Characterization and classification of matrix effects in biological samples analyses

Marchi, Ivano; Viette, Véronique; Badoud, Flavia; Fathi, Marc; Saugy, Martial; Rudaz, Serge; Veuthey, Jean-Luc

How to cite

MARCHI, Ivano et al. Characterization and classification of matrix effects in biological samples analyses. In: Journal of chromatography, 2010, vol. 1217, n° 25, p. 4071–4078. doi: 10.1016/j.chroma.2009.08.061

This publication URL: https://archive-ouverte.unige.ch//unige:8502
Publication DOI: 10.1016/j.chroma.2009.08.061

Journal of Chromatography A, 1217 (2010) 4071–4078

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Characterization and classification of matrix effects in biological samples analyses

Ivano Marchi[a], Véronique Viette[b], Flavia Badoud[a,c], Marc Fathi[b], Martial Saugy[c], Serge Rudaz[a], Jean-Luc Veuthey[a,*]

[a] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Boulevard d'Yvoy 20, 1211 Geneva 4, Switzerland
[b] Laboratory Medicine Service, University Hospitals of Geneva, rue Micheli-Du-Crest 24, 1211 Geneva 14, Switzerland
[c] Swiss Laboratory for Doping Analyses, Institut Universitaire de Médecine Légale, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Chemin des Croisettes 22, 1066 Epalinges, Switzerland

ARTICLE INFO

Article history:
Available online 27 August 2009

Keywords:
Matrix effects
Ion suppression
Plasma
Urine
Sample preparation
SPE
MS

ABSTRACT

An exhaustive classification of matrix effects occurring when a sample preparation is performed prior to liquid-chromatography coupled to mass spectrometry (LC–MS) analyses was proposed. A total of eight different situations were identified allowing the recognition of the matrix effect typology *via* the calculation of four recovery values. A set of 198 compounds was used to evaluate matrix effects after solid phase extraction (SPE) from plasma or urine samples prior to LC–ESI-MS analysis. Matrix effect identification was achieved for all compounds and classified through an organization chart. Only 17% of the tested compounds did not present significant matrix effects.

1. Introduction

Since the introduction of ionization sources working at atmospheric pressure, liquid-chromatography coupled to mass spectrometry (LC–MS) has become the gold standard for the analysis of pharmaceutical compounds in biological matrices such as blood [1,2], plasma [3,4], serum [5] and urine [6]. Direct injection of such samples cannot be performed due to the presence of endogenous compounds leading to various problems such as column clogging [7–9] and MS signal alterations. These alterations were extensively described in electrospray ionization (ESI) after solid phase extraction (SPE) [10–13], online SPE [9], protein precipitation (PP) [13–16] and liquid–liquid extraction (LLE) [17]. APCI signal alterations were also investigated after SPE [10,13,18], online SPE [9] and PP [13–15] but APPI signal alterations were investigated to a lesser extent [9]. The latter alterations are called matrix effects. Not yet well understood, they have been recognized to occur when interfering substances such as proteins, lipids, sugars or salts, which could affect the ionization process, co-elute with analytes [11,19,20]. A sample preparation is therefore mandatory to selectively reduce the amount of these interferents. Various methods are available but the most widely used are PP, LLE and SPE. These sample preparations remove the major part of the endogenous material, but a small amount often remains in the treated sample, possibly inducing matrix effects [18,21]. These matrix effects must therefore be tested during method development to determine their presence and impact on the quantification of analytes. Matrix effects evaluation can be achieved *via* two major techniques providing complementary information. First, qualitative results can be obtained with a post-column infusion system as proposed in 1999 by Bonfiglio et al. [22]. The principle is based on the infusion of a solution of analytes of interest between the column and the MS detector, leading to a constant baseline. Blank samples, extracted with the tested sample preparation procedure, are injected into the system. The presence of matrix effects is highlighted by baseline alteration in a time window. MS responses of analytes eluting within this region will be altered (signal suppression or enhancement), inducing irreproducible and non-quantitative results. The second strategy for matrix effect evaluation was proposed by Matuszewski et al. in 2003 [23] and leads to a quantitative information. This method determines if the presence of matrix interferents causes a problem during the sample preparation step and/or during the analysis, based on the comparison of three different samples: a neat standard, a biological sample spiked prior the extraction and a biological sample spiked after the extraction. Finally, Holcapek et al. proposed in 2004 a third evaluation of matrix effects [24]. The principle was similar to the one proposed by Bonfiglio et al. in 1999. It was also based on the flow injection analysis (FIA) of a solution of the compounds of interest, first in the mobile phase and then in the presence of the mobile phase additives suspected to produce signal alterations. They were quantified by comparing FIA with and without mobile phase additives.

* Corresponding author. Tel.: +41 22 379 63 36; fax: +41 22 379 68 08.
E-mail address: jean-luc.veuthey@unige.ch (J.-L. Veuthey).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.08.061

Since matrix effects can occur during the sample preparation and/or the analysis, various situations can arise. To the best of our knowledge, no exhaustive investigation of the various matrix effects has been published. The aim of this study was therefore to build an exhaustive classification of probable matrix effects encountered when a sample preparation is performed prior to a LC–MS analysis and to test the validity of the proposed model on a set of 198 compounds of pharmaceutical or doping interest.

2. Experimental

The influence of the matrix on the SPE procedure and the LC–MS analysis was evaluated on 198 compounds in urine and plasma samples. Three different SPE and LC–MS procedures were used.

2.1. Chemicals

All standards came from various pharmaceutical companies and were of pharmaceutical purity. A stock solution at 1 mg mL^{-1} was prepared for each substance in a mixture of water/acetonitrile (ACN) (1/1, v/v) and stored at −20 °C. Working solutions were made from the stock solutions, each one containing from two to six analytes at a concentration below the toxic level. Dilution of stock solution was operated with ultra-pure water generated with a Milli-Q Plus water purification system from Millipore (Bedford, USA). Methanol Absolute HPLC-Supra gradient and acetonitrile HPLC-S gradient grade (Biosolve, Valkenswaard, Netherlands), ammonia solution 25% puriss p.a., formic acid puriss p.a. and sodium formate puriss p.a. for HPLC (Fluka, Buchs, Switzerland), and hydrochloric acid fuming 37% puriss p.a. (Merck, Darmstadt, Germany) were used in all experiments. Human plasma and urine samples were obtained from a total of six healthy non-drug-consuming volunteers.

