Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1029 (2004) 153–159
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Countering matrix effects in environmental liquid chromatography–electrospray ionization tandem mass spectrometry water analysis for endocrine disrupting chemicals

Tom Benijts, Riet Dams, Willy Lambert*, André De Leenheer

Laboratory of Toxicology, Ghent University, Harelbekestraat 72, B-9000 Gent, Belgium

Received 19 August 2003; received in revised form 10 December 2003; accepted 11 December 2003

Abstract

In recent years, despite the increasing success of liquid chromatography (LC) coupled to tandem mass spectrometry (MS), reports on matrix susceptibility have shown the limitations of the this powerful analytical technique. Matrix effects (MEs) result from co-eluting residual matrix components affecting the ionization efficiency of target analytes and can lead to erroneous results. The present work evaluates the matrix effect of environmental water samples on 35 endocrine disrupting chemicals (EDCs) in negative and positive LC–ESI-MS/MS. It was shown that mobile-phase additives could significantly influence matrix effects. Addition of acids resulted in a severe signal suppression (average ME%: <65%), and 1 mM ammonium formate increased the average ME% to 84%. The importance of an efficient sample clean-up and internal standardization also was demonstrated. Cleaner extracts resulted in reduced matrix effects (average ME%: 89%) and labeled internal standards proved to have a beneficial effect especially on signal reproducibility (average CV% 4.2% versus 2.6%). The results from the present work indicate that evaluation of matrix effects should become an integrated part of quantitative LC–ESI-MS/MS method development and validation.

Keywords: Water analysis; Matrix effects; Environmental analysis; Endocrine disrupting chemicals

1. Introduction

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) with atmospheric pressure ionization (API) has become a important tool for identification and quantitation of analytes in complex mixtures [1–5]. Undoubtedly, the advent of electrospray ionization (ESI) [6,7] and atmospheric pressure chemical ionization (APCI) [8], which allow for soft ionization of a wide range of substances, is responsible for the increasing success of LC/MS analyses during the last 15 years. One of the limitations, however, is the susceptibility of API interfaces to co-extracted matrix components [9,10]. This matrix effect, defined as the effect of co-eluting residual matrix components on the ionization of the target analyte, typically results in either signal suppression or enhancement. Moreover, interfering matrix components can affect the reproducibility and accuracy of the developed procedure, leading to compromising or erroneous results [10–12].

The mechanism of matrix effect in LC–MS is still not fully resolved. It is assumed that solid analyte precipitation or co-precipitation with other non volatile matrix components causes gas phase ion suppression in APCI [10,13]. In ESI, however, a competition between matrix components and analytes for access to the droplet surface for gas phase emission has been suggested as possible cause of matrix effects. Some key reports have highlighted this phenomenon in bioanalytical [9,11–13,15–27], environmental [28–32], and food analyses [14,33–35].

The objective of the presented experiments was to evaluate the degree of matrix effect originating from co-eluting substances from environmental water samples on 35 endocrine disrupting chemicals (EDCs). Preliminary studies demonstrated highest sensitivity using LC–ESI-MS/MS. During method development for this unique mixture of analytes, the influence of environmental matrix on the electrospray ionization efficiency was assessed by spiking

* Corresponding author. Tel.: +32-9-264-81-35; fax: +32-9-264-81-83.
E-mail address: willy.lambert@ugent.be (W. Lambert).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.12.022

samples post-extraction and comparing signal to injected standards. The present work focused on: (i) the influence of mobile-phase additives (acids and buffers), (ii) the importance of an efficient sample clean-up, and (iii) the role of internal standards to compensate for these matrix effects in LC–ESI-MS/MS.

