Comparison of Matrix Effects in HPLC-MS/MS and UPLC-MS/MS Analysis of Nine Basic Pharmaceuticals in Surface Waters

Jet C. Van De Steene and Willy E. Lambert
Laboratory of Toxicology, Ghent University, Ghent, Belgium

When developing an LC-MS/MS-method matrix effects are a major issue. The effect of co-eluting compounds arising from the matrix can result in signal enhancement or suppression. During method development much attention should be paid to diminishing matrix effects as much as possible. The present work evaluates matrix effects from aqueous environmental samples in the simultaneous analysis of a group of 9 specific pharmaceuticals with HPLC-ESI/MS/MS and UPLC-ESI/MS/MS: flubendazole, propiconazole, pipamperone, cinnarizine, ketoconazole, miconazole, rabeprazole, itraconazole and domperidone. When HPLC-MS/MS is used, matrix effects are substantial and can not be compensated for with analogue internal standards. For different surface water samples different matrix effects are found. For accurate quantification the standard addition approach is necessary. Due to the better resolution and more narrow peaks in UPLC, analytes will co-elute less with interferences during ionisation, so matrix effects could be lower, or even eliminated. If matrix effects are eliminated with this technique, the standard addition method for quantification can be omitted and the overall method will be simplified. Results show that matrix effects are almost eliminated if internal standards (structural analogues) are used. Instead of the time-consuming and labour-intensive standard addition method, with UPLC the internal standardization can be used for quantification and the overall method is substantially simplified.   (J Am Soc Mass Spectrom 2008, 19, 713–718) © 2008 American Society for Mass Spectrometry

Evaluating matrix effects is of the utmost importance when developing a quantitative liquid chromatography/tandem mass spectrometry (LC-MS/MS) method. Coeluting compounds originating from the matrix can cause signal enhancement or suppression. When matrix compounds and analytes enter the ion source at the same time, the ionization efficiency of the analyte might be influenced [1]. In this way, matrix effect can affect the reproducibility and accuracy of the method. Environmental matrices are complex and variable. Matrix effects can arise from unknown and unpredictable compounds. Different authors have studied this phenomenon in environmental applications [2–6]. Special attention has to be made to optimize chromatography and sample preparation. The best option to tackle matrix effects is the use of isotopically labeled internal standards [1, 2, 5, 7]. Lacking those, structural analogues are the second best option. In case of environmental applications, difficulties arise to find structural analogues, such as other drugs of the same class, which are certainly not present in the aquatic environment. Also, the variability of the matrix should be taken into account when examining and diminishing matrix effects; different kinds of matrices should be tested (e.g., different kinds of river water, waste water treatment plant influent and effluent) [1, 5].

In a previously published article, we examined matrix effects in a high performance liquid chromatography (HPLC)-MS/MS analysis of these compounds for different kinds of surface water [6]. Matrix effects were very high and different strategies were tried to diminish these (more extensive sample preparation, lowering the flow-rate and the use of analogue internal standards). Matrix effects were not fully eliminated and were different for different surface water samples. Labeled internal standards, which compensate for (but do not eliminate) matrix effects, were not available for these analytes. The only accurate method for quantification in this manner is standard addition. The drawback is that this method is labor-intensive and time-consuming.

Ultraperformance LC (UPLC) is a new LC-system that is manufactured for the use of columns with sub-2 μm particles. These particles should provide more resolution and more speed [8–10]. Due to better resolution and more narrow peaks, analytes will coelute less with interferences during ionization, so matrix effects could be lower, or even eliminated.

The objective of the present work is to evaluate and

Address reprint requests to Dr. Willy E. Lambert, Laboratory of Toxicology, Ghent University, Harelbekstraat 72, B-9000 Ghent, Belgium. E-mail: Willy.Lambert@Ugent.be

(a)

(b)