2.2. SPE

2.2.1. Oasis HLB

Oasis HLB 30 mg cartridges with 30 μm particles (Waters Corporation, Milford, MA, USA) were employed to prepare plasma samples. An automated ASPEC GX-274 (Gilson, Middletown, USA) system was employed to manage SPE cartridges. One milliliter of methanol (MeOH) and 1 mL of HCl 6N 2% in water was used for sorbent conditioning and equilibration. One milliliter of sample was loaded and washing was performed with 1 mL of a mixture of HCl 120 mM/MeOH (90/10, v/v). Elution was finally carried out with 500 μL of MeOH. Elution solutions were directly transferred to injection vials.

2.2.2. Oasis MAX

Oasis MAX 30 mg cartridges with 30 μm particles (Waters Corporation) were employed to extract acidic compounds from urine samples. They were centrifuged at 2500 × *g* for 5 min and 750 μL of 5% NH_4OH was added to 750 μL of the supernatant. The sorbent was conditioned with 500 μL of MeOH and equilibrated 500 μL of 5% NH_4OH. One milliliter of the basified sample was loaded and washing was carried out with 1 mL of 5% NH_4OH and then with 250 μL of MeOH. Elution was performed with 250 μL of 2% HCOOH in MeOH. Elution solutions were directly transferred to injection vials.

2.2.3. Oasis MCX

Oasis MCX 30 mg cartridges with 30 μm particles (Waters Corporation) were used to extract basic and neutral compounds from urine samples. They were centrifuged at 2500 × *g* for 5 min and 750 μL of HCl 240 mM was added to 750 μL of the collected supernatant. The sorbent was conditioned with 500 μL of MeOH and equilibrated with 500 μL of HCl 120 mM. One milliliter of the acidified sample was loaded and washed with a mixture of HCl 120 mM/MeOH (90/10, v/v). The first elution was done with 250 μL of MeOH and the second elution with the same volume of 5% NH_4OH in MeOH. Both elutions were evaporated to dryness and reconstituted in 50 μL of a mixture of water/MeOH (50/50, v/v) before the analysis.

2.3. Analytical methods

2.3.1. LC–MS

Urine samples extracted on Oasis MCX were analyzed with an Agilent Series 1100 LC system (Agilent Technologies) equipped with an auto-sampler and a binary pump. This system was coupled to an 1100 MSD single quadrupole (Agilent Technologies) with an orthogonal ESI source.

Five microliters of the sample was injected onto an XBridge Shield 100 mm × 2.1 mm, 3.5 μm column (Waters Corporation) kept at 30 °C. The mobile phase consisted of acetate buffer (20 mM pH 5/ACN, 67/33, v/v) and was delivered in the isocratic mode at 300 μL min^{-1}. Nitrogen was used as both the nebulizing (5 L min^{-1}) and drying gas (250 °C). Vaporizer temperature was set at 250 °C, nebulizer pressure at 45 psig and capillary voltage at +2 kV. Detection of protonated analytes was always conducted in the selected ion monitoring (SIM) mode. The Chemstation A.10.03 software (Agilent Technologies) was used for instrument control, data acquisition and data handling.

2.3.2. LC–MS/MS

Plasma samples extracted on Oasis HLB were analyzed with an Agilent Series 1200 LC system (Agilent Technologies, Waldbronn, Germany) equipped with an auto-sampler, a binary pump and a column heater. This system was coupled to a QTrap system (Applied Biosystems, Darmstadt, Germany) with a TurboIonSpray source.

Ten microliters of the sample was injected onto a Hypersil Gold 100 mm × 2.1 mm, 1.9 μm column (Thermo-Scientific, Waltham, MA, USA) kept at 40 °C and equipped with a 2 μm precolumn filter. The mobile phase was delivered at 400 μL min^{-1} in gradient mode. It consisted of (A) 0.1% formic acid and sodium formate 1 μM/(B) ACN. An initial mobile phase composition of 5% A was gradually increased to 70% in 12 min, then to 100% in 2 min and finally held at 100% for 1 min. A 5-min post-run equilibration time was applied after each analysis. Total eluent flow from the HPLC was directed into the turbo ionspray source without any splitting device. MS detection was performed in the positive mode and the needle voltage was +5 kV. The collision cell gas (nitrogen) pressure was set at 5 mTorr. The turbo ion spray heater was maintained at 420 °C with the nebulizer gas and heater gas set at 35 and 65 psi, respectively. Data acquisition, data handling and instrument control were performed with Analyst® version 1.4.

2.3.3. UPLC–MS

Urine samples extracted on Oasis MCX and MAX were analyzed with a Waters Aquity UPLC system (Waters Corporation) equipped with a binary solvent manager and a cooled auto-sampler kept at 4 °C. This system was coupled to a Micromass-Q-Tof Premier® mass spectrometer (Waters Corporation) with an electrospray ionization (ESI) source. Ten microliters of the sample was injected onto a Waters Acquity UPLC BEH C18 2.1 mm × 50 mm, 1.7 μm column (Waters Corporation) kept at 30 °C. The mobile phase consisted of (A) 0.1% formic acid in water/(B) 0.1% formic acid in ACN, linearly programmed from 5% to 95% B in 3 min, with 1.5 min equilibration time and delivered at 400 μL min^{-1}. A Waters Acquity Van Guard BEH C18 2.1 mm × 5 mm, 1.7 μm precolumn (Waters Corporation) was placed in front of the analytical column. Samples extracted on the MCX sorbent were monitored in the positive mode whereas

those extracted on the MAX were monitored in the negative mode. The desolvation gas was delivered at 800 L h^{-1} and 300 °C, and the capillary was set at +3 kV and −2.4 kV in the positive and negative mode, respectively. The micro-channel plates (MCP) were operated at +1.8 kV and −1.7 kV in the positive and negative mode, respectively. The source was adjusted to 100 °C, the cone voltage to 40 V and the cone gas flow to 10 L h^{-1}, the collision energy to 5 eV, and the collision gas flow to 0.32 mL min^{-1} in positive mode and 0.25 mL min^{-1} in the negative mode. Data acquisition, data handling and instrument control were performed with MassLynx® Software (Waters Corporation).

