lead to matrix effects either during iv or po dosing. Conversely, when 0.1% Tween 80 was used as a vehicle, 50-80% ion suppression was observed for both iv and po administration routes (Xu et al., 2005). These results are supported by several other researchers (Larger et al., 2005; Shou and Naidong, 2003; Tong et al., 2002). Moreover, polysorbates including Tween 80 are complex mixtures of components containing polyoxyethylene (POE) sorbitan and POE sorbitan monoesters (Figure 10). POE sorbitan contains approximately twenty ethylene oxide subunits arranged in four chains of various lengths. The oleate monoester accounts for 58–85% of esters in Tween 80, while the remaining esters have alkyl chains from C14 to C18 and include stearate, linoleate, and linolenate esters. Therefore, the composition of Tween 80 is quite variable and depends on the manufacturing process (Hewitt et al., 2011). Additionally, Tween 80 is rapidly hydrolyzed to oleic acid and polyethoxylated sorbitan by esterases in rodent plasma leading to further variability in analyses (Larger et al., 2005; van Tellinghen et al., 1999). Dosing vehicles containing PEG 400 also lead to ionization suppression, in particular, for early eluting compounds (Shou and Naidong, 2003; Tong et al., 2002; Weaver and Riley, 2006; Xu et al., 2005). Co-infusion of PEG 400, Tween 80, or HPCD with an analyte in 70% ACN solution (pH 8) led to significant LC-MS signal suppression in positive and negative ionization modes, while co-infusion with propylene glycol (PG) resulted in the lowest interference among of the four (Tong et al., 2002).

##### Polysorbitan



Number of polyethylene units:  $w+x+y+z = 20$

R = fatty acid alkyl chain

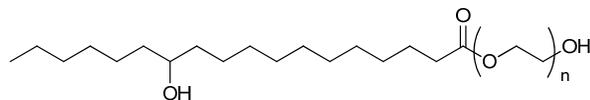
Polysorbate 20 - lauric acid ester

Polysorbate 40 - palmitic acid ester

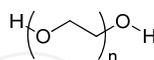
Polysorbate 60 - stearic acid ester

Polysorbate 80 - oleic acid ester

##### Solutol® HS 15



##### Polyethylene glycol 400 (PEG400)



MW 380-420 g/mol

##### Propylene glycol (PG):

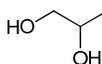


Fig. 10. Commonly used formulation agents.

#### 4.1.4 Cross-talk

Cross-talk occurs with compounds that produce fragments with similar masses. Typically, MRM dwell times and inter scan times are very short. Hence, fragment ions from one transition can still be present in the collision cell when the next MRM transition is monitored, leading to false positive signal. Most commonly cross-talk is observed when structural analogs or SIL-ISTDs are used as internal standards, or when metabolites of the analyte are present in the sample. On rare occasions, structurally unrelated compounds may produce ion fragments with an identical mass leading to cross-talk. Separate analysis of analytes and ISTDs while monitoring all MRM ion transitions is the most reliable method for cross-talk detection. Similar evaluation should be done with known metabolites or analyte degradation products when feasible. The simplest way to eliminate cross talk is to enter a “dummy” or blank MRM transition between mass transitions where cross-talk is observed. The dummy or blank transitions allow the collision cell to completely clear the ions from the previous mass transition. Additionally, cross-talk can be eliminated by reducing the amount of ions entering the collision cell. Therefore, dilution of the analyzed sample, reduced concentrations of ISTDs, or chromatographic resolution of the analyte and the ISTD is typically used to eliminate cross-talk (Morin et al., 2011).

#### 4.1.5 Stable-isotope-labeled internal standards

Stable-isotope-labeled internal standards (SIL-ISTDs) are routinely used in pharmacokinetic analyses and in clinical and forensic toxicology to mitigate matrix effects during LC-MS/MS analysis (Stokvis et al., 2005). SIL-ISTDs are compounds where several atoms of the analyte molecule have been replaced with their stable isotopes. Most commonly hydrogen ( $^1\text{H}$ ) is exchanged for deuterium ( $^2\text{H}$ ) and carbon ( $^{12}\text{C}$ ) is exchanged for carbon ( $^{13}\text{C}$ ), however, nitrogen ( $^{15}\text{N}$ ) and oxygen ( $^{17}\text{O}$ ) labeled SIL-ISTDs also can be used. At least three atoms should be exchanged during the labeling to avoid interference between analyte and SIL-ISTD signals due to cross-talk or signal contribution caused by isotope distribution. Furthermore, SIL-ISTDs should be of high isotopic purity and stable during the analysis. For example, hydrogen-deuterium exchange has been observed in aqueous solutions, therefore  $^{13}\text{C}$  labeling is preferred (Chavez-Eng et al., 2002).

SIL-ISTDs possess nearly identical physicochemical properties compared to their non-labeled counterparts. Therefore, one may expect nearly identical retention times during chromatographic separation and similar behavior in the ion source. Consequently, SIL-ISTD and an analyte should be exposed to the same ionization conditions and normalization relative to SIL-ISTD should minimize variability during the sample analysis. However, on occasion SIL-ISTDs themselves may lead to ion suppression or enhancement of the analyte (Liang et al., 2003; Remane et al., 2010b). To minimize SIL-ISTDs matrix effects, appropriate concentrations of SIL-ISTDs should be employed and linearity of the response should be tested. In addition, there have been reports that SIL-ISTDs are affected differently by matrix effects than the analyte leading to inaccurate quantification (Lindegardh et al., 2008; Jemal et al., 2003; Wang et al., 2007).

#### 4.1.6 In-source fragmentations

Compounds with weak bonds, such as glucuronide- and sulfate-conjugated metabolites, may fragment in the source during the ionization process, thereby regenerating the parent molecule (Figure 11). When the analyte and its metabolite(s) are not resolved via

chromatography, in-source fragmentations may lead to inaccurate quantification of the analyte (Vogeser and Seger, 2010; Vogeser et al., 2001; Yan et al., 2003).

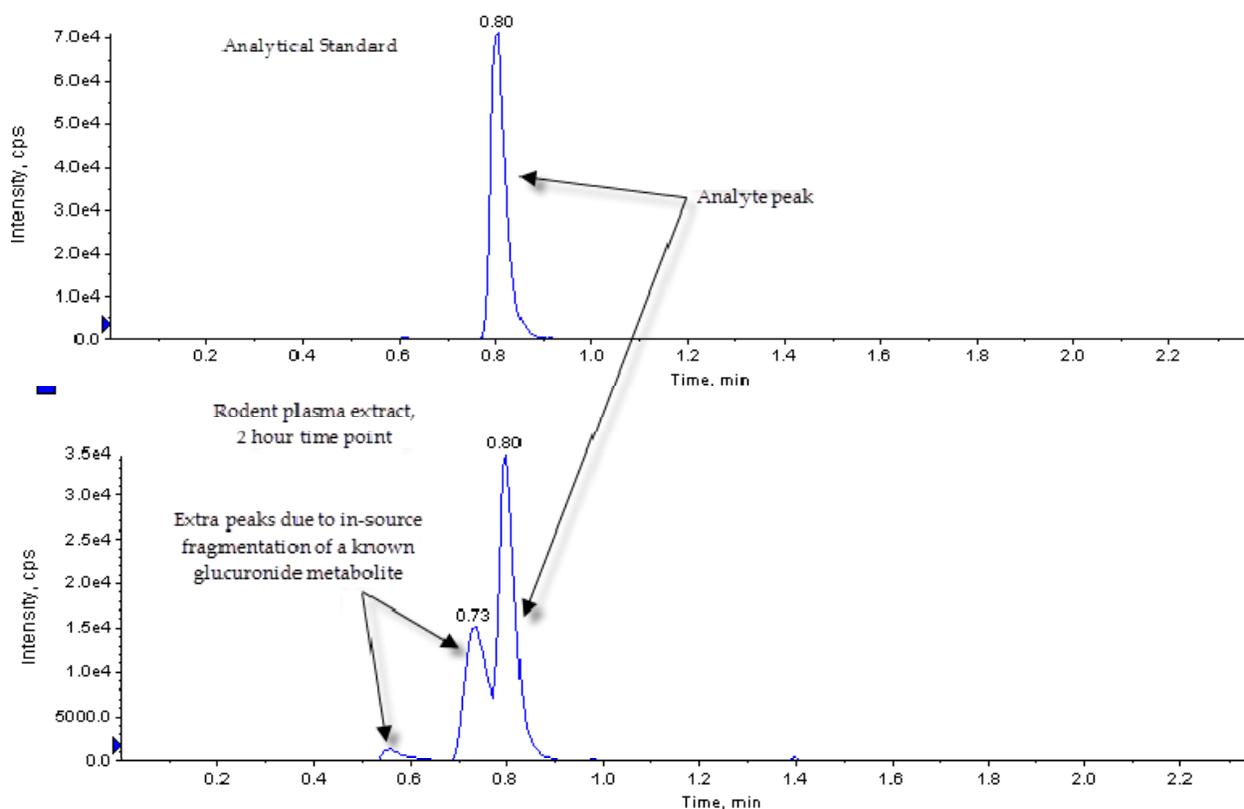


Fig. 11. In-source fragmentation of a glucuronide metabolite resulting in interference of the parent analyte peak.