(c)
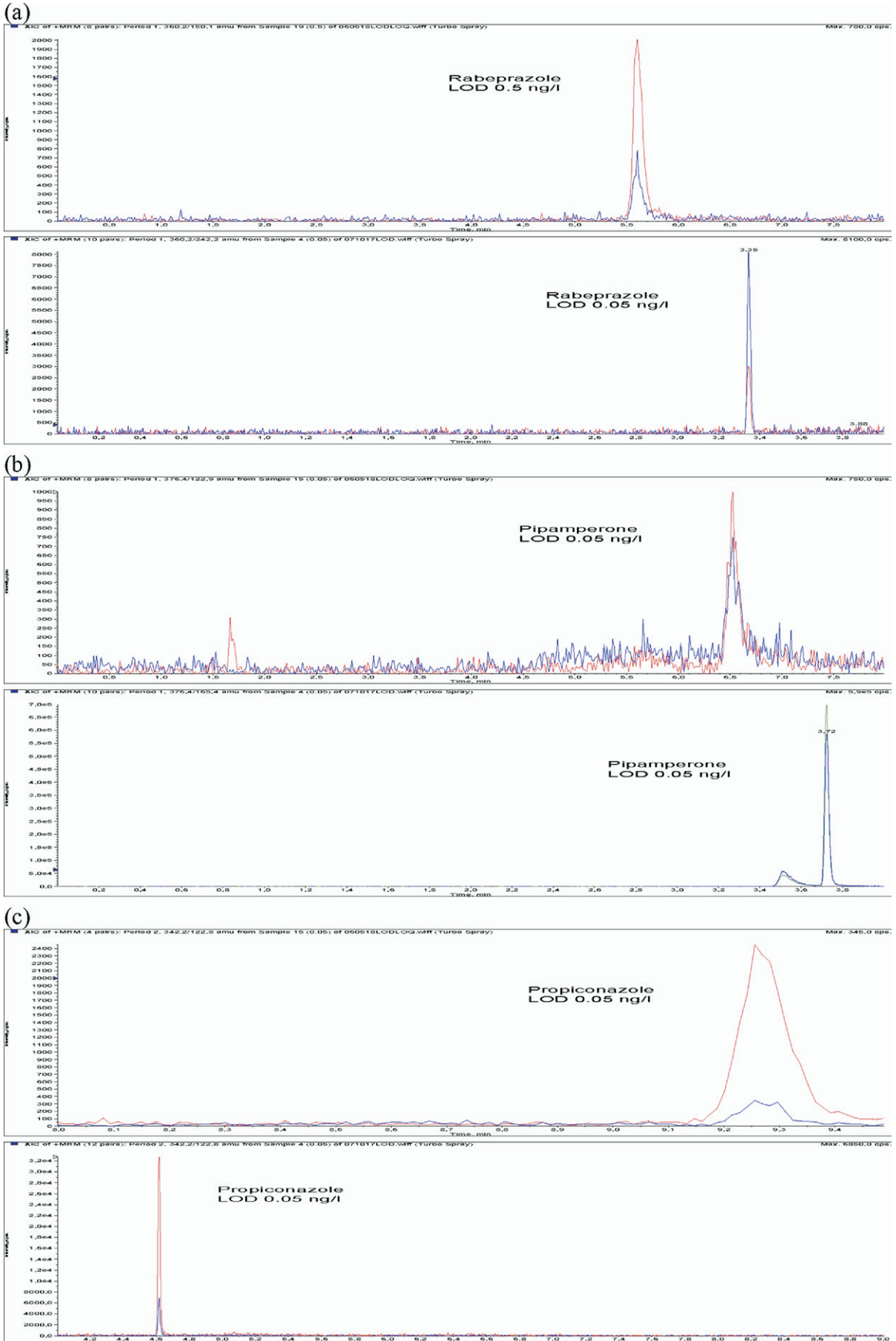
compare matrix effects in the UPLC-MS/MS analysis with the HPLC-MS/MS analysis of nine pharmaceuticals in aqueous environmental samples. If matrix effects are eliminated with this technique, the standard addition method for quantification could be omitted and the overall method could be simplified.

The pharmaceuticals focused on are produced or formulated by one pharmaceutical company. These pharmaceuticals are put on the market in rather high quantities. The predicted environmental concentrations (PEC) of these active pharmaceutical ingredients, calculated based on market volumes and assuming no degradation or absorption (worst-case scenario), are in the range of 0.03 to 0.23 μg/L. According to the EMEA Guideline CHMP/SWP/4447/00, these PECs are above the threshold limit of 0.01 μg/L for investigation of potential environmental damage [11].