3. Results and discussion

As stated in the 2001 FDA Guidelines, "In the case of LC–MS/MS-based procedures, appropriate steps should be taken to ensure the lack of matrix effects throughout the application of the method" [25]. For this purpose, several approaches for the evaluation of matrix effects were described and, among them, Matuszewski et al. [23] proposed a procedure to quantitatively determine in which part of the analytical process matrix effects occur. The quality of the whole analytical process (i.e. *process efficiency*, PE) is related to alterations due to interferences during the sample preparation and analysis (e.g. ionization, ion transmission, etc.). PE was estimated by Matuszewski et al. with the ratio of peak areas from a matrix sample spiked prior to the sample preparation and from a neat standard (Fig. 1).

Residual compounds still present after sample preparation can interfere with the MS ionization process, leading to the well-known signal suppression or enhancement situations. The influence of endogenous compounds on LC–MS (i.e. *matrix effect*, ME) is evaluated by the ratio of peak areas from a matrix sample fortified after the sample preparation and a neat standard (Fig. 1). This is related to the concept of matrix factor (MF), defined as "*a ratio of the analyte peak response in the presence of matrix ions to the analyte peak response in the absence of matrix ions*" in the 2007 Washington workshop/conference report [26].

As previously mentioned, interferents may also affect the sample preparation, leading mostly to a decrease in the extraction yield when compared to standards. The recovery of extraction in the presence of the matrix (i.e. *extraction recovery*, RE) should therefore be determined with the ratio of peak areas from Fig. 1. However, this calculation does not allow for differentiating a low extraction recovery due to interfering compounds from a poor extraction yield due to the sample preparation itself (i.e. *extraction yield*, EY). Since generic procedures are often used in multianalyte determination, a low extraction yield could lead to an RE misevaluation. This modality was therefore included in this study and required only one additional sample, namely, a neat extraction standard. EY was evaluated with the ratio of peak areas from Fig. 1. Thus, a complete investigation of matrix effects required only four samples and the calculation of four ratios. Since RE and ME might present various combinations for a given PE, an exhaustive listing of possibilities is proposed in this work.

Table 1
Summary of observed situations (negative effect (−1), no effect (0), positive effect (+1)).

PE	−1				0		+1	
ME	−1	−1	0	+1	0	+1	+1	+1
RE	−1	0	−1	−1	0	−1	−1	0
	PE^{-}_{-}	PE^{-}_{0}	PE^{0}_{-}	PE^{+}_{-}	PE^{0}_{0}	PE^{+}_{-}	PE^{+}_{-}	PE^{+}_{0}

3.1. Description

As presented in Table 1, PE, being a combination of ME and RE, may be low, good or high (denoted by −1, 0 and +1, respectively). ME can also be divided into three modalities, since signal diminution, no alteration or signal enhancement (−1, 0 and +1, respectively), could be observed when endogenous compounds co-elute with the analytes. RE presents only two modalities since the sample preparation can reasonably either reduce or not influence the extraction recovery (−1 and 0, respectively). Accordingly, eight combinations could be obtained. For the sake of clarity and to define the influence of the matrix on PE, we have used the superscript to express ME while the subscript expresses RE. Therefore, a PE^{-}_{-} indicates that both matrix effect (signal suppression) and a low extraction recovery were observed. As indicated in Table 1, a low PE can result from three situations:

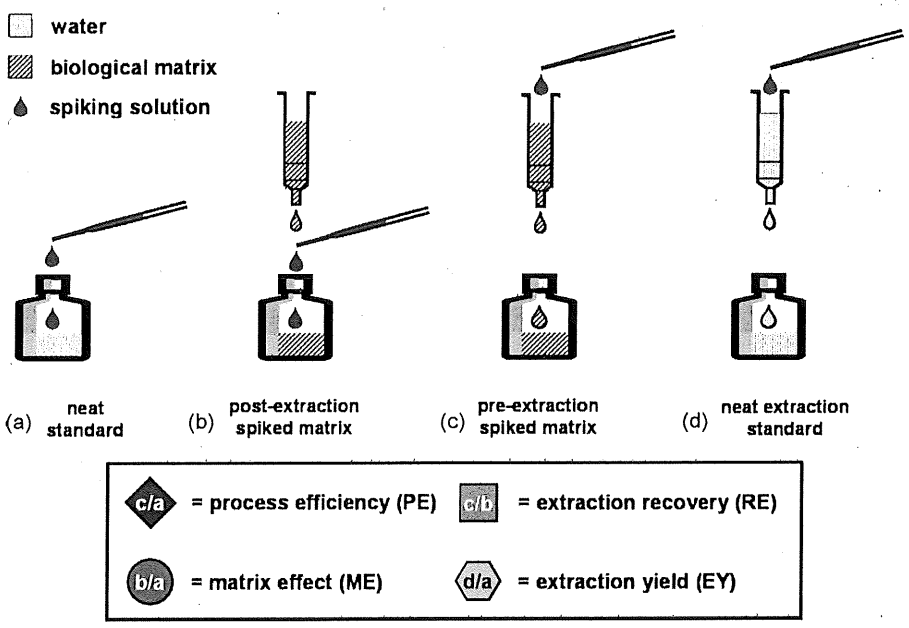

Fig. 1. Representation of samples required for matrix effects evaluation and calculation of the required ratios.