2. Experimental

2.1. Chemicals and reagents

17β-Estradiol, estrone, estriol, 17α-ethynyl estradiol, and diethyl stilbestrol (DES) were a kind gift from Professor Van den Bossche of the Laboratory for Pharmaceutical Chemistry and Drug Analysis (Ghent University, Belgium). 4-*t*-Octylphenol, 4-octylphenol, 4-*t*-butylphenol, 4-*s*-butylphenol, 4-*t*-amylphenol, 4-*n*-nonylphenol, 4-cumylphenol, bisphenol A, bisphenol F, methylparaben, ethylparaben, propylparaben, and benzylparaben were purchased from Sigma–Aldrich Chemicals (Bornem, Belgium). Atrazine-desisopropyl, atrazine-desethyl, hexazinone, simazine, cyanazine, metribuzine, desmetryn, atrazine, ametryn, sebutylazine, propazine, terbutylazine, terbutryn, prometryn, methomyl, aldicarb, and pyrimicarb were obtained from Dr. Ehrerstorfer (Augsburg, Germany). To facilitate discussion for this large mixture of analytes, representatives for each group (Table 1) were chosen to incorporate in the Figs. 1 and 2. Ammonium formate was purchased from Sigma–Aldrich Chemicals (Bornem, Belgium). Water, methanol, and acetonitrile were all of HPLC grade (Merck-Eurolab, Leuven, Belgium).

Stable isotope-labeled surrogate standards 17α-ethynyl estradiol-2,4,16,16-d_4, bisphenol A-d_6, estrone-2,4,16,16-d_4 were purchased from C/D/N Isotopes (Quebec, Canada), diethyl stilbestrol-ring-3,3′,5,5′-diethyl-1,1,1′,1′-d_8 was from Cambridge Isotope Laboratories Inc. (Andover, MA, USA), terbutryn-d_5, simazine-d_{10}, terbutylazine-d_5, 4-*n*-nonylphenol-d_8, atrazine-desethyl-d_6, sebutylazine-d_5, prometryn-d_6, atrazine-d_5, atrazine-desisopropyl-d_5, and propazine-d_6 were from Dr. Ehrerstorfer (Augsburg, Germany), and 17β-estradiol-2,4-d_2 was obtained from Sigma–Aldrich Chemicals (Bornem, Belgium).

Table 1
Target analytes

Group	Analytes[a]
Natural estrogens	Estrone, **estradiol**, estriol
Synthetic estrogens	Ethynyl estradiol, **diethyl stilbestrol** (**DES**)
Parabens	**Methyl-**, ethyl-, propyl-, benzylparaben
Diphenol alkanes	**Bisphenol A**, bisphenol F, 4-cumylphenol
Alkylphenols	4-*t*-Butylphenol, 4-*s*-butylphenol, ***t*-amylphenol**, **4-*t*-octylphenol**, 4-octylphenol, nonylphenol
Carbamates	Methomyl, **aldicarb**, pyrimicarb
Triazines	**Atrazine-desisopropyl**, atrazine-desethyl, hexazinone, simazine, cyanazine, metribuzine, desmetryn, **atrazine**, ametryn, sebutylazine, propazine, terbutylazine, terbutryn, **prometryn**

[a] Representative EDCs in bold.

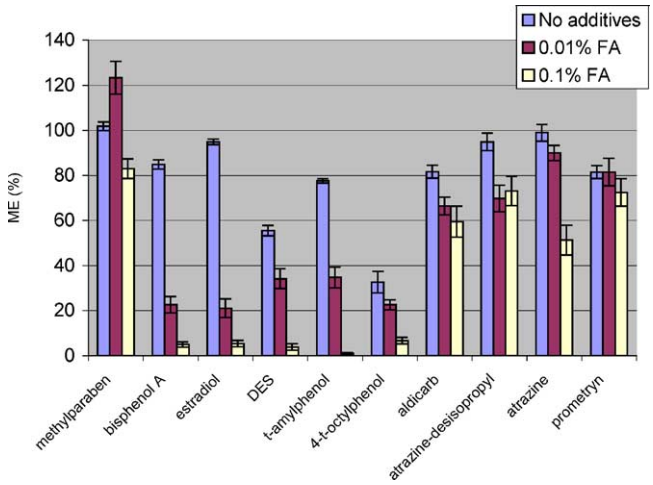

Fig. 1. Mean ME% for 10 representative EDCs obtained after addition of formic acid (FA).