Experimental

Chemicals and Solutions

Flubendazole, pipamperone, cinnarizine, ketoconazole, miconazole, rabeprazole, itraconazole, domperidone, propiconazole (purity all > 99%), and two internal standards [a domperidone analogue (purity > 98%) and an itraconazole analogue (purity > 96%)] were provided by the pharmaceutical company that produces these analytes (structures included in Supplementary Figure a1, which can be found in the electronic version of this article). The other internal standards, hexaconazole (purity > 99%) was purchased from Sigma-Aldrich (Bornem, Belgium) and cambendazole (purity > 99%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Water (H_2O), methanol (MeOH), and acetonitrile (ACN) used during solid-phase extraction were purchased from Merck (Darmstadt, Germany) (HPLC-grade). Chloroform (of technical grade) was from Merck. Water and acetonitrile used for chromatography were of LC-MS-grade and were purchased from Biosolve (Valkenswaard, The Netherlands). Acetic acid and ammonium acetate were from Sigma-Aldrich. Ammonia solution 25% and acetic acid 100% were purchased from Merck.

Stock solutions were prepared in methanol at a concentration of 1 mg/mL and stored at −20 °C. From these stock solutions working solutions were prepared by diluting with methanol. Working solutions were also stored at −20 °C.

HPLC

Chromatography. An HP 1100 Agilent system (Agilent, Avondale, PA) consisting of a membrane degasser, a quaternary gradient pump, an autosampler (10 °C), and a column oven (25 °C) was used for separation. All compounds were separated in one single gradient run.

The separation was performed on a 100 × 4.6 mm i.d. pentafluorophenyl column (PFP-column) with 5 μm particles and a Metaguard precolumn (both Varian, Sint-Katelijne-Waver, Belgium). Eluents were H_2O/ACN [95:5, by vol (A) and 5:95, by vol (B)], both containing 2 mM ammonium acetate and 2 mM acetic acid. The flow rate was 1 mL/min. A postcolumn split was installed (1:5, 200 μL flow to the source). Sample volumes of 20 μL were injected. Gradient conditions were as follows: 0–1.00 min, isocratic 20% B; 1.00–11.50 min, linear from 20% to 100% B; 11.50–16.50 min, isocratic 100% B; 16.50–17.00 min, linear 100% to 20% B; 17.00–24.00 min, isocratic 20% B.

Mass spectrometry. Analytes were detected by multiple reaction monitoring (MRM) using electrospray ionization mass spectrometry (ESI-MS) on an API-4000 (Applied Biosystems/MDS SCIEX, Ontario, Canada), with Turbo Ionspray. During the run the system was in positive ESI-mode.

The source temperature was 550 °C. The curtain gas was 15 psig; the nebulizer and drying gas pressures were both 90 psig. Nitrogen was used both as nebulizing and drying gas. The capillary voltage was 5000 V. For each compound, two MRM-transitions were monitored. MRM-transitions and compound-dependent parameters are summarized in Supplementary Table 1. The MRM-transitions are measured in periods to obtain higher signals. In period 1 (0–7.7 min), flubendazole, pipamperone, rabeprazole, and domperidone are measured with a dwell time of 100 msec. In period 2 (7.7–9.3 min), propiconazole and ketoconazole are measured with a dwell time of 200 msec. In period 3 (9.3–10.3 min), itraconazole is measured with a dwell time of 400 ms. In the last period (10.3–24.0 min), cinnarizine and miconazole are measured with a dwell time of 200 ms.

UPLC

Chromatography. The Acquity Ultra Performance LC-system (Waters, Milford, MA) consisting of a degasser, a binary gradient pump, an autosampler (10 °C) and a column oven (60 °C) were used for separation. All compounds were separated in one single gradient run.

A 100 × 2.1 mm i.d. Acquity UPLC HSS T3 column with 1.8 μm particles (Waters) was used for separation of the compounds. A prefilter was installed before the column. Eluents were H_2O/ACN [95:5, by vol (A) and 5:95, by vol (B)], both containing 2 mM ammonium acetate and 2 mM acetic acid. The flow-rate was 0.5

Figure 1. Extracted ion chromatogram in HPLC analysis at LOD: (**a**, above) rabeprazole (tr = 5.61 min), (**b**, above) pipamperone (tr = 6.50 min), (**c**, above) propiconazole (tr = 9.25 min). Extracted ion chromatogram in UPLC analysis at LOD: (**a**, under) rabeprazole (tr = 3.35 min), (**b**, under) pipamperone (tr = 3.72 min), (**c**, under) propiconazole (tr = 4.60 min).