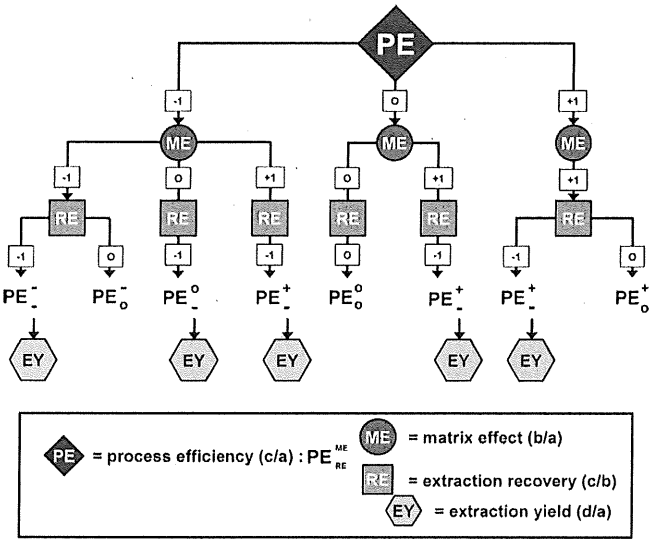

Fig. 2. General scheme for matrix effects attribution.

1. Pure effect; a low ME (e.g. MS signal suppression) or a low RE (PE^{-}_{0} and PE^{0}_{-}, respectively).
2. Synergistic effect; a simultaneous low ME and RE (PE^{-}_{-}) and
3. Antagonistic effect; a low RE partially compensated by a high ME (e.g. MS signal enhancement) (PE^{+}_{-}).

A good PE results from two different cases:

1. Absence of effect; no matrix influence on both ME and RE (PE^{0}_{0}) and
2. Antagonistic effect; a low RE compensated by a high ME (PE^{+}_{-}).

Finally, a high PE always requires a high ME which can or cannot be partially compensated by a low RE (PE^{+}_{-} and PE^{+}_{0}, respectively).

These eight combinations could be determined thanks to the procedure proposed by Matuszewski et al. However, the five cases presenting a low RE require the evaluation of EY for evidence of a possible low extraction yield as indicated in Fig. 2. This general scheme was employed to guide the estimation of the matrix influence and the attribution of cases to tested compounds. PE is first evaluated: the left branch of the scheme corresponds to low PE indicating a significant influence of endogenous compounds on the analytical process. The middle branch represents combinations leading to a good PE but maybe hiding two compensating effects (PE^{+}_{-}). Finally, the right branch reveals, as for low PE, a significant influence of endogenous compounds on the analysis. Then, the second row of the scheme allows refining the matrix effect typology, based on the same principle as discussed for PE. RE is finally used to precisely define the matrix effect typology. Finally, in the case of a low RE, EY should be determined.

3.2. Application

This approach was evaluated with regard to its ability to attribute the matrix effect typology to a set of 198 analytes ($N = 198$). Data were acquired, and all ratios (PE, ME, RE and EY) were determined using two matrices (plasma and urine) with various SPE protocols and LC–MS apparatus. PE, ME and RE were evaluated in triplicate for all compounds and completed with the estimation of EY (Table 2). Since LC–MS variability was around 5%, a reasonable limit was set at a value of ±10% to distinguish between low (+1) and high (−1) effects from good situations (0).

Influence of the biological matrix was successfully attributed to each tested analyte as shown in Fig. 2. However, in a few situations, PE, ME, RE or EY values were close but not within the acceptance limit, involving unclassified cases. In such situations, the closer matrix effect case was therefore selected. For example, cyclizine presented good PE, ME and EY values, while RE was 2% lower than the limit (i.e. 88%). This molecule was thus considered to present a good SPE recovery and was then attributed to the PE^{0}_{0} case.

In order to give a clear overview of the results, the number of analytes per case and their related percentages are reported in Fig. 3. About two-thirds of the analytes (137 cases, 69%) presented a low PE among which 16 compounds (8%) revealed a PE lower than 30%, mainly due to the combination of low RE and low ME. The remaining one-third (58 cases, 29%) showed a good PE almost equally divided between good ME and good RE (34 cases, 17%) and low RE compensated by a high ME (24 cases, 12%). Finally, only three cases (2%) presented a high PE.

It is interesting to note that plasma samples were mainly found in the PE^{-}_{-} and PE^{0}_{-} situation, suggesting a more complex extraction than urine (Fig. 4). However, when plasma extraction is well achieved, good MEs are often encountered (PE^{0}_{0} and PE^{0}_{-}). On the other hand, urine samples dominate PE^{-}_{0} cases, demonstrating that urine endogenous compounds lead to important signal suppressions in LC–ESI-MS even after solid phase extraction. The remaining urine samples are equally distributed between the remaining low and good PE cases. Finally, high PE cases were mostly absent from this set of data, probably due to the use of ESI, known to mainly provide signal suppression rather than signal enhancement.

In general, no influence of the biological matrices was observed for only 17% (34) of the tested analytes (Fig. 5a) and 39% were influenced by only one parameter, 16% of the compounds (30) due to extraction issues (i.e. SPE) and 23% (46) through detector signal alterations. Almost half of the investigated set, 44% (88), suffered from the presence of endogenous compounds in both SPE and LC–MS data, demonstrating the great impact of the biological matrix on the analytical process. Regarding ME (Fig. 5b), about 67% of the compounds exhibited signal alterations (134 cases), 45% presenting signal suppression (91 cases) while the remaining 22% saw signal enhancement (43 cases). As previously mentioned, signal suppressions were expected to be more abundant, since ESI was used. Finally, the RE distribution (Fig. 5c) was quite equilibrated, since 119 cases (60%) showed a low RE equally divided into pure low RE (60 cases, 30%) and low RE emphasized by a low ME (59 cases, 30%). The remaining 40% (80 cases) did not present significant extraction problems.

3.3. Corrective actions

Matrix effects do not necessarily need to be lowered or eliminated, but identified and quantified. Indeed, the 2007 Washington workshop/conference report fixes an acceptable limit for matrix effect variability at 15% for six individual batches of the matrix [26]. When variability is higher than 15%, modifications should be made to the relevant step(s) to reduce the influence of the matrix. Regarding signal alterations during the LC–MS analysis, the use of a deuterated internal standard (I.S.) is recommended. Indeed, as stated in the 2007 Washington workshop/conference report: "*Stable isotope – labeled I.S. minimizes the influence of matrix effects most effectively since the matrix effects observed for stable isotope – labeled I.S. are generally similar to those observed for the matching analyte*". If a deuterated I.S. is not available, an "*Analog I.S. may also compensate for matrix effects; however, the stable isotope – labeled internal standards are most effective and should be used whenever possible*" [26].