2.2. LC–MS/MS

Liquid chromatography was carried out with an HP-1100 HPLC system (Agilent, Palo Alto, CA, USA). The analytes were chromatographed at ambient temperature under gradient conditions on a C18 Luna column (100 mm × 2 mm, 3 μm) fitted with a guard column with the same stationary phase (4 mm × 2 mm, particle size 3 μm) (Phenomenex, Torrance, CA, USA). Eluent flow rate was set at 200 μl/min. An LC/MSD Trap VL mass selective detector was equipped with an ESI interface (Agilent, Palo Alto, CA, USA). Parameters were optimized by continuous infusion of standards. Analytes were divided in two groups according

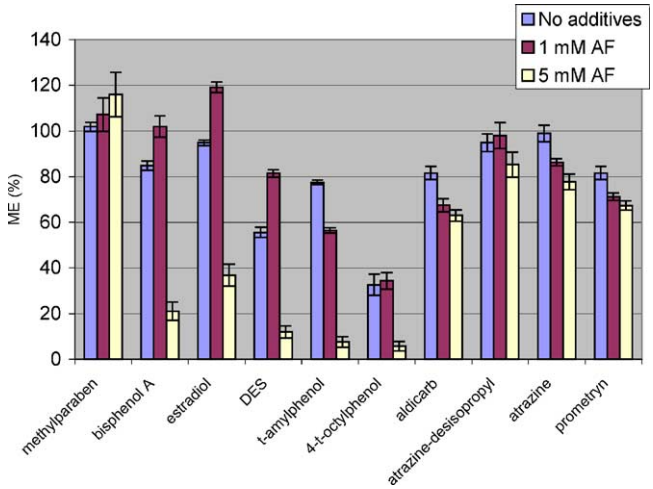

Fig. 2. Mean ME% for 10 representative EDCs obtained after addition of ammonium formate (AF).

ionization polarity (negative and positive ionization). The following optimized ESI parameters were applied: drying gas flow rate, 8 l min^{-1}; drying gas temperature, 350 °C; nebulizing gas pressure 30 psi; capillary voltage (NI/PI), +3861/−3811 V. The electron multiplier and dynode voltage were set at 1740 V and 7.0 kV, respectively. A switch valve was used to divert the column effluent to waste 2 min prior to and 1 min following elution of the analytes.

2.3. Sample preparation

Water samples were filtered over a precombusted GF/F (0.7 μm nominal) glass fiber filter and a membrane filter (0.45 μm nominal) (Whatman, Maidstone, England). The filtrate was subjected to an off-line solid-phase extraction (SPE) using Oasis HLB (6 ml, 200 mg) columns (Waters, Milford, MA, USA).

2.3.1. Basic SPE procedure

Columns were preconditioned sequentially with 6 ml methyl-*t*-butyl ether (MTBE), 6 ml methanol, and 6 ml water. Subsequent to sample loading (500 ml at approximately 10 ml/min), the column was rinsed with 3 ml of 5% methanol in water and dried under vacuum for 30 min. Analytes were eluted with two 3 ml portions of MTBE.

2.3.2. Extended SPE procedure

Conditioning of the cartridges was performed with 6 ml *i*-propanol–MTBE (10/90, v/v), 6 ml methanol, and 6 ml water. Samples were loaded onto the cartridges and rinsed sequentially with 3 ml of a mixture of water and methanol (70/30, v/v), 3 ml of water, and 3 ml 2% ammonia-methanol (90/10, v/v, pH 11.5). Analytes were eluted from the SPE cartridge with two 3 ml portions of *i*-propanol–MTBE (10/90, v/v).

The obtained extracts were evaporated to dryness in a TurboVap LV evaporator from Zymark (Hopkinton, MA, USA) and reconstituted in 200 μl water–acetonitrile (80/20, v/v).