#### 4.2 Matrix effects caused by endogenous components

Endogenous matrix effects are caused by components which naturally occur in the matrices (Table 3). Plasma and urine are the most commonly used biological samples for LC-MS/MS analysis, however, feces, saliva, bile, and tissue homogenates are occasionally analyzed as well. Each of these biological matrices contains distinct endogenous components that may lead to different matrix effects and require specific protocols for sample preparation. Furthermore, substantial variations in matrix effects can be observed in samples from different individual subjects due to genetic variation, disease state and/or the presence of other xenobiotic compounds (Remane et al., 2010a). Detection of endogenous substances, compounds with limited stability in biological matrices, and trace analytes are particularly prone to matrix effects.

In general, polar compounds are affected more by endogenous matrix effects than non-polar compounds, because many components of biological matrices and environmental samples are polar, water-soluble compounds and are eluted early during a reverse phase (RP) chromatography (Bonfiglio et al., 1999; Muller et al., 2002).

Matrix	Components	Amount
Blood	Plasma	~55%
	Red blood cells	~45%
	White blood cells	>1%
Plasma	Water	~90%
	Protein	~8%
	Inorganic salts	0.9%
	Organic substances (lipids, hormones, vitamins etc.)	1.1%
Urine	Water	95%
	Urea	9.3 g/L
	Chloride	1.87 g/L
	Sodium	1.17 g/L
	Potassium	0.75 g/L
	Creatine	0.67 g/L
	Other ions and compounds	lesser amounts
Liver bile*	Water	90-95%
	Total solids	5-10%
	Bile salts (bile acids)	3-45 mM
	Bilirubin	1-2 mM
	Phospholipids	150-800 mg/dL
	Free Cholesterol	80-200 mg/dL
	Protein (total)	2-20 mg/dL
	Glutathione	0-5 mM
	Sodium	140-170 mM
	Potassium	2.7-6.7 mM
	Calcium	2.5-6.4 mM
	HCO <sub>3</sub> <sup>-</sup>	12-55 mM

\*Data adopted from (Dancygier, 2010)

Table 3. General composition of biological matrices.

#### 4.2.1 Plasma

Plasma contains dissolved proteins, amino acids, peptides, glucose, carbohydrates, vitamins, electrolytes, hormones and lipids, all of which may lead to matrix effects. Serum protein is a major component of plasma with concentrations ranging from 6.0 to 8.3 g/dL. Albumins, globulins, and fibrinogens constitute approximately 60%, 18% and 4% of the total serum plasma protein, respectively. Albumin serves as a transport protein for carrying large organic anions, such as fatty acids, bilirubin, drugs, and hormones, such as cortisol and thyroxine. Albumin concentrations may vary among individual subjects and are affected by dehydration, protein malnutrition, kidney and liver disease, etc. Non-specific binding of analytes to albumin and globulins or specific binding to particular plasma proteins may impact analyte recovery during the analytical sample preparation. High affinity, specific binding may be of particular concern during analysis of naturally occurring compounds, their analogues, and highly potent drugs. For example, analysis of vitamins D2, D3, and their metabolites requires disruption of protein binding and longer chromatography methods to avoid matrix effects (Casetta et al., 2010; Hollis, 2007; Vogeser and Seger, 2010).

In most cases, analyte binding to proteins can be disrupted by organic solvent, acid, inorganic salt, or metal ion treatment. In MS/MS analysis acids, salts, or metal ions may lead to undesired ion suppression on their own and therefore are rarely used. Organic solvent based PPT efficiently removes most of the protein and does not require post-precipitation processing of samples. In a study conducted by Polson et al., a volume ratio of 2.5:1 of precipitant to plasma was found to maximize protein removal while minimizing sample dilution. Under these conditions, ACN precipitation removed >97% of protein, while methanol removed ~94% of protein (Polson et al., 2003). LLE and SPE also rely on use of organic solvents to efficiently disrupt analyte-protein interactions and remove proteins from the samples; therefore, proteins rarely lead to matrix effects in LC-MS/MS analysis of small molecules. However, endogenous amino acids and peptides can interfere with protein, peptide and peptidomimetic analysis. Albumins and immunoglobulins along with other abundant plasma proteins lead to significant ion suppression in proteomics and interfere with the detection of less abundant proteins (Ahmed et al., 2003; Lo et al., 2009). Recently, significant efforts have been devoted to the development of LC-MS/MS-based biomarker assays for diagnostic purposes (Ahmed et al., 2003; Apweiler et al., 2009; Pusch et al., 2003). Affinity chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-page) can be utilized to deplete plasma samples of highly abundant proteins and improve detection limits of biomarkers (Ahmed et al., 2003; Borg et al., 2011; Liu et al., 2009).

Other plasma matrix components such as salts, sugars, amino acids, lipids, and vitamins are not removed as efficiently by PPT, while LLE or SPE are better suited for this purpose as will be discussed in section 5.1. The highly polar components of plasma such as electrolytes, amino acids, glucose, and vitamins rarely interfere with analysis of non-polar organic compounds typically encountered in the drug development process, as they are easily separated by HPLC. However, they may lead to matrix effects when very rapid LC methods are used.

Lipids are considered the main culprit of matrix effects in blood and plasma samples. Plasma contains thousands of distinct lipids comprised of six main categories including fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterols, and prenols (Figure 12) (Quehenberger et al., 2010). Phospholipids are present in plasma at extremely high concentrations, with glycerophosphocholines and lysophospholipids constituting up to 70% and 10% of the total plasma phospholipids, respectively. Phospholipids have a polar head group that contains an ionizable negatively charged phosphate group and a positively charged amine group that are responsible for strong ion suppression in both positive and negative ionization modes (Chambers et al., 2007). In addition, phospholipids contain one or two fatty acid esters that are responsible for the hydrophobicity of phospholipids. In general, ACN extracts contain significantly lower amounts of residual lipids than methanol or acetone extracts (Chambers et al., 2007; Ismaiel et al., 2010). LLE can provide cleaner samples, but lipid separation depends heavily on pH and organic solvents used for the extraction (Chambers et al., 2007; Ismaiel et al., 2010; Muller et al., 2002). Non-polar lipids such as triacylglycerols, cholesterol, and cholesterol esters have good solubility in hexane, chloroform, and ethers and will be extracted with these solvents from plasma. On the other hand, polar lipids, such as phospholipids, are more soluble in polar solvents, such as methanol and ethyl acetate. Therefore, it is important to determine which lipids lead to matrix effects and select solvents for LLE or SPE that will not extract these lipids. Since phospholipids are more likely to lead to pronounced matrix effects, the use of less polar

solvents, such as methyl tert-butyl ether (MTBE) and MTBE-hexane mixtures for LLE can help to minimize matrix effects caused by phospholipids (Bennett and Liang, 2004; Ismaiel et al., 2010). Similar considerations apply to SPE sample clean-up, but derivatized solid-phase columns can provide efficient phospholipid removal (Aurand and Trinh, 2009; Chambers et al., 2007).