Table 1. Matrix effects based on areas and area ratios for the analytes in three different water samples (A, B, and C) with HPLC and UPLC

	ME (%) without internal standards			ME (%) with internal standards		
	HPLC			HPLC		
	A	B	C	A	B	C
Flubendazole(4)	78.2	82.5	96.6	130.0	132.7	137.5
Pipamperone(2)	40.0	42.8	51.4	85.4	78.4	73.8
Rabeprazole(4)	37.8	41.7	53.5	63.5	67.3	76.1
Domperidone(2)	57.8	62.0	69.1	122.4	113.1	97.1
Propiconazole(3)	60.9	65.7	83.7	95.2	99.0	92.4
Ketoconazole(3)	37.5	39.3	62.6	58.7	59.1	69.0
Itraconazole(1)	12.3	11.7	57.2	148.7	140.8	122.6
Cinnarizine(1)	2.6	4.8	22.4	26.5	55.8	55.7
Miconazole(1)	5.5	4.8	30.9	49.2	77.4	66.8
	UPLC			UPLC		
	A	B	C	A	B	C
Flubendazole(4)	105.4	96.7	98.2	115.4	112.4	97.4
Pipamperone(4)	93.1	96.9	101.7	102.3	112.8	101.1
Rabeprazole(4)	94.5	94.0	102.4	103.5	109.2	101.9
Domperidone(4)	92.4	72.9	88.2	101.7	85.1	87.6
Propiconazole(3)	101.2	93.8	104.6	101.1	97.4	103.0
Ketoconazole(3)	112.8	86.8	146.5	112.1	90.0	126.4
Itraconazole(1)	33.2	27.5	74.8	110.1	128.5	120.8
Cinnarizine(1)	23.5	13.1	16.5	97.8	94.7	99.3
Miconazole(1)	38.8	33.4	53.7	110.5	129.9	105.2

Internal standards used: (1) itraconazole analogue; (2) domperidone analogue; (3) hexaconazole; (4) cambendazole.

mL/min. Sample volumes of 4 μL were injected. Gradient conditions were as follows: 0–4.38 min, linear from 20% to 100% B; 4.38–6.46 min, isocratic 100% B; 6.46–6.67 min, linear from 100% to 20% B; 6.67–9.59 min, isocratic 20% B.

Mass spectrometry. Analytes were detected by MRM using electrospray ionization mass spectrometry (ESI-MS) on an API-4000 (Applied Biosystems/MDS SCIEX), with Turbo Ionspray. During the run the system was in positive ESI-mode.

The source temperature was 550 °C. The curtain gas was 15 psig; the nebulizer gas pressure was 90 psig, and drying gas pressure was 60 psig. Nitrogen was used both as nebulizing and drying gas. The capillary voltage was 2500 V. For each compound 2 MRM-transitions were monitored. MRM-transitions and compound-dependent parameters are summarized in Supplementary Table 1. The MRM-transitions are measured in two periods to obtain higher signals. In period 1 (0–4 min), flubendazole, pipamperone, rabeprazole, and domperidone are measured with a dwell time of 20 ms; in period 2 (4–9.59 min), ketoconazole, itraconazole, cinnarizine, miconazole, and propiconazole are measured with a dwell time of 20 ms.

Sample Preparation

Surface water samples were collected and filtered onto a Metrigard glass fibre filter (0.5 μm) in a SolVac holder (both from Pall, Ann Arbor, MI). Filters were washed

with methanol (5 mL per L of the sample). The water samples were stored at 4 °C until extraction. Before extraction, the pH of the water samples was adjusted to 7 using a 5% ammonia-solution or a 2% acetic acid-solution.