2.4. Evaluation of matrix effect

In correspondence to the strategy applied by Matuszewski et al. [36], matrix effects were evaluated by comparing the MS/MS responses of known amounts of working standards (*A*) with those measured in a blank water extract spiked with the same analyte amount after extraction (*B*). Differences observed in MS/MS response could thus be attributed to the effect of sample matrix on the ionization efficiency only. The ratio ($B/A \times 100$) is defined as absolute matrix effect (ME%). The absence of absolute matrix effect is indicated by a value of 100%, i.e. the response in the mobile phase and in the extract is the same. A value of >100% indicates an ionization enhancement and a value of <100% indicates an ionization suppression. All samples were spiked at the same concentration (50 ng/l). Initial experiments were conducted in fivefold on blank surface water samples, collected from a local brook originating from the river Schelde.

3. Results and discussion

3.1. Mobile-phase additives

The influence of mobile-phase composition on ionization efficiency is a well described phenomenon in LC–MS [32,35,37–42]. Less illustrated is the effect of mobile-phase additives on matrix-induced ionization suppression/enhancement of analytes. Choi et al. [33] demonstrated an improved correlation between signals of standard and matrix samples when mobile-phase additives, such as formic acid, ammonium formate or ammonium hydroxide were used. This study investigated the influence on matrix effect of two acids (acetic acid and formic acid) at two concentration levels (0.01 and 0.1%, v/v) and two buffers (ammonium acetate and ammonium formate) at two different concentrations (1 and 5 mM). The MS/MS response ratio between standards and spiked extracts (basic SPE procedure) was used to express matrix effect. Fig. 1 illustrates the results obtained with formic acid for the 10 representative analytes. With the exception of the group of parabens, the addition of acid to the mobile phase had a drastic effect on the ionization efficiency of NI-analytes in the presence of matrix components. Both acetic and formic acid suppressed the responses significantly at a concentration of 0.01% (v/v). A concentration of 0.1% (v/v) even led to a signal suppression of over 90% (ME% < 10%). In contrast to the rest of the NI-analytes, methyl-, ethyl-, propyl-, and benzylparaben (represented by methylparaben) were less influenced by the addition of acids to the mobile phase. Mild acidic conditions even resulted in a slight signal enhancement. The effect of acidic conditions in PI-mode were less manifest. Although a predominantly suppressive effect was observed for both acids at the investigated concentration levels, ME% was never less than 50%.

A different trend was observed when supplementing buffers to the mobile phase. Depending on the nature and concentration of the buffer, differences in ME were observed. Fig. 2 shows the effect of ammonium formate on ionization efficiency of a spiked sample matrix (basic SPE procedure). As can be seen, the effects in NI are predominantly positive and much more pronounced as compared to those in PI. With increasing concentration, mobile-phase buffers exhibited a suppressive effect on analyte intensity during standard and matrix analysis. At a concentration of 1 mM, for a number of analytes, a slight ionization enhancement was observed, most likely due to changing spray conditions and/or ion–molecule reactions. The addition of 1 mM of ammonium formate even results in a signal enhancement for estriol (ME%: 172%). However, further increase of buffer concentrations to 5 mM resulted in a severe suppression of the analyte signal. The latter effect was probably due to an increased number of ions (matrix

and additives) in the spray. This could reduce access of the target analyte to the droplet surface, eventually leading to complete droplet saturation and suppression of target analyte ionization. Again the four parabens behaved differently as compared to the rest of the NI-analytes. Increasing the buffer concentration up to 5 mM, resulted for both ammonium formate as for ammonium acetate in a slight signal enhancement. In general, for both NI and PI, relative responses using ammonium formate were slightly favorable to those obtained with ammonium acetate (data not shown). Based on these findings, we concluded to add 1 mM of ammonium formate to the HPLC-solvent.

It must be noted that the use of the aforementioned mobile-phase additives only slightly influenced the elution profile of the target analytes. Prior to LC–MS/MS a full scan LC–MS analysis was performed in order to adjust MS/MS time segments. Full scan mass spectra did not reveal any adduct formation arising from mobile-phase additives.

3.2. Solid-phase extraction

Although considered to be a very effective separation technique, in many cases liquid chromatography alone has been shown to be insufficient in handling matrix effect.