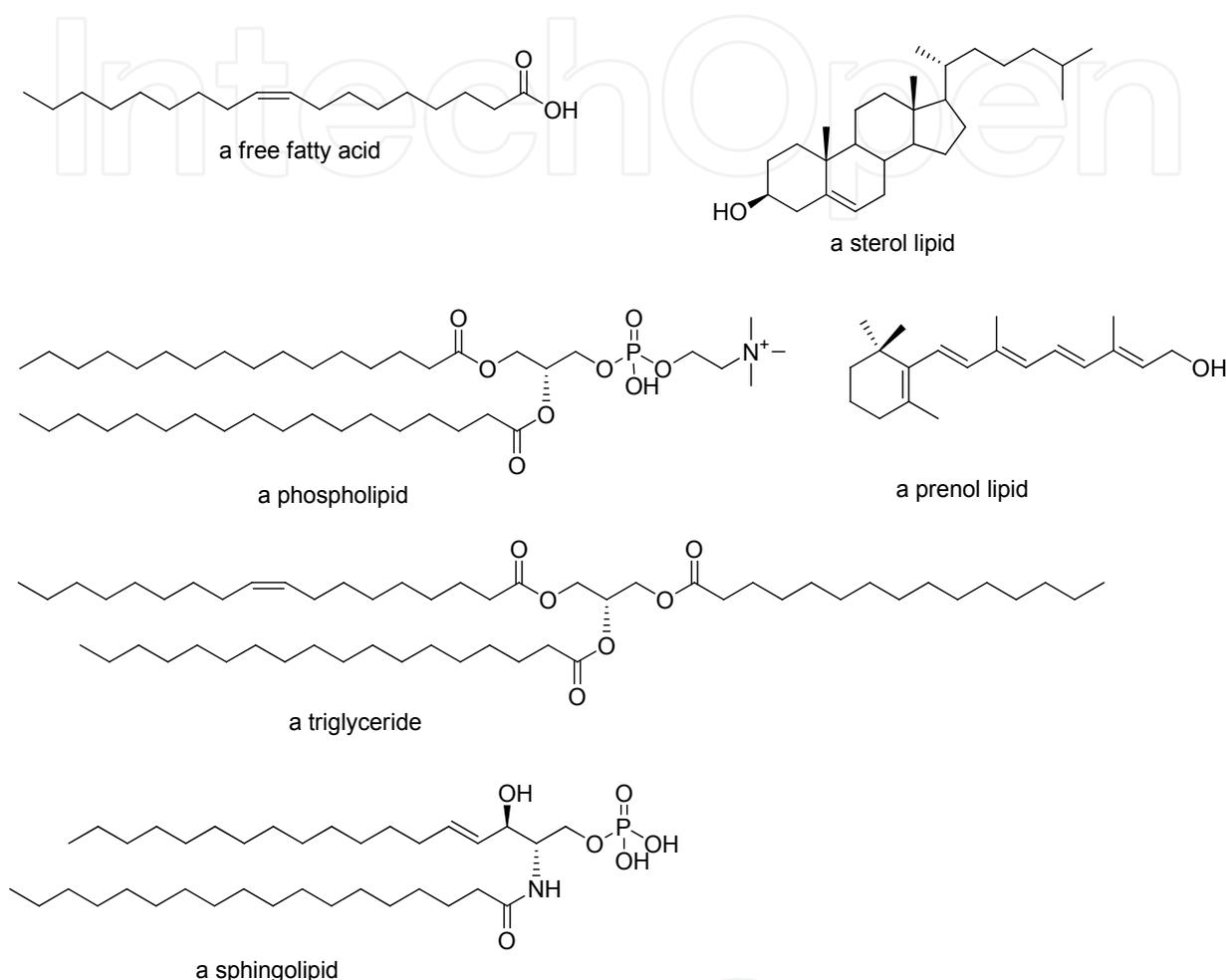


Fig. 12. Representative structures of endogenous lipid classes in human plasma (Fahy et al., 2005).

Chromatographic resolution of non-polar analytes from phospholipids is complicated by several aspects. Among them, elution of phospholipids from RP columns requires long run times and high organic solvent concentrations (60-90%) which may lead to co-elution with many non-polar drug-like compounds. Moreover, plasma contains numerous phospholipids that elute at various retention times collectively leading to broad ion suppression regions. Finally, lipid and phospholipid concentrations and composition in plasma can vary greatly between individuals leading to high variability in the observed matrix effects. When rapid gradients and short LC methods are used, phospholipids are retained on the column leading to accumulation and subsequent degradation of the column, as well as sporadic elution of lipids. Ultimately, a more thorough sample preparation/clean-up method may be required to remove these lipids.

### 4.2.2 Urine

In comparison to plasma, urine has much lower protein content and fewer matrix components. It primarily contains highly polar urea, inorganic salts, creatinine, and low quantities of carbohydrates, hormones, proteins, and metabolites of endogenous compounds (e.g. steroid metabolites, 2-oxoglutarate, succinate, citrate, dimethylglycine, trimethylaminoxide and taurine) and xenobiotics (e.g. xenobiotic glucuronides). Therefore, matrix effects are observed less frequently and simple sample dilution is sufficient to resolve observed matrix effects (Bell et al., 2011; Schreiber et al., 2007; Schreiber and Formal, 2007). However, it is important to note that, the composition and pH of urine is considerably more variable compared to plasma and is affected by age, gender, disease state, and dietary habits. Thus, pH adjustment of samples may be needed and calibration samples may not be representative of the matrix from individual samples.

## 5. Overcoming matrix effects

Strategies for overcoming issues associated with matrix effects will be reviewed in this section. Figure 13 and Table 4 summarize the various method modifications that can be applied to eliminate matrix effects.

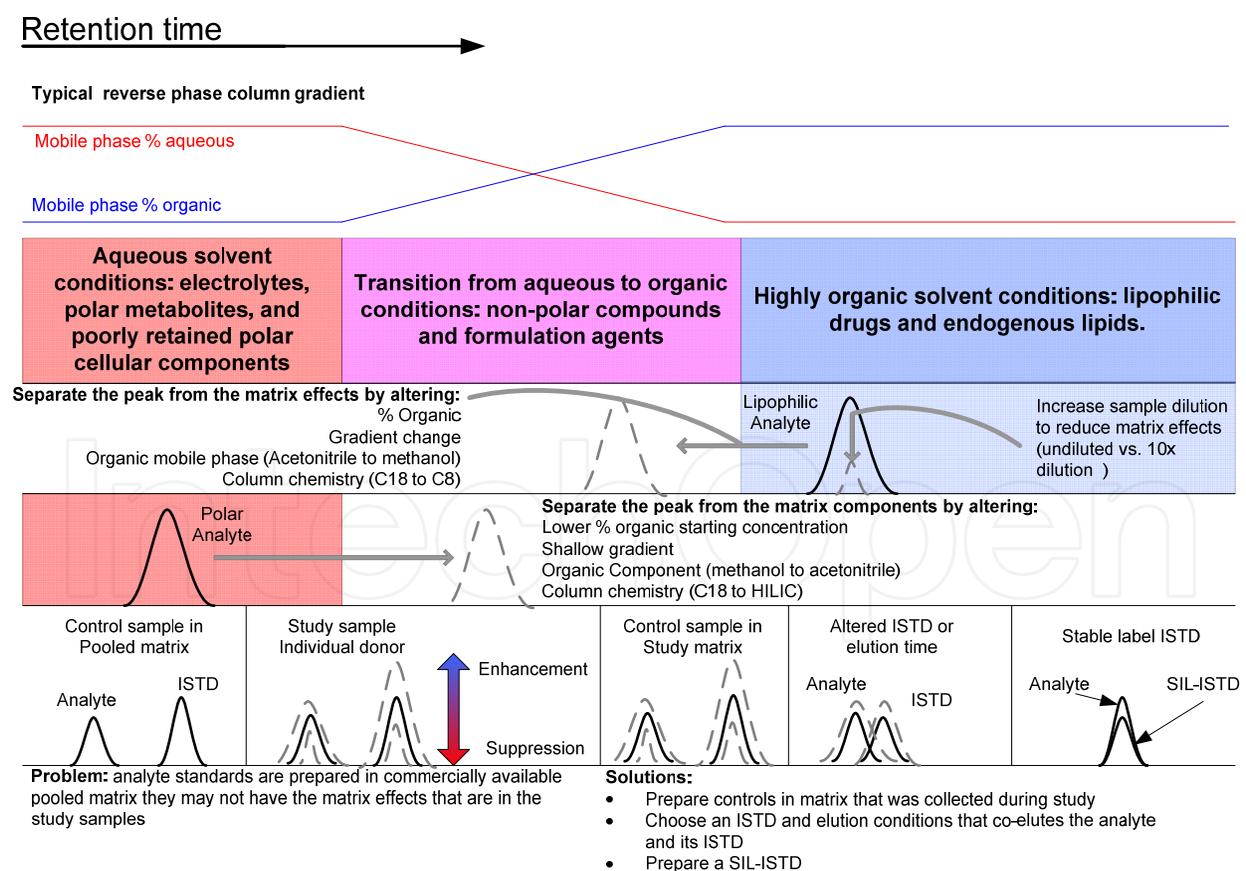


Fig. 13. Addressing matrix effects.