Table 2
List of the hundred and ninety-eight investigated compounds with their respective Process Efficiency (PE), Matrix Effect (ME), Extraction Recovery (RE) and Extraction Yield (EY).

Compound	Matrix	Apparatus	PE (%)	ME (%)	RE (%)	EY (%)
Alprazolam	Plasma	LC–MS/MS	45	49	90	82
Amfepramone	Plasma	LC–MS/MS	93	92	103	101
Amisulpride	Plasma	LC–MS/MS	93	132	74	91
Amitriptyline	Plasma	LC–MS/MS	54	61	91	100
Amphetamine	Plasma	LC–MS/MS	78	91	86	89
Aripirazole	Plasma	LC–MS/MS	80	102	78	99
Atenolol	Plasma	LC–MS/MS	44	92	48	34
Bisoprolol	Plasma	LC–MS/MS	101	99	102	96
Bromazepam	Plasma	LC–MS/MS	79	89	91	91
Buprenorphine	Plasma	LC–MS/MS	89	109	84	96
Bupropion	Plasma	LC–MS/MS	109	134	83	91
Chlordiazepoxide	Plasma	LC–MS/MS	93	91	103	95
Chloroquine	Plasma	LC–MS/MS	107	129	84	88
Chlorprothixène	Plasma	LC–MS/MS	36	53	71	90
Citalopram	Plasma	LC–MS/MS	90	109	82	94
Clobazam	Plasma	LC–MS/MS	38	40	94	66
Clomipramine	Plasma	LC–MS/MS	62	95	65	101
Clonazepam	Plasma	LC–MS/MS	34	41	83	86
Clonidine	Plasma	LC–MS/MS	95	128	76	100
Clotiapine	Plasma	LC–MS/MS	29	33	90	105
Clozapine	Plasma	LC–MS/MS	92	104	89	82
Cocaïne	Plasma	LC–MS/MS	92	97	96	93
Codeine	Plasma	LC–MS/MS	85	105	81	16
Cotinine	Plasma	LC–MS/MS	2	32	6	8
Cyclizine	Plasma	LC–MS/MS	95	109	88	94
Desipramine	Plasma	LC–MS/MS	47	45	105	119
Desmethyl-chlordiazepoxide	Plasma	LC–MS/MS	3	7	47	7
Dextrometrophane	Plasma	LC–MS/MS	77	89	86	99
Dimetindène	Plasma	LC–MS/MS	46	65	71	109
Diphenhydramine	Plasma	LC–MS/MS	84	111	77	56
Duloxetine	Plasma	LC–MS/MS	29	42	75	71
EDDP	Plasma	LC–MS/MS	76	88	85	83
Flumazenil	Plasma	LC–MS/MS	88	110	81	93
Flunitrazepam	Plasma	LC–MS/MS	28	43	66	72
Fluoxetine	Plasma	LC–MS/MS	53	63	85	58
Fluphenazine	Plasma	LC–MS/MS	73	84	87	101
Fluvoxamine	Plasma	LC–MS/MS	74	103	72	98
Haloperidol	Plasma	LC–MS/MS	89	96	93	96
Heroïne	Plasma	LC–MS/MS	107	248	44	93
9-Hydroxy-risperidone	Plasma	LC–MS/MS	92	107	86	83
Imipramime	Plasma	LC–MS/MS	36	73	50	104
Indomethacine	Plasma	LC–MS/MS	24	55	44	57
Lamotrigine	Plasma	LC–MS/MS	89	116	77	102
Levopromazine	Plasma	LC–MS/MS	14	26	56	59
Lidocaïne	Plasma	LC–MS/MS	93	114	83	84
Loperamide	Plasma	LC–MS/MS	81	109	76	102
LSD	Plasma	LC–MS/MS	55	67	82	90
Maprotiline	Plasma	LC–MS/MS	56	68	82	80
MDMA (extasy)	Plasma	LC–MS/MS	89	121	74	95
Methadone	Plasma	LC–MS/MS	43	83	52	87
Methaqualone	Plasma	LC–MS/MS	37	48	80	94
Metoclopramide	Plasma	LC–MS/MS	99	104	96	90
Metoprolol	Plasma	LC–MS/MS	84	106	79	94
Mianserine	Plasma	LC–MS/MS	111	128	89	98
Mirtazapine	Plasma	LC–MS/MS	80	102	79	99
Moclobemide	Plasma	LC–MS/MS	85	96	89	90
6-Mono-acetyl-morphine	Plasma	LC–MS/MS	103	102	103	89
Morphine	Plasma	LC–MS/MS	15	33	45	23
N-Desmethyl-citalopram	Plasma	LC–MS/MS	86	114	78	97
N-Desmethyl-clomipramine	Plasma	LC–MS/MS	68	89	76	98
N-Desmethyl-clozapine	Plasma	LC–MS/MS	100	121	82	93
N-Desmethyl-mirtazapine	Plasma	LC–MS/MS	67	96	71	78
Nefazodone	Plasma	LC–MS/MS	70	92	76	86
Nicotine	Plasma	LC–MS/MS	8	25	33	2
Nitrazepam	Plasma	LC–MS/MS	62	73	86	89
Nordiazepam	Plasma	LC–MS/MS	55	63	87	99
Norfluoxetine	Plasma	LC–MS/MS	34	57	59	91
Nortriptyline	Plasma	LC–MS/MS	29	33	89	90
Noscapine	Plasma	LC–MS/MS	96	115	84	90
O-desmethyl-venlafaxine	Plasma	LC–MS/MS	95	96	101	99
Olanzapine	Plasma	LC–MS/MS	106	110	100	88
Opipramol	Plasma	LC–MS/MS	113	138	82	83
Orphenadrine	Plasma	LC–MS/MS	71	86	82	104
Oxazepam	Plasma	LC–MS/MS	58	82	72	81
Papaverine	Plasma	LC–MS/MS	101	123	83	94