Table 2
Influence of sample clean-up and internal standardization on ME% ($n = 5$)

	Basic SPE procedure		Extended SPE procedure		Internal standardization	
	ME%	CV%	ME%	CV%	ME%	CV%
Negative mode						
Estriol	172	21.0	110	5.7	99	2.6
Methylparaben	107	6.8	101	4.5	98	3.6
Ethylparaben	110	3.8	105	3.6	98	2.2
Bisphenol F	81	6.0	92	5.0	92	3.7
Propylparaben	114	1.0	103	2.6	100	1.8
Bisphenol A	102	4.5	100	3.0	99	1.8
Estradiol	119	2.0	107	3.8	98	2.5
Ethynyl estradiol	86	1.7	94	2.1	97	2.4
Estrone	76	7.3	88	4.2	95	2.9
Benzylparaben	106	5.1	97	6.7	97	3.1
4-*t*-Butylphenol	110	5.1	95	6.5	93	3.7
DES	82	2.0	88	3.8	96	2.8
4-*s*-Butylphenol	89	2.7	94	2.8	90	3.0
4-*t*-Amylphenol	57	2.1	79	7.4	85	3.8
4-Cumylphenol	80	5.9	90	5.8	93	2.5
4-*t*-Octylphenol	34	10.4	55	9.7	68	4.9
4-Octylphenol	12	9.9	31	7.6	45	5.8
4-*n*-Nonylphenol	32	7.1	50	7.6	77	2.6
Mean[a]			88	5.1	90	3.1
Mean[b]			88	4.1	94	2.5
Positive mode						
Metomyl	61	5.5	81	3.9	85	2.7
Atrazine-desisopropyl	98	5.8	97	5.2	98	1.7
Atrazine-desethyl	90	2.3	95	2.9	99	2.1
Hexazinone	98	3.8	99	2.5	103	1.4
Aldicarb	68	4.3	81	5.0	87	3.4
Simazine	95	4.6	95	4.7	97	2.2
Cyanazine	92	4.2	96	3.9	99	2.0
Metribuzine	96	2.9	96	2.1	95	1.4
Pyrimicarb	90	4.9	94	2.5	97	2.2
Desmetryn	75	1.5	88	2.0	94	1.6
Atrazine	86	1.8	93	2.4	100	1.6
Ametryn	76	1.1	87	1.8	104	2.2
Sebutylazine	77	1.1	81	3.5	95	2.2
Propazine	74	3.5	92	3.3	95	2.0
Terbutylazine	64	3.3	86	2.4	94	1.3
Terbutryn	60	4.5	89	2.5	98	2.0
Prometryn	71	2.2	90	3.2	97	1.7
Mean[a]			91	3.2	96	2.0
Mean[b]			90	3.2	97	1.8

[a] Mean of all analytes.
[b] Mean of analytes with IS.

Recent studies on matrix effect have highlighted the importance of an efficient sample clean-up in quantitative LC–ESI-MS/MS analysis [9,15,17,18,30]. The effect of SPE on ionization efficiency was evaluated for two SPE extraction procedures using Oasis HLB columns. Both procedures differ in extent of the washing step. The basic SPE procedure includes a classical low organic wash (5% methanol), while the extended SPE procedure consists of a three-step wash, adjusting organic concentration (30%, v/v, methanol) and pH (11.5). This extended washing procedure is mainly focused on interfering humic acids and other organic material, typically present in environmental water samples. Table 2 depicts the ME obtained with both extraction procedures. As can be seen, the influence of an extended washing procedure is substantial (values closer to 100%). In both ionization modes, increased ME% values were observed for practically all compounds. Apparently, a majority of interfering matrix components could be eliminated. Except for 4-*t*-octylphenol (55%), 4-octylphenol (31%), and nonylphenol (50%) the ME% for all analytes was acceptable, exceeding 79%. Apparently, ionization of late eluting alkylphenols suffers heavily from hydrophobic matrix components, insufficiently removed by SPE extraction. These findings are in accordance with previously published data obtained for nonylphenol, where a suppression up to 50% was noticed [29,43].