Source of Matrix Effect		Example	Impact	Potential solutions	References
Endogenous	Lipids	Lyso-Phosphatidylcholine 18:0	Suppression; late elution (if not fully eluted it can impact subsequent runs)	Adjust gradient	King et al., 2000
	Polar matrix components	K <sup>+</sup> , Ca <sup>++</sup>	Suppression, especially early in the run	HILIC column	Hsieh, 2008; Ji et al. 2008
Exogenous	Formulation components	Tween 80, PEG 400	Suppression, especially for early eluters	Increase mobile phase pH	Weaver & Riley, 2006
	Labware	Plasticizers	Suppression, later in run, if not fully eluted it can impact subsequent runs	Lengthen LC run; change labware supplier	Guo, 2006; Mei, 2003; van Hout, 2003
	In-source fragmentation	Glucuronide- and sulfate-conjugate metabolites	Enhancement if it co-elutes with parent	Modify source parameters (temperature or voltage); Additionally, changes can be made to the LC gradient or column temperature	Vogeser, 2001; Vogeser, 2010; Yan, 2003

Table 4. Examples of matrix effects, their impact, and suggested solutions.

### 5.1 Sample preparation

Protein precipitation (PPT) is a rapid, nonspecific method that can be utilized for sample clean-up in a high-throughput, automated manner. PPT-based purification relies on reduced solubility of proteins and highly polar matrix components in aqueous-organic solvent solutions. Acetonitrile, methanol or acetonitrile-methanol mixtures are most commonly used for PPT. More than 90% of plasma proteins can be removed from samples using PPT when the plasma to organic solvent ratio is at least 1 to 2.5 (Chambers et al., 2007; Polson et al., 2003). Unfortunately, many matrix components such as lipids, formulation agents, and other substances remain in the supernatant following centrifugation. These components often cause ion suppression, leading to increased variability between samples. Alternative sample

preparation and extraction techniques include SPE and LLE. However, these extraction methods are much more labor intensive and therefore they are not typically used in early drug discovery. A comprehensive discussion of LLE and SPE is beyond the scope of this manuscript, and only a brief description of each will follow.

Liquid-liquid extraction (LLE) is based on the partitioning of an analyte into two separate liquids. The technique works by taking advantage of the differential solubility of an analyte in two immiscible liquids. One of the phases usually is water or a buffer solution, while the other is an organic solvent such as toluene, diethyl ether, hexane, dichloromethane, or MTBE. Selection of the proper organic solvent to obtain maximum recovery should be based on the analyte's solubility in the particular solvent. Chambers et al. compared LLE methods to several SPE and PPT methods in terms of each technique's overall cleanliness, matrix effects, and analyte recovery. They found that using MTBE and basified MTBE with a single extraction technique resulted in clean extracts that were similar to those obtained from cation exchange SPE and were better than PPT. However, analyte recovery using these two LLE methods significantly decreased compared to cation exchange SPE with basified methanol - average % recovery values for MTBE LLE, basified MTBE LLE, and cation exchange SPE with basified methanol were 43%, 38%, and 94%, respectively. Yet, when they used basified MTBE with a two step extraction procedure, both the cleanliness and analyte recovery (average of 87%) increased (Chambers et al., 2007). While LLE provides clean extracts and decent analyte recovery, it is much more labor intensive than PPT. Furthermore, as evidenced by the Chambers' investigation, multiple extractions may be necessary to obtain a sufficient quantity of analyte, decreasing overall efficiency that is essential for high-throughput sample clean-up and analysis in early drug discovery.

Solid phase extraction (SPE) methods rely on the affinity of an analyte for a stationary phase and are often used to isolate analyte(s) of interest from a wide range of matrices including urine, blood, tissue homogenates, etc. Depending on the properties of the analyte and the solid phase, either the analyte of interest is retained while the unwanted matrix components elute with the solvent wash. Or the unwanted matrix components are retained and the analyte elutes with the solvent wash. In the first case, the retained analyte is subsequently eluted with a different solvent. There are numerous SPE stationary phases available, including normal phase, reversed phase, and ion exchange (Chambers et al., 2007; Supelco, 1998). In addition, more specialized solid supports such as HILIC, mixed-mode resins, and zirconium coated particles for phospholipid removal are also commercially available. Table 5 shows typical analyte, matrix, and stationary phase/sorbent examples. The stationary phase and eluent can be adjusted to achieve the optimal sample clean-up and analyte recovery. For example, extraction of primary, secondary, and tertiary amines from biological fluids would be best accomplished by using strong cation exchange (SCX stationary phase); whereas extraction of large, hydrophobic molecules from biological matrices or water should be performed via reversed phase SPE with a C18-T (wide pore) stationary phase (Phenomenex, 2009). Optimal SPE conditions depend upon physicochemical properties of analytes and matrix components in the samples and require extensive method development. Therefore, SPE is less useful for a high-throughput analysis of a diverse set of compounds encountered in the early stages of the drug discovery but is widely used for clinical sample analysis. A more thorough discussion on LLE and SPE can be found elsewhere (Wilson et al., 2000).

SPE Phase	Analyte Properties	Eluting Solvent Properties	Stationary Phase / Sorbent
Normal Phase	Moderate to highly polar	Mid- to non-polar organic solvent (hexane, toluene, chloroform)	Polar (CN, NH <sub>2</sub> , EPH, Silica, Alumina-N)
Reversed Phase	Low to moderately polar, hydrophobic	Non-polar or polar organic solvents with or without water, buffer, and/or strong acid or base	Non-polar (C18, C8, SDB-L, Phenyl, CN)
Ion Exchange	Ionized/charge compounds	Aqueous & low ionic strength buffers (biological fluids plus buffers)	Polar (cation exchange: WCX, SCX, Screen-C; anion exchange: WAX/NH <sub>2</sub> , SAX, Screen-A)

Table 5. Analyte, matrix, and stationary phase properties associated with three commonly used SPE methods (Phenomenex, 2009; Supelco, 1998).

Relatively quick and simple approaches to overcome ion suppression are available and include reducing the volume of the sample injected and/or diluting the samples prior to injection. Sample dilution and reducing the injection volume simply decreases the amount of interfering matrix components introduced into the ion source (Choi et al., 2001; Taylor, 2005). Schuhmacher et al. has demonstrated that dilution of samples with mobile phase results in reduced ion suppression. In their study, dilution from 10- to 100-fold allowed investigators to identify that the matrix effects were caused by the vehicles (PEG 400 and Solutol) used for dosing (Schuhmacher et al., 2003).

Some commonly encountered matrix effects are caused by formulation agents. The easiest approach for reducing these matrix effects is to avoid formulation agents that are known to lead to significant ionization suppression (e.g. Tween 80, PEG 400, Solutol). Alternatively, modifying chromatographic conditions and/or implementing SPE can be used to overcome formulation agent-caused matrix effects. One rather unique approach for eliminating vehicle-related matrix effects was reported by Schuhmacher et al. in which drugs for iv studies were formulated in the respective species' plasma (Schuhmacher et al., 2003).

Column overload is caused by accumulation of matrix components which are not adequately removed during sample clean-up and is a common occurrence when a column has been used extensively and these components are not sufficiently eluted by the HPLC method. While reducing the injection volume may fix the issue for a short period of time, a better long term solution is to add a highly (95-100%) organic wash step after analyte elution. In extreme cases where washing does not resolve the problem, the LC column may need to be replaced. Figure 14 shows chromatograms before and after column replacement. The top two chromatograms show peak tailing caused by column overload. After the LC column was replaced, the bottom two chromatograms showed much sharper analyte and ISTD peaks. Additionally, column replacement increased the analyte signal for both the compound of interest and the ISTD.