Table 2 (*Continued*)

Compound	Matrix	Apparatus	PE (%)	ME (%)	RE (%)	EY (%)
Paroxetine	Plasma	LC–MS/MS	79	102	79	90
Pentazocine	Plasma	LC–MS/MS	95	98	98	85
Propanolol	Plasma	LC–MS/MS	83	92	91	86
Propofol	Plasma	LC–MS/MS	44	62	71	82
Protriptyline	Plasma	LC–MS/MS	49	74	67	103
Quinine	Plasma	LC–MS/MS	32	37	88	118
Quetiapine	Plasma	LC–MS/MS	93	107	88	105
Reboxetine	Plasma	LC–MS/MS	97	108	90	87
Risperidone	Plasma	LC–MS/MS	88	96	92	90
Sertindole	Plasma	LC–MS/MS	99	122	82	103
Sertraline	Plasma	LC–MS/MS	49	73	68	87
Sotalol	Plasma	LC–MS/MS	17	98	18	60
Tamoxifène	Plasma	LC–MS/MS	58	102	57	82
Thioridazine	Plasma	LC–MS/MS	95	91	116	86
Tramadol	Plasma	LC–MS/MS	89	98	93	96
Triazolam	Plasma	LC–MS/MS	78	79	99	99
Trimipramine	Plasma	LC–MS/MS	32	48	68	86
Venlafaxine	Plasma	LC–MS/MS	98	119	83	105
Verapamil	Plasma	LC–MS/MS	63	75	85	97
Zolpidem	Plasma	LC–MS/MS	106	109	97	101
Zopiclone	Plasma	LC–MS/MS	80	102	81	91
Acetazolamide	Urine	UPLC–MS	65	57	115	108
Amfepramone	Urine	UPLC–MS	87	95	92	90
Amfetaminil	Urine	UPLC–MS	47	83	57	33
Amiloride	Urine	UPLC–MS	62	59	106	83
Anastrozole	Urine	UPLC–MS	82	102	80	98
Atenolol	Urine	UPLC–MS	62	96	65	87
Bendroflumethiazide	Urine	UPLC–MS	99	153	64	90
Benzoylecgonine	Urine	UPLC–MS	111	100	111	87
Bromantan	Urine	UPLC–MS	100	349	29	95
Buprenorphine	Urine	UPLC–MS	105	145	72	47
Bupropion	Urine	UPLC–MS	120	108	111	114
Caffeine	Urine	UPLC–MS	77	124	63	100
Cathine	Urine	UPLC–MS	89	179	50	80
Celiprolol	Urine	UPLC–MS	119	99	120	101
Chlorothiazide	Urine	UPLC–MS	46	71	64	59
Chlorphentermine	Urine	UPLC–MS	91	106	85	119
Chlorthalidone	Urine	UPLC–MS	43	41	105	92
Clopamide	Urine	UPLC–MS	81	74	110	74
Crothetamide	Urine	UPLC–MS	47	218	22	115
Dextromoramide	Urine	UPLC–MS	97	121	80	85
Dichlorphenamide	Urine	UPLC–MS	57	48	118	87
Dimetamphetamine	Urine	UPLC–MS	94	108	87	101
Ephedrine	Urine	UPLC–MS	78	72	108	73
Etafedrine	Urine	UPLC–MS	63	125	50	76
Ethacrynic acid	Urine	UPLC–MS	68	65	105	69
Ethylamphetamine	Urine	UPLC–MS	81	156	52	71
Etilefrine	Urine	UPLC–MS	88	77	115	108
Fenfluramine	Urine	UPLC–MS	113	160	71	113
Fenproporex	Urine	UPLC–MS	65	121	54	83
Fentanyl	Urine	UPLC–MS	105	105	100	109
Hydrochlorothiazide	Urine	UPLC–MS	20	31	63	66
Indapamide	Urine	UPLC–MS	77	78	99	108
Isometheptene	Urine	UPLC–MS	72	157	46	83
MDA	Urine	UPLC–MS	56	105	54	72
MDMA	Urine	UPLC–MS	97	153	63	107
Mefenorex	Urine	UPLC–MS	104	183	57	104
Metamphetamine	Urine	UPLC–MS	71	93	76	67
Methadone	Urine	UPLC–MS	61	57	108	84
Methylamphetamine	Urine	UPLC–MS	81	89	91	78
Methylecgonine	Urine	UPLC–MS	6	83	8	15
Methylphenidate	Urine	UPLC–MS	94	166	57	101
Metipranolol	Urine	UPLC–MS	100	114	87	114
Metolazone	Urine	UPLC–MS	46	229	20	87
Metoprolol	Urine	UPLC–MS	101	86	117	97
Nadolol	Urine	UPLC–MS	100	92	109	106
Nikethamide	Urine	UPLC–MS	62	75	83	76
Norbuprenorphine	Urine	UPLC–MS	37	70	53	46
Norfentanyl	Urine	UPLC–MS	105	219	48	119
Oxilofrine	Urine	UPLC–MS	81	97	84	102
Pemoline	Urine	UPLC–MS	81	100	81	64
Pentetrazol	Urine	UPLC–MS	31	47	66	75
Phendimetrazine	Urine	UPLC–MS	81	95	85	90
Phenpromethamine	Urine	UPLC–MS	93	204	46	110
Phentermine	Urine	UPLC–MS	88	213	41	96
Phenylpropanolamine	Urine	UPLC–MS	85	105	81	110

Table 2 (*Continued*)