3.3. Internal standards

To compensate the phenomenon of matrix effect internal standards have been shown to be an effective tool [21,29]. An important prerequisite, however, is that analyte and internal standard have very similar characteristics, and identical, or at least very close, retention times. Both compounds should be affected by the co-eluted matrix to the same extent. In this respect isotopically labeled internal standards offer the best solution. However, since the use of stable isotopes is generally very cost prohibitive, and commercial availability

Table 3
Influence of sample origin on ME% ($n = 5$)

	Surface water ME%	Rain water ME%	Ground water ME%	Channel water ME%	WWTP ME%	Industrial effluents ME%
Neagtive mode						
Estriol	99	95	96	95	94	95
Methylparaben	98	96	97	101	98	97
Ethylparaben	98	99	97	99	97	94
Bisphenol F	92	95	95	93	92	91
Propylparaben	100	93	101	95	96	95
Bisphenol A	99	102	100	98	99	101
Estradiol	98	99	100	98	100	98
Ethynyl estradiol	97	98	98	97	100	100
Estrone	95	96	97	100	99	96
Benzylparaben	97	95	97	98	98	93
4-*t*-Butylphenol	93	97	95	97	94	91
DES	96	98	94	98	97	93
4-*s*-Butylphenol	90	91	92	91	94	90
t-Amylphenol	85	91	87	89	90	85
4-Cumylphenol	93	96	95	97	93	95
4-*t*-Octylphenol	68	72	61	66	68	67
4-Octylphenol	45	55	52	49	45	43
Nonylphenol	77	82	80	79	79	74
Positive mode						
Metomyl	85	89	88	85	89	84
Desisopropyl atrazine	98	100	102	99	100	102
Desethyl atrazine	99	99	100	100	101	98
Hexazinone	103	101	99	101	98	97
Aldicarb	87	91	93	89	87	86
Simazine	97	99	99	97	95	95
Cyanazine	99	101	101	98	97	96
Metribuzine	95	96	97	98	96	93
Pyrimicarb	97	98	98	95	96	93
Desmetryn	94	96	96	93	93	92
Atrazine	100	101	100	99	99	98
Ametryn	104	100	100	101	98	100
Sebutylazine	95	98	98	94	94	92
Propazine	95	100	98	97	96	95
Terbuthylazine	94	99	96	96	94	93
Terbutryn	98	100	102	97	98	96
Prometryn	97	99	99	97	95	95

is often limited, in many cases structural analogs offer a valuable alternative. In this study, for the 35 EDCs under investigation, as much as 15 isotopically labeled internal standards were used to compensate for matrix effects. In the absence of a commercially available stable isotope, the closest eluting isotopically labeled compound was assigned as internal standard for the remaining target analytes. Table 2 summarizes the ME% obtained with internal standardization using the extended SPE extraction. The mean ME% is given for all target analytes and for those having an identical isotopically labeled analogue. As can be seen, internal standards bring ME% values closer to 100%, and this beneficial trend is noticeable for practically all analytes (with or without isotopically labeled analogue). Unfortunately, for 4-octylphenol and 4-*t*-octylphenol the assigned labeled internal standard (4-*n*-nonylphenol-d_8) could not compensate for the substantial matrix suppression. ME% for both analytes is still far away from the target value of 100% (45 and 68%, respectively) when compared to all other analytes (>77%). However, although the loss in sensitivity associated with matrix suppression was not compensated, applying internal standardization had a positive effect on reproducibility of subsequent measurements with coefficients of variation ($n = 5$) never exceeding 5.8% for all analytes. This compensating effect of internal standards on precision and reliability of the quantitative LC–MS/MS method is in agreement with results reported by Matuszewski et al. [36].

3.4. Environmental water samples

Various types of environmental water samples, including surface water, rain water, ground water, channel water, wastewater treatment plant (WWTP) effluents, and industrial effluents, were collected and analyzed to evaluate matrix impact on the final LC–ESI-MS/MS method. As can be seen from Table 3, although for WWTP and industrial effluents ME% is slightly lower (more suppression), sample origin has a limited impact on ME%. This phenomenon is thought to be primarily the result of the extensive sample clean-up used in this method. The extended SPE procedure's three-step wash effectively removed most of the interfering humic acids and other organic material from the environmental water samples, leading to comparable ME% values. Additionally, possible differences were also compensated for by the labeled internal standards.