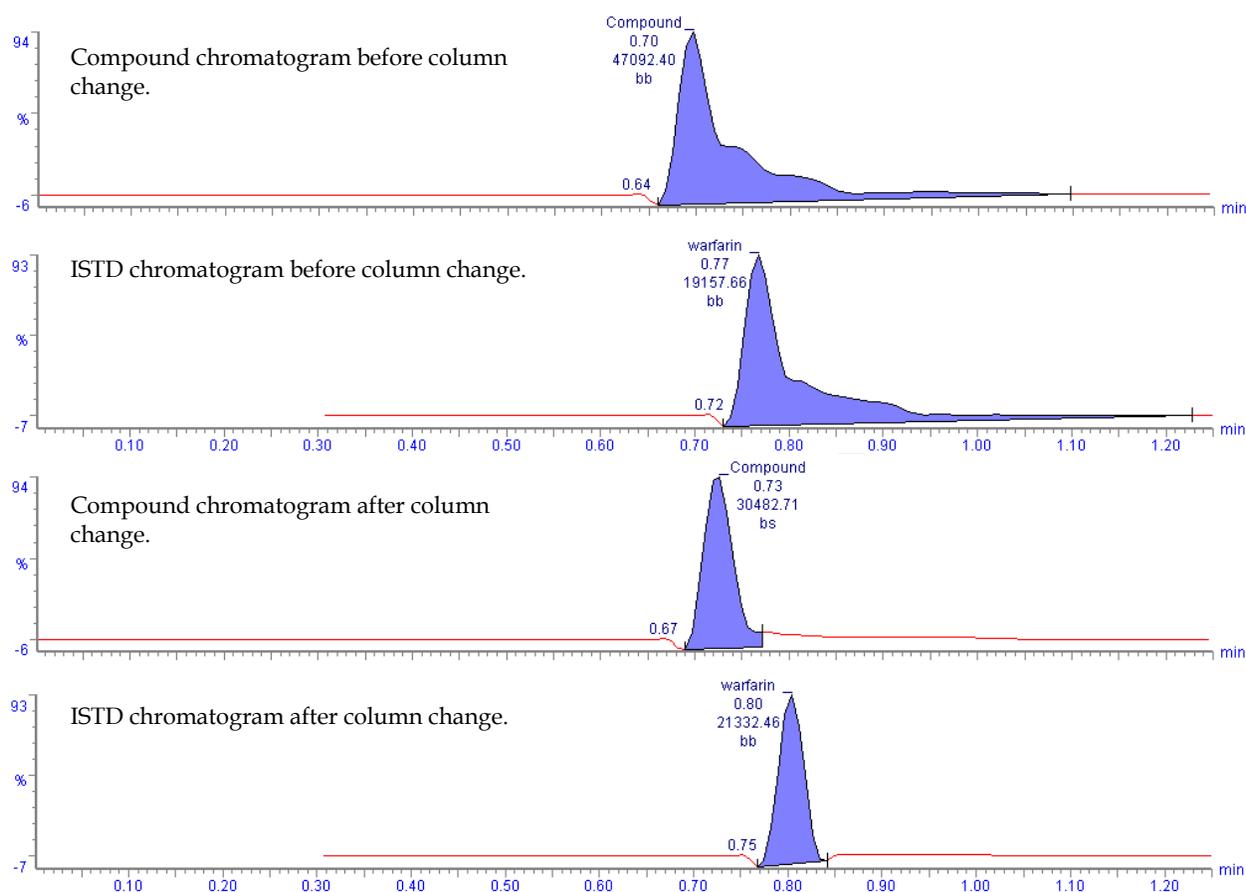


Fig. 14. Chromatograms illustrating an example of column matrix overload, and the subsequent column replacement to overcome matrix effects.

## 5.2 Chromatographic conditions

Another widely used strategy for the reduction of matrix effects is the optimization of chromatographic conditions. In most cases, ion-suppression is caused by the co-elution of the matrix components with the analyte of interest. Therefore, with increased chromatographic separation between the analyte(s) and the matrix components, fewer matrix effects are likely to be encountered.

Optimizing a gradient method involves modification of chromatographic parameters, such as initial and final eluent strength and gradient duration, all of which may prove advantageous for chromatographic resolution of matrix components away from the analyte (Schellinger and Carr, 2006). When Gallart-Ayala et al. developed the analytical method for determining bisphenols in soft drinks using LC-ESI-MS/MS, they observed that the responses of the analytes were 80-95% lower in the matrix than those obtained from a standard solution at the same concentration level. They then monitored the samples through both LC-UV and LC-MS/MS, and found that signal suppression was likely caused by the co-elution of the matrix components with the analytes. To reduce the ionization suppression, several strategies were successfully applied: 1) extension of the linear gradient duration from 1 min to 3 min; 2) lowering of the initial mobile phase organic content from 50% to 15% methanol; 3) adjustment of the final mobile phase composition from 100% to 80% methanol. The chromatographic peaks of the analytes were almost completely resolved from the matrix components and the matrix effects were greatly reduced (Gallart-Ayala et

al., 2011). A shorter gradient duration normally leads to greater matrix effects due to the reduction of chromatographic resolution between analytes and matrix components (Chambers et al., 2007). When matrix effects are caused by co-elution of matrix components and the analyte of interest, changing to another column type or manufacturer, increasing the column length and/or extending the gradient duration will typically resolve the issue. Alternatively, fine tuning the mobile phase solvent strength can prove to be effective to achieve chromatographic separation. Ye et al. used a mixture of methanol and ACN as the organic mobile phase, to minimize phospholipid-related matrix effects and maintain high sample throughput (Ye et al., 2011).

Recently, an ultra-fast gradient approach also referred to as a "ballistic" gradient, allowed for analysis cycle times of 2 minutes or less (Romanyshyn and Tiller, 2001; Romanyshyn et al., 2000). Tiller and Romanyshyn studied matrix effects encountered using ultra-fast gradients and fast isocratic methods. They demonstrated that while both methods are subject to similar matrix effects, gradient elution had several advantages in reducing matrix effects. Gradient elution gave better separation of the analyte from the poorly retained polar components, and a more complete elution of highly retained contaminants from the column, thereby reducing the potential matrix effects during sample analysis. Moreover, the early and late chromatographic regions can be diverted to waste, thus reducing matrix components that can foul the MS source (Tiller and Romanyshyn, 2002).

Ultra Performance Liquid Chromatography (UPLC) provides advantages over traditional HPLC in speed, sensitivity, and resolution of analytes (Novakova et al., 2006; Wren and Tchelitcheff, 2006). When analyzing nine different drugs in three surface water samples, Van De Steene and Lambert observed severe matrix effects using HPLC. After implementing analogue ISTDs, significant matrix effects were still observed. However, through UPLC implementation, these matrix effects were significantly reduced, and accurate quantitation of all nine compounds using analogue ISTDs became feasible (Van De Steene and Lambert, 2008). With improved sensitivity and resolution, UPLC generally encounters fewer matrix effects and affords a more robust analytical method than HPLC.

Mobile phase pH can influence the retention times of ionizable analytes (basic or acidic) by changing the ionization equilibrium. Under HPLC conditions with an acidified mobile phase, basic compounds are present as charged species. As a result, they are poorly retained on the column and elute early with a highly aqueous mobile phase (Chambers et al., 2007). Under acidic conditions, basic compounds may encounter matrix effects from salts and highly polar, poorly retained matrix components. Conversely, at basic pH, basic compounds stay neutral, are better retained, elute with high organic content mobile phase, and generate stronger MS signals. In a study of matrix effects caused by PEG 400, the basic analyte co-eluted directly with PEG 400 under acidic mobile phase conditions. To overcome this, the mobile phase pH was increased to 10.5, and improved separation between the analyte and PEG 400 was achieved. This resulted in greatly reduced matrix effects and allowed for more accurate quantification of the analyte (Weaver and Riley, 2006).

Analytical columns of the same stationary phase (for example C18) from different manufacturers are generally not equivalent and will often result in slightly different separation of sample components. Hence, the degree of a matrix effect may differ when columns from different manufacturers are used. For example, in developing a ballistic gradient, De Nardi & Bonelli compared matrix effects after using an ACE C18-300 30x2.1mm (5 $\mu$ ) and a POLARITY dC18 30x2.1mm (5 $\mu$ ) analytical column. The analyte peaks had no

overlap with the PEG 400 peaks when using the ACE column, while partial overlap occurred when using the POLARITY column (De Nardi and Bonelli, 2006).

A relatively new column, the hydrophilic interaction LC (HILIC) column, consists of bare silica or a polar phase (amino, diol, cyano, etc.) bonded to silica and provides more favorable conditions for polar compound retention and ionization (Hsieh, 2008) (Ji et al., 2008). With HILIC chromatography, polar analytes have prolonged retention times and elute at high organic mobile phase content; therefore, they are separated from ion suppressing-early eluters and have high ionization efficiencies. Ji et al. developed a quantitation method for doxazosin using HILIC-MS/MS with a mobile phase consisting of ACN/ammonium formate (100 mM, pH 4.5) (93:7 v/v). The method was free of matrix effects assessed by post extraction analyte spiking (Ji et al., 2008). However, it is important to note that under HILIC conditions, certain polar endogenous components could be strongly retained on a HILIC column, potentially causing matrix effects that are not observed in RP conditions (Jian et al., 2010). Although water is usually used as the strong solvent in HILIC, by switching from water to a weaker solvent (methanol, ethanol, propanol etc.), HILIC columns can be used to effectively separate less polar compounds (Jian et al., 2010; Xu et al., 2007).