Compound	Matrix	Apparatus	PE (%)	ME (%)	RE (%)	EY (%)
Pholedrine	Urine	UPLC–MS	78	86	91	95
Probenecide	Urine	UPLC–MS	77	450	17	93
Prolintane	Urine	UPLC–MS	105	183	57	85
Propylhexedrine	Urine	UPLC–MS	77	152	51	79
Pseudoephedrine	Urine	UPLC–MS	80	75	107	89
Ritalinic acid	Urine	UPLC–MS	48	100	49	90
RSR 14	Urine	UPLC–MS	85	124	69	73
Salmeterol	Urine	UPLC–MS	94	99	95	78
Sibutramine	Urine	UPLC–MS	109	382	28	87
Strychnine	Urine	UPLC–MS	55	79	70	81
Xipamide	Urine	UPLC–MS	94	115	82	91
Acebutolol	Urine	LC–MS	59	65	90	93
Acetazolamide	Urine	LC–MS	26	28	92	31
Adrafinil	Urine	LC–MS	54	55	98	98
Atenolol	Urine	LC–MS	48	53	91	86
Bendroflumethiazide	Urine	LC–MS	69	72	96	106
Benzoylecgonine	Urine	LC–MS	46	49	91	92
Bethamethasone	Urine	LC–MS	77	72	106	105
Bumetanide	Urine	LC–MS	82	86	95	84
Canrenone	Urine	LC–MS	69	71	99	89
Carteolol	Urine	LC–MS	64	64	100	93
Celiprolol	Urine	LC–MS	71	79	90	98
Chlorothiazide	Urine	LC–MS	24	26	91	91
Chlortalidone	Urine	LC–MS	75	65	114	86
Clopamide	Urine	LC–MS	35	38	91	93
Dexamethasone	Urine	LC–MS	79	80	98	109
Dichlorphenamide	Urine	LC–MS	86	78	109	107
Esmolol	Urine	LC–MS	10	9	109	95
Ethacrinic acid	Urine	LC–MS	52	56	93	96
Fentanyl	Urine	LC–MS	91	97	94	91
Finasteride	Urine	LC–MS	73	75	94	95
Furosemide	Urine	LC–MS	83	98	85	84
Gestrinone	Urine	LC–MS	74	74	100	99
Hydrochlorothiazide	Urine	LC–MS	62	55	113	106
Indapamide	Urine	LC–MS	97	94	98	103
Methylphenidate	Urine	LC–MS	78	95	83	92
Metipranolol	Urine	LC–MS	56	61	93	99
Metolazone	Urine	LC–MS	33	26	111	98
Metoprolol	Urine	LC–MS	27	30	89	94
Modafinil	Urine	LC–MS	96	83	107	100
Nadolol	Urine	LC–MS	54	53	97	94
Piretanide	Urine	LC–MS	51	46	93	108
Probenecide	Urine	LC–MS	93	100	93	102
Sotalol	Urine	LC–MS	58	51	109	97
Strychnine	Urine	LC–MS	36	39	94	96
Torasemide	Urine	LC–MS	57	66	85	87
Xipamide	Urine	LC–MS	68	67	101	109

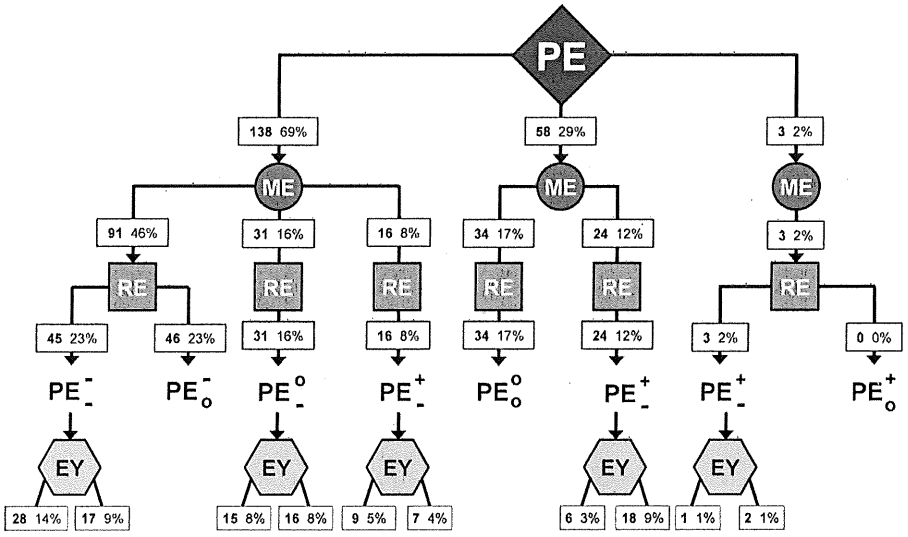

Fig. 3. General distribution of matrix effects.

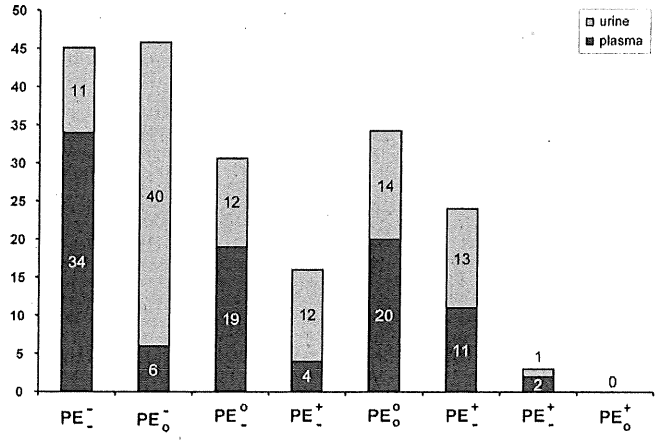

Fig. 4. Matrix distribution among the eight cases.

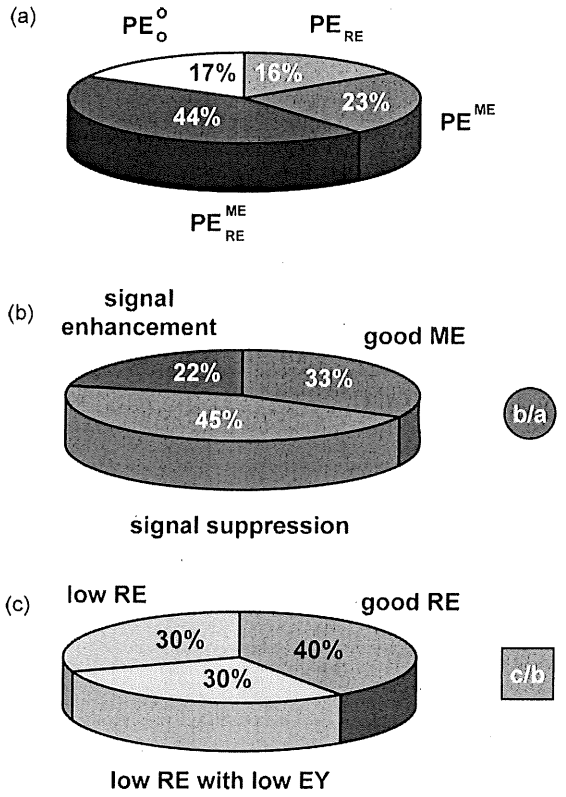

Fig. 5. (a) Relative distribution of ME and RE on the whole set of data. PE^0_0 indicates cases with good ME and RE, PEME is related to cases with low or high ME, PERE refers to cases with low RE and PE^{ME}_{RE} is related to cases with low or high ME and low RE. (b) Relative distribution of low, good and high ME. (c) Relative distribution of low and good RE.

Chromatographic conditions can also be improved to bring out the analyte from the matrix effect window using the procedure proposed by Bonfiglio et al. [22]. This method allows directly identifying the chromatographic region experiencing matrix effects. When endogenous compounds prevent a satisfactory extraction of the analytes with the selected procedure, protocol (re-)optimization could be reconsidered. In this regard, a sample pre-treatment (e.g. protein precipitation) can be of great help in removing the major part of endogenous compounds prior to a dedicated extraction [18].

Finally, the use of an alternative ionization source can strongly reduce matrix effects problems. Indeed, APCI and APPI have been shown to be less prone to signal alterations with MS detection [11,13,19,27]. In spite of the lower ionization yield of polar compounds, background noise is less intense, possibly leading to similar or higher signal-to-noise ratios. Finally, the use of direct-electron ionization (direct-EI) has also been recently proposed to overcome matrix effects encountered with MS detection for the analysis of pharmaceutical compounds in biological and environmental samples [28].

4. Conclusion

An overall classification of potential matrix effects was proposed in the case of a sample preparation followed by a LC–MS analysis. A total of eight different cases were evidenced.

Based on this classification, a schema was suggested for the attribution of the matrix effect typology. Only four samples (aqueous and matrix samples fortified prior or after sample preparation) were required, allowing the calculation of four ratios, namely PE, ME, RE, and EY. In order to illustrate the proposed method, a total of 198 analytes were used as model compounds to evaluate three different SPE and LC–MS protocols. All compounds were attributed to one of the eight possibilities. Eighty-three percent of the investigated molecules underwent matrix effects, the major part suffering from the influence of endogenous compounds during both SPE and LC–MS steps.

References

[1] W.M.A. Niessen, J. Chromatogr. A 794 (1998) 407.
[2] W.M.A. Niessen, A.P. Tinke, J. Chromatogr. A 703 (1995) 37.
[3] H.H. Maurer, J. Chromatogr. B 713 (1998) 3.
[4] P. Marquet, G. Lachatre, J. Chromatogr. B 733 (1999) 93.
[5] C.K. Lim, G. Lord, Biol. Pharm. Bull. 25 (2002) 547.
[6] J. Abian, J. Mass Spectrom. 34 (1999) 157.
[7] S. Souverain, S. Rudaz, J.L. Veuthey, J. Chromatogr. A 1058 (2004) 61.
[8] S. Souverain, S. Rudaz, J.-L. Veuthey, J. Pharm. Biomed. Anal. 35 (2004) 913.
[9] I. Marchi, S. Rudaz, M. Selman, J.L. Veuthey, J. Chromatogr. B 845 (2007) 244.
[10] J. Schuhmacher, D. Zimmer, F. Tesche, V. Pickard, Rapid Commun. Mass Spectrom. 17 (2003) 1950.
[11] L.E. Sojo, G. Lum, P. Chee, Analyst 128 (2003) 51.
[12] C.R. Mallet, Z. Lu, J.R. Mazzeo, Rapid Commun. Mass Spectrom. 18 (2004) 49.
[13] R. Dams, M.A. Huestis, W.E. Lambert, C.M. Murphy, J. Am. Soc. Mass Spectrom. 14 (2003) 1290.
[14] Y. Hsieh, M. Chintala, H. Mei, J. Agans, J.M. Brisson, K. Ng, W.A. Korfmacher, Rapid Commun. Mass Spectrom. 15 (2001) 2481.
[15] H. Mei, Y. Hsieh, C. Nardo, X. Xu, S. Wang, K. Ng, W.A. Korfmacher, Rapid Commun. Mass Spectrom. 17 (2003) 97.
[16] P.R. Tiller, L.A. Romanyshyn, Rapid Commun. Mass Spectrom. 16 (2001) 92.
[17] B.K. Choi, D.M. Hercules, A.I. Gusev, J. Chromatogr. A 907 (2001) 337.
[18] M.W.J. van Hout, H.A.G. Niederlander, R.A. de Zeeuw, G.J. de Jong, Rapid Commun. Mass Spectrom. 17 (2003) 245.
[19] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882.
[20] J. Henion, E. Brewer, G. Rule, Anal. Chem. 70 (1998) 650A.
[21] C. Polson, P. Sarkar, B. Incledon, V. Raguvaran, R. Grant, J. Chromatogr. B 785 (2003) 263.
[22] R. Bonfiglio, R.C. King, T.V. Olah, K. Merkle, Rapid Commun. Mass Spectrom. 13 (1999) 1175.
[23] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[24] M. Holcapek, K. Volna, P. Jandera, L. Kolarova, K. Lemr, M. Exner, A. Cırkva, J. Mass Spectrom. 39 (2004) 43.
[25] Food and Drug Administration, Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), 2001.
[26] C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, Pharm. Res. 24 (2007) 1962.
[27] Y. Hsieh, G. Wang, Y. Wang, S. Chackalamannil, J. Brisson, K. Ng, W.A. Korfmacher, Rapid Commun. Mass Spectrom. 16 (2002) 944.
[28] A. Cappiello, G. Famiglini, P. Palma, E. Pierini, V. Termopoli, H. Trufelli, Anal. Chem. 80 (2008) 9343.