4. Conclusion

Ion suppression and enhancement originating from matrix components is a common phenomenon associated with pneumatically assisted electrospray ionization mass spectrometry. The present work evaluated matrix effect on 35 EDCs in environmental water by comparing responses of standards and post-extraction spiked matrix samples. A clear compound and ionization mode dependence was observed. Although both ionization modes (NI and PI) experienced matrix effect, NI-ESI was clearly more influenced by co-eluting matrix components as compared to PI-ESI.

It has been demonstrated that mobile-phase additives can have a significant influence on matrix effects. Ion suppression or enhancement of the target analyte by co-elution of matrix components is a critical aspect in LC–MS analysis and should be an important consideration during method development and validation.

Supplementing acids to the HPLC-solvent resulted for most analytes in a considerable signal suppression (average ME%: <65%). Conversely, depending on the concentration, buffers demonstrated both beneficial and disadvantageous effects. The addition of 1 mM of ammonium formate resulted in notably improved ME% values (average ME%: 80% versus 84%).

In an effort to compensate for matrix effects, an extended sample clean-up and the use of labeled internal standards was evaluated. Expanding the SPE wash step, and thus eliminating a considerable amount of co-eluting matrix components, improved signals considerably (average ME%: 89%). In addition, labeled internal standards improved signal reproducibility (average CV%: 4.2% versus 2.6%).

Acknowledgements

T.B. gratefully acknowledges the Flemish Institute for the Promotion of Scientific and Technological Research in the Industry (IWT), Flanders, Belgium for his study grant. This work was partly supported by the European Community through grant Life 98 ENV/B/000260. The authors thank ECCA Laboratories (Merelbeke, Belgium) for providing LC–MS/MS resources.

References

[1] A.M. Krstulovic, C.R. Lee, S. Firmin, G. Jacquet, C.N. Van Dau, D. Tessier, LC–GC Eur. 15 (2002) 31.
[2] J. Simal-Gandara, A.P. Damant, L. Castle, Crit. Rev. Anal. Chem. 32 (2002) 47.
[3] P. Marquet, Ther. Drug Monit. 24 (2002) 255.
[4] R. Thomas, Spectroscopy 16 (2001) 28.
[5] E. Hogendoorn, P. van Zoonen, J. Chromatogr. A 892 (2000) 435.
[6] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Mass Spectrom. Rev. 9 (1990) 37.
[7] A.P. Bruins, J. Chromatogr. A 794 (1998) 345.
[8] J.D. Henion, B.A. Thomson, P.H. Dawson, Anal. Chem. 54 (1982) 451.
[9] D.L. Buhrman, P.I. Price, P.J. Rudewicz, J. Am. Soc. Mass Spectrom. 7 (1996) 1099.
[10] R. King, R. Bonfiglio, C. Fernandez-Metzler, C. Miller-Stein, T. Olah, J. Am. Soc. Mass Spectrom. 11 (2000) 942.
[11] I. Fu, E.J. Woolf, B.K. Matuszewski, J. Pharm. Biomed. Anal. 18 (1998) 347.
[12] H. Mei, Y.S. Hsieh, C. Nardo, X.Y. Xu, S.Y. Wang, K. Ng, W.A. Korfmacher, Rapid Commun. Mass Spectrom. 17 (2003) 97.