As discussed earlier, cleaner samples are obtained from SPE or multiple LLE. However, with the ever-increasing pace in drug discovery study, minimal sample pretreatment and automation are desired. Consequently, on-line 2-D chromatography with valve switching technology has seen more and more use lately (Mullett, 2007; Pascoe et al., 2001; Pol and Hyotylainen, 2008). Two-dimensional chromatography involves the transfer of a fraction or fractions of sample from a primary column to a secondary column for further separation. In orthogonal 2-D chromatography two columns with different stationary phases are utilized to increase the separation between the analyte and the matrix components. Dodgen et al. developed a simple method for simultaneous quantitation of dextromethorphan, omeprazole, and their metabolites in plasma for the phenotypic profiling of cytochrome P450 2D6 and 2C19. In this study, the supernatant generated from PPT was loaded directly to a SPE capture column for purification. After the initial unretained matrix components eluted, a switching valve directed the trapped analytes to the analytical column. The primary SPE column removed almost all ion suppression/enhancement effects caused by the matrix components. The total run time was 6 min (Dodgen et al., 2011).

### 5.3 Calibration with internal standards

Perhaps the most common method used to compensate for matrix effects involves the appropriate choice of ISTDs. When performing PPT, the ISTD is typically included with the organic solvent that is used to precipitate the protein. It is added to the precipitating solvent so that there is a uniform concentration throughout the samples to be analyzed. For an ISTD to effectively compensate for matrix effects it should have a retention time similar to the analyte of interest. The analyte response can be normalized to the ISTD peak simply by dividing the analyte peak area by the ISTD peak area. Since the ISTD concentration is equal across all of the samples, comparing the analyte's peak area to the ISTD's peak area serves to normalize the data and compensate for signal response variability caused by matrix effects. Ideally, a stable-isotope-labeled analyte analog can be used as an ISTD. Since this SIL-ISTD would have nearly identical chemical and structural properties as the analyte of interest, the two compounds should behave similarly during sample preparation and LC-MS analysis

(Jessome and Volmer, 2006). However, availability and cost concerns can severely limit the extent to which SIL-ISTDs are employed (Schuhmacher et al., 2003; Xu et al., 2007).

## 6. Regulatory perspectives

A complete discussion regarding the regulatory perspective on matrix effects is beyond the scope of this review and has been discussed in detail elsewhere (Viswanathan et al., 2007). In short, analysis of samples from GLP and clinical studies require a validated LC-MS/MS method. The Food and Drug Administration (FDA) has provided a guidance document for validating methods for the analysis of bioanalytical samples (FDA, 2001). This guidance provides details on the parameters required for a complete validation including method linearity, precision, accuracy, sample stability, evaluation of matrix effects, etc. The FDA recently required incurred sample analysis in which a subset of samples (~10%) from clinical and GLP studies are reanalyzed. The reanalysis results are compared to the original results, and that at least two-thirds of the reanalysis batch results must be within 20% of the original sample values. Failure to meet this criterion will initiate an investigation to determine the source of the discrepancy in the data.

Although the parameters described above almost always lead to a robust, validated method, there are certain situations where matrix effects may persist. In such cases, sample reanalysis generally leads to widely different results from what was originally reported. These cases are frequently associated with unknown matrix effects, which are often unique to a particular patient's sample(s). As mentioned previously, clinical trial samples are distinctive in their composition, thus a universal LC-MS/MS method will not always remove or compensate for the components that may interfere with analyte quantitation. Further, method validation across different patients' samples is impractical due to this inherent uniqueness. These situations, when they occur, are handled on a case by case basis. Typically, an investigation into the discrepancy is conducted and documented and the reporting of the data is specified by Standard Operating Procedures (SOPs) that are written to handle such situations.

## 7. Conclusion

Matrix effects can be a complicated and time consuming challenge for the analytical chemist charged with developing robust, reproducible, and accurate analytical methods. There are a diversity of sample and system conditions that lead to matrix effects, and an equally diverse set of potential options to remedy them.

The mechanisms by which matrix components cause ionization suppression (or enhancement) are still not well-understood. This serves as a testament to the challenge they provide to the analytical chemist. In the years since the first published articles describing matrix effects, analytical chemists have recognized the importance of understanding and mitigating matrix effects.

## 8. References

- Annesley, T.M. (2007). Methanol-associated matrix effects in electrospray ionization tandem mass spectrometry. *Clin Chem* 53, 1827-1834.
- Apweiler, R., Aslanidis, C., Deufel, T., Gerstner, A., Hansen, J., Hochstrasser, D., Kellner, R., Kubicek, M., Lottspeich, F., Maser, E., *et al.* (2009). Approaching clinical proteomics:

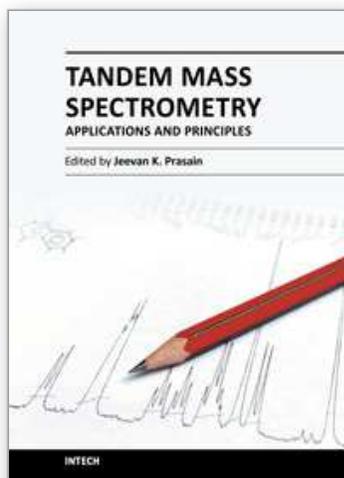
- current state and future fields of application in fluid proteomics. *Clin Chem Lab Med* 47, 724-744.
- Aurand, C., and Trinh, A. (2009). Selective Depletion of Phospholipids in Bioanalysis Using HybridSPE-PPT Technology. *Chromatography Today*, 38-41.
- Bell, C., George, C., Kicman, A.T., and Traynor, A. (2011). Development of a rapid LC-MS/MS method for direct urinalysis of designer drugs. *Drug Test Anal* 3, 496-504.
- Benijts, T., Dams, R., Lambert, W., and De Leenheer, A. (2004). Countering matrix effects in environmental liquid chromatography-electrospray ionization tandem mass spectrometry water analysis for endocrine disrupting chemicals. *J Chromatogr A* 1029, 153-159.
- Bennett, P., and Liang, H. (2004). Overcoming Matrix Effects Resulting from Biological Phospholipids Through Selective Extraction in Quantitative LC/MS/MS. In ASMS Conference (Nashville, TN, Tandem Labs).
- Bonfiglio, R., King, R.C., Olah, T.V., and Merkle, K. (1999). The effects of sample preparation methods on the variability of the electrospray ionization response for model drug compounds. *Rapid Commun Mass Spectrom* 13, 1175-1185.
- Borg, J., Campos, A., Diema, C., Omenaca, N., de Oliveira, E., Guinovart, J., and Vilaseca, M. (2011). Spectral counting assessment of protein dynamic range in cerebrospinal fluid following depletion with plasma-designed immunoaffinity columns. *Clin Proteomics* 8, 6.
- Casetta, B., Jans, I., Billen, J., Vanderschueren, D., and Bouillon, R. (2010). Development of a method for the quantification of 1 $\alpha$ ,25(OH) $_2$ -vitamin D $_3$  in serum by liquid chromatography tandem mass spectrometry without derivatization. *Eur J Mass Spectrom (Chichester, Eng)* 16, 81-89.
- Chambers, E., Wagrowski-Diehl, D.M., Lu, Z., and Mazzeo, J.R. (2007). Systematic and comprehensive strategy for reducing matrix effects in LC/MS/MS analyses. *J Chromatogr B Analyt Technol Biomed Life Sci* 852, 22-34.
- Chavez-Eng, C.M., Constanzer, M.L., and Matuszewski, B.K. (2002). High-performance liquid chromatographic-tandem mass spectrometric evaluation and determination of stable isotope labeled analogs of rofecoxib in human plasma samples from oral bioavailability studies. *J Chromatogr B Analyt Technol Biomed Life Sci* 767, 117-129.
- Chiu, M.L., Lawi, W., Snyder, S.T., Wong, P.K., Liao, J.C., and Gau, V. (2010). Matrix Effects - A Challenge Toward Automation of Molecular Analysis. *Journal of the Association for Laboratory Automation* 15, 233-242.
- Choi, B.K., Hercules, D.M., and Gusev, A.I. (2001). Effect of liquid chromatography separation of complex matrices on liquid chromatography-tandem mass spectrometry signal suppression. *J Chromatogr A* 907, 337-342.
- Dams, R., Benijts, T., Gunther, W., Lambert, W., and De Leenheer, A. (2002). Influence of the eluent composition on the ionization efficiency for morphine of pneumatically assisted electrospray, atmospheric-pressure chemical ionization and sonic spray. *Rapid Commun Mass Spectrom* 16, 1072-1077.
- Dancygier, H. (2010). *Clinical Hepatology: Principles and Practice of Hepatobiliary Diseases, Vol 1* (New York, NY, Springer-Verlag).
- De Nardi, C., and Bonelli, F. (2006). Moving from fast to ballistic gradient in liquid chromatography/tandem mass spectrometry pharmaceutical bioanalysis: Matrix effect and chromatographic evaluations. *Rapid Commun Mass Spectrom* 20, 2709-2716.
- Dodgen, T.M., Cromarty, A.D., and Pepper, M.S. (2011). Quantitative plasma analysis using automated online solid-phase extraction with column switching LC-MS/MS for characterising cytochrome P450 2D6 and 2C19 metabolism. *J Sep Sci* 34, 1102-1110.