Solid-phase extractions were performed on Speedisk phenyl cartridges (100 mg; 3 mL) of J. T. Baker (Achrom NV, Machelen, Belgium). The cartridges were conditioned with 3 mL of MeOH and 3 mL of H_2O using −60 kPa. Then, the 100 mL surface water sample was applied using −30 kPa. The wash step consisted of 3 mL of H_2O/MeOH (60:40, by vol) (−30 kPa). Elution occurred with 2 × 0.5 mL of MeOH. The NH_2-column was conditioned with 5 mL of $CHCl_3$/MeOH (80:20, by vol) using −60 kPa. The extract eluted from the phenyl column was diluted with 4 mL of $CHCl_3$, applied onto the NH_2-column and directly collected. The extract was evaporated to dryness under a gentle nitrogen stream at 40 °C and redissolved in 100 μL (HPLC-extract) or 50 μL (UPLC-extract) of H_2O/ACN (80/20, by vol); 20 μL (HPLC) or 4 μL (UPLC) was injected.

Matrix Effects

Experiments to evaluate matrix effects were in correspondence to the strategy applied by Matuszewski et al. [12]. MS/MS areas of known amounts of standards (A) were compared with those measured in a blank water extract spiked, after extraction, with the same analyte amount (B). The ratio (B/A × 100) is defined as the absolute matrix effect (ME%). A value of 100% indicates

that there is no absolute matrix effect. There is signal enhancement if the value is >100% and signal suppression if the value is <100%. Tests were conducted in triplicate on blank surface water samples, originating from a local brook, and spiked to obtain a concentration of 20 ng/L of each pharmaceutical for the HPLC analysis, and 400 ng/L of each pharmaceutical for the UPLC analysis. Water samples were first examined for the presence of possible contaminants.

Results and Discussion

Optimization of UPLC-MS/MS

When changing from HPLC- to UPLC analysis, MS parameters need to be altered to obtain the highest MS signals possible. The flow to the source was changed from 0.2 to 0.5 mL/min, so source parameters needed to be adjusted. Dwell times were diminished to obtain enough data points.

In Figure 1, the extracted ion chromatograms of the HPLC- and UPLC analysis at the limit of detection of rabeprazole, pipamperone, and propiconazole are shown. The extracted ion chromatograms of all the analytes are shown in Supplementary Figure a3. Total run time is diminished from 24 min to 9.59 min. Resolution is improved in the UPLC-chromatogram and peaks are more narrow compared with the HPLC chromatogram. Analysis speed is improved, and solvent consumption is diminished.

Matrix Effects

Matrix effects were calculated on three different surface water samples, taken from three different brooks. The samples for HPLC analysis were spiked with a standard mix to obtain a concentration of 20 ng/L; the samples for UPLC analysis were spiked with a standard mix to obtain a concentration of 400 ng/L. Itraconazole cannot be detected with a similar sensitivity in the UPLC analysis so the concentration was elevated above the limit of quantification of itraconazole. In Table 1, matrix effects without internal standards for the HPLC analysis and for the UPLC analysis are shown (for absolute areas obtained by HPLC and UPLC, see Supplementary Table 2). The results demonstrate that matrix effect is substantially reduced when the UPLC analysis is performed. Matrix interferences coelute less with the analytes than in HPLC analysis, and signal suppression is diminished drastically (see also Supplementary Figure 2).

Also in Table 1, matrix effects with internal standards are shown (for absolute area ratio, see Supplementary Table 2). Normally, the internal standard with the highest structural similarity to the analyte is chosen. This is in most cases also the one that elutes the closest. If not, the two internal standards (in the same period) are compared with what extent they compensate best for the matrix effects. Matrix effects are only minor or

Table 2. Limits of detection and quantification of the analytes, with HPLC and UPLC

	LOD HPLC (ng/L)	LOQ HPLC (ng/L)	LOD UPLC (ng/L)	LOQ UPLC (ng/L)
Flubendazole	<0.05	0.05	<0.05	<0.05
Pipamperone	0.05	1	<0.05	<0.05
Rabeprazole	0.5	0.5	<0.05	<0.05
Domperidone	<0.05	0.5	<0.05	<0.05
Propiconazole	0.05	0.05	<0.05	<0.05
Ketoconazole	5	10	5	10
Itraconazole	1	10	325	350
Cinnarizine	<0.05	<0.05	10	10
Miconazole	<0.05	10	<0.05	<0.05

even totally eliminated in UPLC analysis compared with HPLC analysis. As a result, the labor-intensive and time-consuming standard addition method can be omitted. Internal standardization can be used, so the overall method is simplified.

In the UPLC analysis, cambendazole is used as internal standard for domperidone instead of the domperidone analogue, because cambendazole elutes more closely to domperidone than the domperidone analogue.