[13] M.W.J. van Hout, H.A.G. Niederlander, R.A. de Zeeuw, G.J. de Jong, Rapid Commun. Mass Spectrom. 17 (2003) 245.
[14] R. Pascoe, J.P. Foley, A.I. Gusev, Anal. Chem. 73 (2001) 6014.
[15] J. Smeraglia, S.F. Baldrey, D. Watson, Chromatographia 55 (2002) S95.
[16] Y.S. Hsieh, M. Chintala, H. Mei, J. Agans, J.M. Brisson, K. Ng, W.A. Korfmacher, Rapid Commun. Mass Spectrom. 15 (2001) 2481.
[17] R. Bonfiglio, R.C. King, T.V. Olah, K. Merkle, Rapid Commun. Mass Spectrom. 13 (1999) 1175.
[18] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882.
[19] Y. Deng, J.T. Wu, T.L. Lloyd, C.L. Chi, T.V. Olah, S.E. Unger, Rapid Commun. Mass Spectrom. 16 (2002) 1116.
[20] A.T. Murphy, M.J. Berna, J.L. Holsapple, B.L. Ackermann, Rapid Commun. Mass Spectrom. 16 (2002) 537.
[21] R. Kitamura, K. Matsuoka, E. Matsushima, Y. Kawaguchi, J. Chromatogr. B 754 (2001) 113.
[22] J.T. Wu, H. Zeng, M.X. Qian, B.L. Brogdon, S.E. Unger, Anal. Chem. 72 (2000) 61.
[23] X.C. Tong, I.E. Ita, J.Y. Wang, J.V. Pivnichny, J. Pharm. Biomed. Anal. 20 (1999) 773.
[24] M.J. Avery, Rapid Commun. Mass Spectrom. 17 (2003) 197.
[25] C. Muller, P. Schafer, M. Stortzel, S. Vogt, W. Weinmann, J. Chromatogr. B 773 (2002) 47.
[26] K.A. Mortier, K.M. Clauwaert, W.E. Lambert, J.F. Van Bocxlaer, E.G. Van den Eeckhout, C.H. Van Peteghem, A.P. De Leenheer, Rapid Commun. Mass Spectrom. 15 (2001) 1773.
[27] J.V. Sancho, O.J. Pozo, F.J. Lopez, F. Hernandez, Rapid Commun. Mass Spectrom. 16 (2002) 639.
[28] E. Dijkman, D. Mooibroek, R. Hoogerbrugge, E. Hogendoorn, J.V. Sancho, O. Pozo, F. Hernandez, J. Chromatogr. A 926 (2001) 113.
[29] P.L. Ferguson, C.R. Iden, B.J. Brownawell, Anal. Chem. 72 (2000) 4322.
[30] R. Steen, A.C. Hogenboom, P.E.G. Leonards, R.A.L. Peerboom, W.P. Cofino, U.A.Th. Brinkman, J. Chromatogr. A 857 (1999) 157.
[31] A.C. Hogenboom, W.M.A. Niessen, U.A.Th. Brinkman, J. Sep. Sci. 24 (2001) 331.
[32] R.B. Geerdink, A. Kooistra-Sijpersma, J. Tiesnitsch, P.G.M. Kienhuis, U.A.Th. Brinkman, J. Chromatogr. A 863 (1999) 147.
[33] B.K. Choi, D.M. Hercules, A.I. Gusev, Fresenius J. Anal. Chem. 369 (2001) 370.
[34] S. Ito, K. Tsukada, J. Chromatogr. A 943 (2002) 39.
[35] A. DiCorcia, C. Crescenzi, A. Lagana, J. Agric. Food Chem. 44 (1996) 1930.
[36] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[37] R. Dams, T. Benijts, W. Gunther, W. Lambert, A. De Leenheer, Rapid Commun. Mass Spectrom. 16 (2002) 1072.
[38] T. Benijts, R. Dams, W. Gunther, W. Lambert, A. De Leenheer, Rapid Commun. Mass Spectrom. 16 (2002) 1358.
[39] D. Temesi, B. Law, LC–GC N. Am. 17 (1999) 626.
[40] R. Kostianinen, A.P. Bruins, Rapid Commun. Mass Spectrom. 10 (1996) 1393.
[41] S.L. Zhou, M. Hamburger, Rapid Commun. Mass Spectrom. 9 (1995) 1516.
[42] W.H. Schaefer, F. Dixon, J. Am. Soc. Mass Spectrom. 7 (1996) 1059.
[43] M. Petrovic, D. Barcelo, J. Mass Spectrom. 36 (2001) 1173.