- Fahy, E., Subramaniam, S., Brown, H.A., Glass, C.K., Merrill, A.H., Jr., Murphy, R.C., Raetz, C.R., Russell, D.W., Seyama, Y., Shaw, W., *et al.* (2005). A comprehensive classification system for lipids. *J Lipid Res* 46, 839-861.
- FDA (2001). Guidance for Industry: Bioanalytical Method Validation (Rockville, MD, Department of Health and Human Services, FDA, Center for Drug Evaluation and Research).
- Gallart-Ayala, H., Moyano, E., and Galceran, M.T. (2011). Analysis of bisphenols in soft drinks by on-line solid phase extraction fast liquid chromatography-tandem mass spectrometry. *Anal Chim Acta* 683, 227-233.
- Gosetti, F., Mazzucco, E., Zampieri, D., and Gennaro, M.C. (2010). Signal suppression/enhancement in high-performance liquid chromatography tandem mass spectrometry. *J Chromatogr A* 1217, 3929-3937.
- Guo, X., Bruins, A.P., and Covey, T.R. (2006). Characterization of typical chemical background interferences in atmospheric pressure ionization liquid chromatography-mass spectrometry. *Rapid Commun Mass Spectrom* 20, 3145-3150.
- Gustavsson, S.A., Samskog, J., Markides, K.E., and Langstrom, B. (2001). Studies of signal suppression in liquid chromatography-electrospray ionization mass spectrometry using volatile ion-pairing reagents. *J Chromatogr A* 937, 41-47.
- Hewitt, D., Alvarez, M., Robinson, K., Ji, J., Wang, Y.J., Kao, Y.H., and Zhang, T. (2011). Mixed-mode and reversed-phase liquid chromatography-tandem mass spectrometry methodologies to study composition and base hydrolysis of polysorbate 20 and 80. *J Chromatogr A* 1218, 2138-2145.
- Hollis, B.W. (2007). Assessment of circulating 25(OH)D and 1,25(OH)2D: emergence as clinically important diagnostic tools. *Nutr Rev* 65, S87-90.
- Hsieh, Y. (2008). Potential of HILIC-MS in quantitative bioanalysis of drugs and drug metabolites. *J Sep Sci* 31, 1481-1491.
- Ismail, O.A., Zhang, T., Jenkins, R.G., and Karnes, H.T. (2010). Investigation of endogenous blood plasma phospholipids, cholesterol and glycerides that contribute to matrix effects in bioanalysis by liquid chromatography/mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 878, 3303-3316.
- Jemal, M., Schuster, A., and Whigan, D.B. (2003). Liquid chromatography/tandem mass spectrometry methods for quantitation of mevalonic acid in human plasma and urine: method validation, demonstration of using a surrogate analyte, and demonstration of unacceptable matrix effect in spite of use of a stable isotope analog internal standard. *Rapid Commun Mass Spectrom* 17, 1723-1734.
- Jessome, L.L., and Volmer, D.A. (2006). Ion Suppression: A Major Concern in Mass Spectrometry. *LCGC North America* 24, 498-510.
- Ji, H.Y., Park, E.J., Lee, K.C., and Lee, H.S. (2008). Quantification of doxazosin in human plasma using hydrophilic interaction liquid chromatography with tandem mass spectrometry. *J Sep Sci* 31, 1628-1633.
- Jian, W., Edom, R.W., Xu, Y., and Weng, N. (2010). Recent advances in application of hydrophilic interaction chromatography for quantitative bioanalysis. *J Sep Sci* 33, 681-697.
- King, R., Bonfiglio, R., Fernandez-Metzler, C., Miller-Stein, C., and Olah, T. (2000). Mechanistic investigation of ionization suppression in electrospray ionization. *J Am Soc Mass Spectrom* 11, 942-950.
- Kostiainen, R., and Kauppila, T.J. (2009). Effect of eluent on the ionization process in liquid chromatography-mass spectrometry. *J Chromatogr A* 1216, 685-699.
- Larger, P.J., Breda, M., Fraier, D., Hughes, H., and James, C.A. (2005). Ion-suppression effects in liquid chromatography-tandem mass spectrometry due to a formulation

- agent, a case study in drug discovery bioanalysis. *J Pharm Biomed Anal* 39, 206-216.
- Liang, H.R., Foltz, R.L., Meng, M., and Bennett, P. (2003). Ionization enhancement in atmospheric pressure chemical ionization and suppression in electrospray ionization between target drugs and stable-isotope-labeled internal standards in quantitative liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 17, 2815-2821.
- Lindegardh, N., Annerberg, A., White, N.J., and Day, N.P. (2008). Development and validation of a liquid chromatographic-tandem mass spectrometric method for determination of piperazine in plasma stable isotope labeled internal standard does not always compensate for matrix effects. *J Chromatogr B Analyt Technol Biomed Life Sci* 862, 227-236.
- Little, J.L., Wempe, M.F., and Buchanan, C.M. (2006). Liquid chromatography-mass spectrometry/mass spectrometry method development for drug metabolism studies: Examining lipid matrix ionization effects in plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 833, 219-230.
- Liu, A., Tweed, J., and Wujcik, C.E. (2009). Investigation of an on-line two-dimensional chromatographic approach for peptide analysis in plasma by LC-MS-MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 877, 1873-1881.
- Mallet, C.R., Lu, Z., and Mazzeo, J.R. (2004). A study of ion suppression effects in electrospray ionization from mobile phase additives and solid-phase extracts. *Rapid Commun Mass Spectrom* 18, 49-58.
- Marchi, I., Viette, V., Badoud, F., Fathi, M., Saugy, M., Rudaz, S., and Veuthey, J.L. (2010). Characterization and classification of matrix effects in biological samples analyses. *J Chromatogr A* 1217, 4071-4078.
- Matuszewski, B.K. (2006). Standard line slopes as a measure of a relative matrix effect in quantitative HPLC-MS bioanalysis. *J Chromatogr B Analyt Technol Biomed Life Sci* 830, 293-300.
- Matuszewski, B.K., Constanzer, M.L., and Chavez-Eng, C.M. (2003). Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal Chem* 75, 3019-3030.
- Mei, H. (2005). Matrix Effects: Causes and Solutions. In *Using Mass Spectrometry For Drug Metabolism Studies*, W.A. Korfmacher, ed. (CRC Press), pp. 103-149.
- Mei, H., Hsieh, Y., Nardo, C., Xu, X., Wang, S., Ng, K., and Korfmacher, W.A. (2003). Investigation of matrix effects in bioanalytical high-performance liquid chromatography/tandem mass spectrometric assays: application to drug discovery. *Rapid Commun Mass Spectrom* 17, 97-103.
- Morin, L.P., Mess, J.N., Furtado, M., and Garofolo, F. (2011). Reliable procedures to evaluate and repair crosstalk for bioanalytical MS/MS assays. *Bioanalysis* 3, 275-283.
- Muller, C., Schafer, P., Stortzel, M., Vogt, S., and Weinmann, W. (2002). Ion suppression effects in liquid chromatography-electrospray-ionisation transport-region collision induced dissociation mass spectrometry with different serum extraction methods for systematic toxicological analysis with mass spectra libraries. *J Chromatogr B Analyt Technol Biomed Life Sci* 773, 47-52.
- Mullett, W.M. (2007). Determination of drugs in biological fluids by direct injection of samples for liquid-chromatographic analysis. *J Biochem Biophys Methods* 70, 263-273.
- Novakova, L., Solichova, D., and Solich, P. (2006). Advantages of ultra performance liquid chromatography over high-performance liquid chromatography: comparison of different analytical approaches during analysis of diclofenac gel. *J Sep Sci* 29, 2433-2443.