LOD and LOQ

Limits of detection and quantification (S/N > 3 and S/N > 10, respectively) in surface water are shown in Table 2. Only for itraconazole and cinnarizine, the limits of detection and/or quantification are higher in the UPLC analysis than in the HPLC analysis. Part of an explanation for this might be that dwell time is diminished drastically in UPLC analysis, so data points are diminished and sensitivity is negatively influenced.

Conclusions

Matrix effects were examined in surface water analysis for nine basic pharmaceuticals, using HPLC-MS/MS and UPLC-MS/MS. In the HPLC-MS/MS analysis, matrix effects are severe, even with analogue internal standards, and for accurate quantification the standard addition method is necessary. When using UPLC-MS/MS, with the aid of analogue internal standards, matrix effects are minor or eliminated. In this way, the standard addition method for quantification can be omitted. Thanks to UPLC, internal standardization can be used and the overall method is simplified.

References

1. Taylor, P. J. Matrix Effects: The Achilles Heel of Quantitative High-Performance Liquid Chromatography-Electrospray-Tandem Mass Spectrometry. Clin. Biochem. **2005**, *38*, 328–334.
2. Benijts, T.; Dams, R.; Lambert, W.; De Leenheer, A. Countering Matrix Effects in Environmental Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry Water Analysis for Endocrine Disrupting Chemicals. *J. Chromatogr. A* **2004**, *1029*, 153–159.

3. Kloepfer, A.; Quintana, J. B.; Reemtsma T. Operational Options to Reduce Matrix Effects in Liquid Chromatography-Electrospray Ionization-Mass Spectrometry Analysis of Aqueous Environmental Samples. *J. Chromatogr. A.* **2005**, *1067,* 153–160.

4. Choi, B. K.; Hercules D. M.; Gusev A. I. LC-MS/MS Signal Suppression Effects in the Analysis of Pesticides in Complex Environmental Matrices. *Fresenius J. Anal. Chem.* **2001** *369,* 370–377.

5. Petrovic, M.; Hernando, M. D.; Diaz-Cruz, M. S.; Barcelo, D. Liquid Chromatography-Tandem Mass Spectrometry for the Analysis of Pharmaceutical Residues in Environmental Samples: A Review. *J. Chromatogr. A* **2005**, *1067,* 1–14.

6. Van De Steene, J. C.; Mortier, K. A.; Lambert, W. E. Tackling Matrix Effects During Development of a Liquid Chromatographic-Electrospray Ionization Tandem Mass Spectrometric Analysis of Nine Basic Pharmaceuticals in Aqueous Environmental Samples. *J. Chromatogr. A.* **2006**, *1123,* 71–81.

7. Matuszewski, B. K. Standard Line Slopes as a measure of a Relative Matrix Effect in Quantitative HPLC-MS Bioanalysis. *J. Chromatogr. B. Anal. Technol. Biomed. Life Sci.* **2006**, *830,* 293–300.

8. Leandro, C. C.; Hancock, P.; Fussell, R. J.; Keely, B. J. Comparison of Ultraperformance Liquid Chromatography and High-Performance Liquid Chromatography for the Determination of Priority Pesticides in Baby Foods by Tandem Quadrupole Mass Spectrometry. *J. Chromatogr. A.* **2006**, *1103,* 94–101.

9. Novakova, L.; Matysova, L.; Solich, P. Advantages of Application of UPLC in Pharmaceutical Analysis. *Talanta* **2006**, *68,* 908–918.

10. Churchwell, M. I.; Twaddle, N. C.; Meeker, L. R.; Doerge, D. R. Improving LC-MS Sensitivity Through Increases in Chromatographic Performance: Comparisons of UPLC-ES/MS/MS to HPLC-ES/MS/MS. *J. Chromatogr. B. Anal. Technol. Biomed. Life Sci.* **2005**, *825,* 134–143.

11. EMEA. Note for Guidance on Environmental Risk Assessment of Medicinal Products for Human Use. CMPC/SWP/444 (The European agency for the evaluation of medicinal products). 2005.

12. Matuszewski, B. K.; Constanzer, M. L.; Chavez-Eng, C. M. Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC-MS/MS. *Anal. Chem.* **2003**, *75,* 3019–3030.