- Pascoe, R., Foley, J.P., and Gusev, A.I. (2001). Reduction in matrix-related signal suppression effects in electrospray ionization mass spectrometry using on-line two-dimensional liquid chromatography. *Anal Chem* 73, 6014-6023.
- Phenomenex, I. (2009). Chromatography Product Guide 09/10. In HPLC, GC, SPE (Torrance, CA, Phenomenex, Inc.).
- Pol, J., and Hyotylainen, T. (2008). Comprehensive two-dimensional liquid chromatography coupled with mass spectrometry. *Anal Bioanal Chem* 391, 21-31.
- Polson, C., Sarkar, P., Incedon, B., Raguvaran, V., and Grant, R. (2003). Optimization of protein precipitation based upon effectiveness of protein removal and ionization effect in liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 785, 263-275.
- Pusch, W., Flocco, M.T., Leung, S.M., Thiele, H., and Kostrzewa, M. (2003). Mass spectrometry-based clinical proteomics. *Pharmacogenomics* 4, 463-476.
- Quehenberger, O., Armando, A.M., Brown, A.H., Milne, S.B., Myers, D.S., Merrill, A.H., Bandyopadhyay, S., Jones, K.N., Kelly, S., Shaner, R.L., *et al.* (2010). Lipidomics reveals a remarkable diversity of lipids in human plasma. *J Lipid Res* 51, 3299-3305.
- Remane, D., Meyer, M.R., Wissenbach, D.K., and Maurer, H.H. (2010a). Ion suppression and enhancement effects of co-eluting analytes in multi-analyte approaches: systematic investigation using ultra-high-performance liquid chromatography/mass spectrometry with atmospheric-pressure chemical ionization or electrospray ionization. *Rapid Commun Mass Spectrom* 24, 3103-3108.
- Remane, D., Wissenbach, D.K., Meyer, M.R., and Maurer, H.H. (2010b). Systematic investigation of ion suppression and enhancement effects of fourteen stable-isotope-labeled internal standards by their native analogues using atmospheric-pressure chemical ionization and electrospray ionization and the relevance for multi-analyte liquid chromatographic/mass spectrometric procedures. *Rapid Commun Mass Spectrom* 24, 859-867.
- Romanyshyn, L., Tiller, P.R., and Hop, C.E. (2000). Bioanalytical applications of 'fast chromatography' to high-throughput liquid chromatography/tandem mass spectrometric quantitation. *Rapid Commun Mass Spectrom* 14, 1662-1668.
- Romanyshyn, L.A., and Tiller, P.R. (2001). Ultra-short columns and ballistic gradients: considerations for ultra-fast chromatographic liquid chromatographic-tandem mass spectrometric analysis. *J Chromatogr A* 928, 41-51.
- Schellinger, A.P., and Carr, P.W. (2006). Isocratic and gradient elution chromatography: a comparison in terms of speed, retention reproducibility and quantitation. *J Chromatogr A* 1109, 253-266.
- Schuhmacher, J., Zimmer, D., Tesche, F., and Pickard, V. (2003). Matrix effects during analysis of plasma samples by electrospray and atmospheric pressure chemical ionization mass spectrometry: practical approaches to their elimination. *Rapid Commun Mass Spectrom* 17, 1950-1957.
- Shou, W.Z., and Naidong, W. (2003). Post-column infusion study of the 'dosing vehicle effect' in the liquid chromatography/tandem mass spectrometric analysis of discovery pharmacokinetic samples. *Rapid Commun Mass Spectrom* 17, 589-597.
- Supelco (1998). Guide to Solid Phase Extraction. In Bulletin 910 (Bellefonte, PA, Supelco).
- Taylor, P.J. (2005). Matrix effects: the Achilles heel of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometry. *Clin Biochem* 38, 328-334.
- Tiller, P.R., and Romanyshyn, L.A. (2002). Implications of matrix effects in ultra-fast gradient or fast isocratic liquid chromatography with mass spectrometry in drug discovery. *Rapid Commun Mass Spectrom* 16, 92-98.

- Trufelli, H., Palma, P., Famigliani, G., and Cappiello, A. (2011). An overview of matrix effects in liquid chromatography-mass spectrometry. *Mass Spectrom Rev* 30, 491-509.
- Van De Steene, J.C., and Lambert, W.E. (2008). Comparison of matrix effects in HPLC-MS/MS and UPLC-MS/MS analysis of nine basic pharmaceuticals in surface waters. *J Am Soc Mass Spectrom* 19, 713-718.
- van Hout, M.W., Niederlander, H.A., de Zeeuw, R.A., and de Jong, G.J. (2003). Ion suppression in the determination of clenbuterol in urine by solid-phase extraction atmospheric pressure chemical ionisation ion-trap mass spectrometry. *Rapid Commun Mass Spectrom* 17, 245-250.
- van Tellingen, O., Beijnen, J.H., Verweij, J., Scherrenburg, E.J., Nooijen, W.J., and Sparreboom, A. (1999). Rapid esterase-sensitive breakdown of polysorbate 80 and its impact on the plasma pharmacokinetics of docetaxel and metabolites in mice. *Clin Cancer Res* 5, 2918-2924.
- Viswanathan, C.T., Bansal, S., Booth, B., DeStefano, A.J., Rose, M.J., Sailstad, J., Shah, V.P., Skelly, J.P., Swann, P.G., and Weiner, R. (2007). Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. *Pharm Res* 24, 1962-1973.
- Vogeser, M., and Seger, C. (2010). Pitfalls associated with the use of liquid chromatography-tandem mass spectrometry in the clinical laboratory. *Clin Chem* 56, 1234-1244.
- Vogeser, M., Zchoval, R., Spohrer, U., and Jacob, K. (2001). Potential lack of specificity using electrospray tandem-mass spectrometry for the analysis of mycophenolic acid in serum. *Ther Drug Monit* 23, 722-724.
- Wang, S., Cyronak, M., and Yang, E. (2007). Does a stable isotopically labeled internal standard always correct analyte response? A matrix effect study on a LC/MS/MS method for the determination of carvedilol enantiomers in human plasma. *J Pharm Biomed Anal* 43, 701-707.
- Weaver, R., and Riley, R.J. (2006). Identification and reduction of ion suppression effects on pharmacokinetic parameters by polyethylene glycol 400. *Rapid Commun Mass Spectrom* 20, 2559-2564.
- Wilson, I.D., Adlard, E.R., Cooke, M., and Poole, C.F. (2000). *Encyclopedia of Separation Science*, I.D. Wilson, Adlard, E.R., Cooke, M., and Poole, C.F., ed. (San Diego, Academic Press).
- Wren, S.A., and Tchelitcheff, P. (2006). Use of ultra-performance liquid chromatography in pharmaceutical development. *J Chromatogr A* 1119, 140-146.
- Xu, R.N., Fan, L., Rieser, M.J., and El-Shourbagy, T.A. (2007). Recent advances in high-throughput quantitative bioanalysis by LC-MS/MS. *J Pharm Biomed Anal* 44, 342-355.
- Xu, X., Mei, H., Wang, S., Zhou, Q., Wang, G., Broske, L., Pena, A., and Korfmacher, W.A. (2005). A study of common discovery dosing formulation components and their potential for causing time-dependent matrix effects in high-performance liquid chromatography tandem mass spectrometry assays. *Rapid Commun Mass Spectrom* 19, 2643-2650.
- Yan, Z., Caldwell, G.W., Jones, W.J., and Masucci, J.A. (2003). Cone voltage induced in-source dissociation of glucuronides in electrospray and implications in biological analyses. *Rapid Commun Mass Spectrom* 17, 1433-1442.
- Ye, Z., Tsao, H., Gao, H., and Brummel, C.L. (2011). Minimizing matrix effects while preserving throughput in LC-MS/MS bioanalysis. *Bioanalysis* 3, 1587-1601.
- Zheng, J.J., Lynch, E.D., and Unger, S.E. (2002). Comparison of SPE and fast LC to eliminate mass spectrometric matrix effects from microsomal incubation products. *J Pharm Biomed Anal* 28, 279-285.



## **Tandem Mass Spectrometry - Applications and Principles**

Edited by Dr Jeevan Prasain

ISBN 978-953-51-0141-3

Hard cover, 794 pages

**Publisher** InTech

**Published online** 29, February, 2012

**Published in print edition** February, 2012

Tandem Mass Spectrometry - Applications and Principles presents comprehensive coverage of theory, instrumentation and major applications of tandem mass spectrometry. The areas covered range from the analysis of drug metabolites, proteins and complex lipids to clinical diagnosis. This book serves multiple groups of audiences; professional (academic and industry), graduate students and general readers interested in the use of modern mass spectrometry in solving critical questions of chemical and biological sciences.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Terence G. Hall, Inese Smukste, Karen R. Bresciano, Yunxia Wang, David McKearn and Ronald E. Savage (2012). Identifying and Overcoming Matrix Effects in Drug Discovery and Development, Tandem Mass Spectrometry - Applications and Principles, Dr Jeevan Prasain (Ed.), ISBN: 978-953-51-0141-3, InTech, Available from: <http://www.intechopen.com/books/tandem-mass-spectrometry-applications-and-principles/identifying-and-overcoming-matrix-effects-in-drug-discovery-and-development>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen