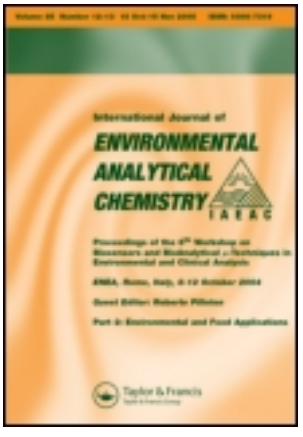

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/geac20

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Available online: 05 Oct 2011

To cite this article: Jet C. Van De Steene & Willy E. Lambert (2011): Determination of four basic pharmaceuticals and one pesticide in surface water with UPLC-ESI-MS/MS, International Journal of Environmental Analytical Chemistry, 91:12, 1218-1226

To link to this article: http://dx.doi.org/10.1080/03067311003778664

Intern. J. Environ. Anal. Chem.
Vol. 91, No. 12, October 2011, 1218–1226

Taylor & Francis
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Determination of four basic pharmaceuticals and one pesticide in surface water with UPLC-ESI-MS/MS

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(*Received 9 December 2009; final version received 16 February 2010*)

This paper describes the optimisation and validation of an ultra performance liquid chromatography- tandem mass spectrometry (UPLC-MS/MS) method for the analysis of four pharmaceuticals (flubendazole, pipamperone, rabeprazole and domperidone) and one pesticide (propiconazole) in surface water. Sample preparation was reduced substantially as compared to a previously published high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, thanks to the use of UPLC. In addition, internal standards could now be used for quantification instead of the standard addition method. Extraction was performed on a Speedisk phenyl solid-phase extraction tube. A Waters Acquity HSS T3 UPLC column (100×2.1 mm i.d.; 1.8-μm particles) was used for separation and an API 4000 triple quadrupole was used as a detector. Total run time was 8.59 minutes. Matrix effects were examined on different surface water samples. Limits of detection and quantification in surface water samples were between 100 and 500 pg/l. Validation was performed on surface water. The method showed good precision ($<15\%$) and accuracy (85–120%). This method is less time-consuming and labour-intensive than a previously published HPLC-procedure without compromising validation parameters.

1. Introduction

Pharmaceuticals are a major group of environmental contaminants, excreted by humans or animals in large quantities. Wastewater treatment plants are only capable of limited removal, so there is a continuous input of pharmaceuticals in the environment [1].

Research towards pharmaceutical occurrence in the environment or removal in wastewater treatment plants is necessary owing to potential toxicity problems. Pharmaceuticals are made to interact with living creatures. Acute toxicity towards aquatic plants and organisms cannot be excluded [2]. Also continuously low inflow of pharmaceuticals in surface water could cause subtle changes in organisms which are difficult to detect, but can become clear over a long period of time [1]. Mixtures of pharmaceuticals can also give rise to an unexpected negative impact on fauna and flora [1,2].

The pharmaceuticals and pesticide focused on flubendazole, pipamperone, rabeprazole, domperidone and propiconazole are produced or formulated by one pharmaceutical company. In Table 1, relevant information data on these pharmaceuticals is summarised.

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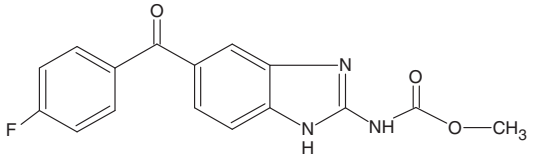
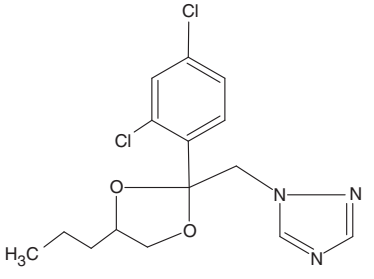
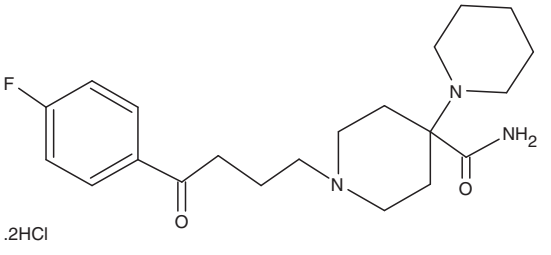
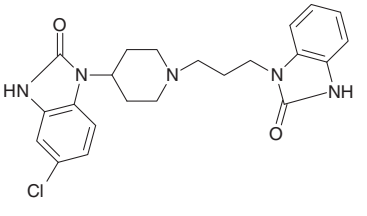
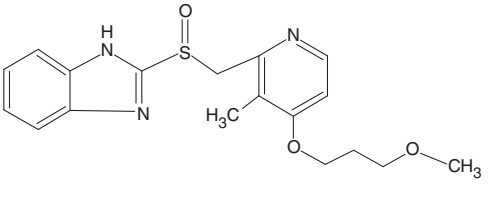
ISSN 0306–7319 print/ISSN 1029–0397 online

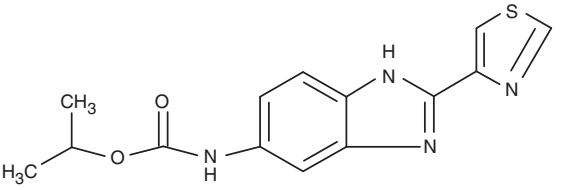
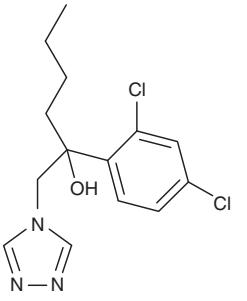
http://dx.doi.org/10.1080/03067311003778664
http://www.tandfonline.com

Table 1. Structures and characteristics of the analytes.

Analyte-Structure	Parameters
Flubendazole 	CAS number: 31430-15-6 MW: 313.29 pKa_1: 3.6 (protonated form) pKa_2: 9.6 log P_{ow}: 3.00 Use: antihelmintic
Propiconazole 	CAS number: 60207-90-1 MW: 342.22 pKa: <3 (protonated form) log P_{ow}: 3.68 Use: pesticide
Pipamperone 	CAS number: 2448-68-2 MW: 448.41 (375.49+72.92) pKa_1: 8.4 (protonated form) pKa_2: 4.8 (protonated form) log P_{ow}: 2.56 Use: neuroleptic
Domperidone 	CAS number: 57808-66-9 MW: 425.92 pKa_1: 7.6 (protonated form) pKa_2: 11.1 pKa_3: 11.8 log P_{ow}: 3.96 Use: neuroleptic
Rabeprazole 	CAS number: 117976-89-3 MW: 359.44 pKa_1: 8.5 pKa_2: 4.4 (protonated form) log P_{ow}: 1.46 Use: protonpumpinhibitor

(*continued*)

Table 1. Continued.

Analyte-Structure	Parameters
IS 1: cambendazole 	
IS 2: hexaconazole 	

The Predicted Environmental Concentrations (PEC) of these ingredients (for surface water; for Belgium), calculated based on market volumes and assuming no degradation or absorption (worst-case scenario), are in the range 0.03–0.23 μg/l. According to the EMEA Guideline (European Medicines Agency) CHMP/SWP/4447/00 [3], these PECs are above the threshold limit of 0.01 μg/l for environmental risk assessment.

For three individual compounds (propiconazole, domperidone, and flubendazole), analytical methods in environmental waters or wastewater have been published. For propiconazole, solid-phase extraction (SPE) on a C18 sorbent is used and analysis is done with liquid chromatography combined with either a single quadrupole mass spectrometer or a quadrupole-ion trap-MS for surface water [4]. Alternatively, for surface water analysis liquid/liquid extraction is used combined with GC-NPD or GC-ECD [5]. Ochiai *et al*. also examined propiconazole in surface water with dual stir bar sorptive extraction- thermal desorption- low thermal mass gas chromatography-mass spectrometry [6]. Twenty-two triazole compounds, of which propiconazole was one, were analysed in raw and tap water by a combination of C18-SPE, mixed-mode cationic SPE and mixed-mode anionic SPE columns, combined with LC-MS/MS [7]. Kahle *et al*. examined the degradation of azole fungicides including propiconazole in wastewater treatment plants [8]. Domperidone was analysed using C18-SPE and HPLC combined with a UV-detector in wastewater [9] and using the same extraction method but with differential pulse voltammetry as detection mode also in wastewater [10]. Flubendazole was analysed in seepage water with solid-phase extraction (Chromabond Easy) and LC-MS/MS by Weiss *et al*. [11].

An offline double SPE-method coupled to high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) was already developed for these analytes

in surface water and wastewater [12]. This paper reports a simplified method for these pharmaceuticals of different classes, based on a single solid-phase extraction followed by ultra performance liquid chromatography-electrospray-tandem mass spectrometry (UPLC-MS/MS). This allows the identification and quantification of these pharmaceuticals in surface water in a less labour-intensive and in a quicker way without compromising validation parameters, including matrix effects.

2. Experimental

2.1 *Chemicals and solutions*

Flubendazole (99.00% purity), pipamperone (99.93% purity), rabeprazole (99.43% purity), domperidone (99.86% purity) and propiconazole (100% purity) were provided as a gift by the pharmaceutical company. Hexaconazole was purchased from Sigma-Aldrich (Bornem, Belgium) and cambendazole, 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). 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 (Bornem, Belgium). Ammonia solution 25% and acetic acid 100% were purchased from Merck (Darmstadt, Germany).

Stock solutions were prepared in methanol at a concentration of 1 mg/ml and stored at −20°C (and were stable for at least 6 months). From these stock solutions, working solutions were prepared by dilution with methanol. Working solutions were also stored at −20°C.

2.2 *Sample preparation*

The surface water samples were grab samples taken from a brook near the laboratory. They were filtered onto Metrigard glass fibre filter (0.5 µm pore size) in a SolVac holder (both from Pall, Ann Arbor, MI, USA). Filters were washed with methanol (5 ml per litre 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) from J.T. Baker (Boom NV, Diegem, Belgium). The cartridges were conditioned with 3 ml of MeOH and 3 ml of H_2O using −60 kPa. Then, a 100-ml sample was loaded using −30 kPa. The wash step consisted of 3 ml of H_2O/MeOH (60 : 40, v/v) (−30 kPa). Elution occurred with 2 × 0.5 ml of MeOH (no vacuum applied).

The extract was evaporated to dryness under a gentle nitrogen stream at 40°C and redissolved in 50 µl of H_2O/ACN (80/20, v/v). After centrifugation, an aliquot was transferred to a vial and 4 µl was injected.

2.3 *Chromatography*

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

A 100 × 2.1-mm I.D. Acquity HSS T3 column with 1.8-μm particles (Waters, Milford, USA) was used for separation of the compounds. A VanGuard Pre-Column Acquity UPLC HSS T3 (5 × 2.1-mm I.D., 1.8-μm particles) was installed in front of the analytical 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 (pH aqueous phase: 4.75). The flow-rate was 0.5 ml/min. Sample volumes of 4 μl were injected. Gradient conditions were as follows: 0.00–1.00 min: 20%B, 1.00–4.38 min: 20 → 100% B, 4.38–6.46 min: 100%B, 6.46–6.67 min: 100% → 20%B, 6.67–8.59 min: 20%B.

2.4 *Mass spectrometry*

Analytes were detected by selected reaction monitoring (SRM) using electrospray ionisation mass spectrometry (ESI-MS) on an API-4000 (Applied Biosystems/MDS Sciex, Ontario, Canada), with Turbo Ionspray. During the analysis the MS was working in the positive mode. The source temperature was 550°C. The curtain gas was 15 psig; the nebuliser gas pressure was 90 psig, and drying gas pressure was 60 psig. Nitrogen was used both as nebulising and drying gas. The capillary voltage was 2500 V. For each compound 2 SRM-transitions and their ratio were monitored. For identification of the analytes, not only should the 2 SRM-transitions be present, but also the ratio of the transitions is compared with that of the standard. The deviation should be below 20%. SRM-transitions and compound-dependent parameters are summarised in Table 2. The SRM-transitions are measured in 2 periods. In period 1 (0–4 min), flubendazole, pipamperone, rabeprazole and domperidone are measured with a dwell time of 20 msec; in period 2 (4–8.59 min), propiconazole is measured also with a dwell time of 20 msec. The internal standards cambendazole (measured in period 1) and hexaconazole (measured in period 2) function as internal standard for the compounds eluting in that period.

Table 2. SRM-transitions and compound-dependent parameters.

Compound	SRM-transitions (m/z)	Declustering potential (V)	Entrance potential (V)	Collision energy (V)	Collision cell exit potential (V)
Flubendazole	314.20 → 281.90	71	9	29	16
(IS1)*	314.20 → 122.80	71	4	51	8
Propiconazole	342.16 → 159.30	76	9	41	12
(IS2)*	342.16 → 122.80	76	8	81	10
Pipamperone	376.40 → 165.40	76	5	41	14
(IS1)*	376.40 → 122.90	76	5	65	10
Rabeprazole	360.20 → 242.20	36	9	17	18
(IS1)*	360.20 → 195.10	36	10	27	16
Domperidone	426.20 → 175.10	96	11	37	16
(IS1)*	426.20 → 147.10	96	10	59	10
Internal standard 1 (IS1): cambendazole	303.21 → 217.30	76	10	37	14
Internal standard 2 (IS2): hexaconazole	315.30 → 69.90	66	10	49	4

*Hexaconazole is used as an internal standard for propiconazole only. For the other compounds, cambendazole is used.

2.5 *Validation*

Validation was done on surface water, sampled from a brook near the laboratory. For validation, the following parameters were evaluated: matrix effects, recovery, sensitivity, linearity, accuracy and precision.

Matrix effects were determined for each analyte at low (20 ng/l) and high (400 ng/l) concentration, in fivefold on two surface water samples taken from different locations. Five replicates of each surface water sample were spiked after extraction. Experiments to evaluate matrix effects corresponded with the strategy applied by Matuszewski *et al.* [13]. 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 ($B/A \times 100$) 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 $<100\%$.

Recovery was determined on surface water at low (20 ng/l) and high (400 ng/l) concentration. Surface water samples were spiked before (C) and after extraction (B). The ratio of $C/B \times 100$ is defined as the recovery. Recoveries were calculated based on areas and based on area ratios (compensation by internal standards).

Two SRM transitions were monitored per analyte. For the LOD, both transitions need a $S/N > 3$. For the LOQ, the most sensitive transition needs a $S/N > 10$, and RSD% for five replicates has to be lower than 20%.

Seven point calibration lines were constructed in surface water at concentrations ranging from 0.5 to 250 ng/l, and were evaluated by the method of least squares and expressed by the coefficient of correlation (r^2) [14]. Precision was evaluated at three different concentration levels, i.e. low (0.5 ng/l), medium (7.5 ng/l) and high (250 ng/l). Precision was assessed by five determinations per concentration in one day. Replicates were made by spiking five blank surface water samples before extraction. Precision was expressed as RSD%. Accuracy was evaluated with separately prepared stock and working solutions of all standards at three different concentration levels, i.e. low (0.5 ng/l), medium (7.5 ng/l) and high (250 ng/l). Stability tests had already been performed [12].

3. Results and discussion

3.1 *Development of a less time-consuming and labour-intensive method*

Sample preparation was optimised in view of recovery of the analytes and matrix effects, as has been already discussed extensively [15]. We have investigated the impact of the matrix on the ionisation efficiency of the analytes with UPLC in another paper [16]. There we proved that the UPLC-method was less prone to matrix effects than the HPLC-method. With the use of structural analogues as internal standards, matrix effects are substantially reduced, which is not the case when HPLC is used for analysis. Unfortunately, isotopically labelled internal standards were not available for these analytes. Compared with the HPLC-MS/MS method where standard addition was necessary in view of the remaining matrix effects [12,15], this UPLC-MS/MS method is less time-consuming and labour-intensive.

In this paper, we could improve the method even more by reducing the sample preparation. The previous sample clean-up step based on NH_2-SPE [12] is now eliminated. For all five analytes we could limit sample preparation to only one SPE-step. This means a reduction in processing time of about 45 minutes. Recoveries in the old and new

sample preparation method are comparable, and are all above 85%. Matrix effects were eliminated, or reduced to a minimum even without a second sample clean-up step. Matrix effect data at low and high concentration levels in surface water are shown in Table 3. To address matrix variability, a second surface water sample, taken from another brook, was also evaluated for matrix effects. Absolute matrix effects are calculated from the peak area of the analytes, and relative matrix effects are calculated from the peak area ratio (area analyte divided by the area of the respective internal standard). In both samples, matrix effects were compensated for by internal standards (structural analogues).

3.2 *Validation*

Recoveries at low and high concentrations (20 and 400 ng/l), detection limits and quantification limits are presented in Table 4. Quantitative determination is possible down

Table 3. Matrix effects at low (20 ng/l) and high (400 ng/l) concentration on surface water.

Matrix effect $n=5$ % (*RSD*%)	Sample 1 low *absolute*	*relative*	Sample 2 low *absolute*	*relative*	Sample 1 high *absolute*	*relative*	Sample 2 high *absolute*	*relative*
Flubendazole	89.6(8)	106.8(6)	93.3(5)	110.6(2)	105.1(1)	102.2(2)	83.4(6)	97.8(3)
Pipamperone	59.5(13)	70.5(9)	61.0(2)	74.5(6)	97.0(5)	94.8(12)	74.3(7)	89.6(5)
Rabeprazole	91.5(7)	108.6(2)	88.2(6)	104.6(2)	103.7(1)	101.0(1)	88.0(7)	103.2(4)
Domperidone	61.8(13)	81.1(7)	75.1(7)	89.1(4)	109.5(8)	110.8(10)	66.3(8)	90.7(7)
Propiconazole	74.3(8)	83.1(5)	76.6(3)	83.2(3)	95.8(5)	80.2(5)	75.0(8)	86.8(10)

Table 4. Detection and quantification limits in surface water. Recovery in surface water at low (20 ng/l) and high (400 ng/l) concentration ($n=5$) (%RSD).

	LOD (*ng/l*)	LOQ (*ng/l*)	Recovery (%) Low *area*	*area ratio*	Recovery (%) High *area*	*area ratio*
Flubendazole	0.5	0.5	101.1(2)	105.0(4)	95.0(2)	124.6(1)
Pipamperone	0.5	0.5	91.9(5)	85.9(6)	93.6(7)	109.9(3)
Rabeprazole	0.5	0.5	99.8(3)	103.9(5)	95.7(1)	125.5(1)
Domperidone	0.1	0.5	93.7(4)	95.2(3)	95.5(7)	97.5(7)
Propiconazole	0.5	0.5	101.5(3)	95.5(4)	95.5(5)	95.8(4)

Table 5. Calibration (r^2), precision (within-day) and accuracy data on a surface water sample ($n=5$) (low: 0.5 ng/l, mid: 7.5 ng/l, high: 250 ng/l). Analytical results for a surface water sample are given in the last column.

	r^2	Precision (%) low	mid	high	Accuracy (%) low	mid	high	Surface water (ng/l)
Flubendazole	0.998	13	4	4	119	120	114	29
Pipamperone	0.999	9	9	8	122	106	105	10
Rabeprazole	0.998	6	1	3	103	97	90	<LOD
Domperidone	0.999	5	8	13	96	99	101	21
Propiconazole	0.998	14	6	5	88	97	107	<LOD

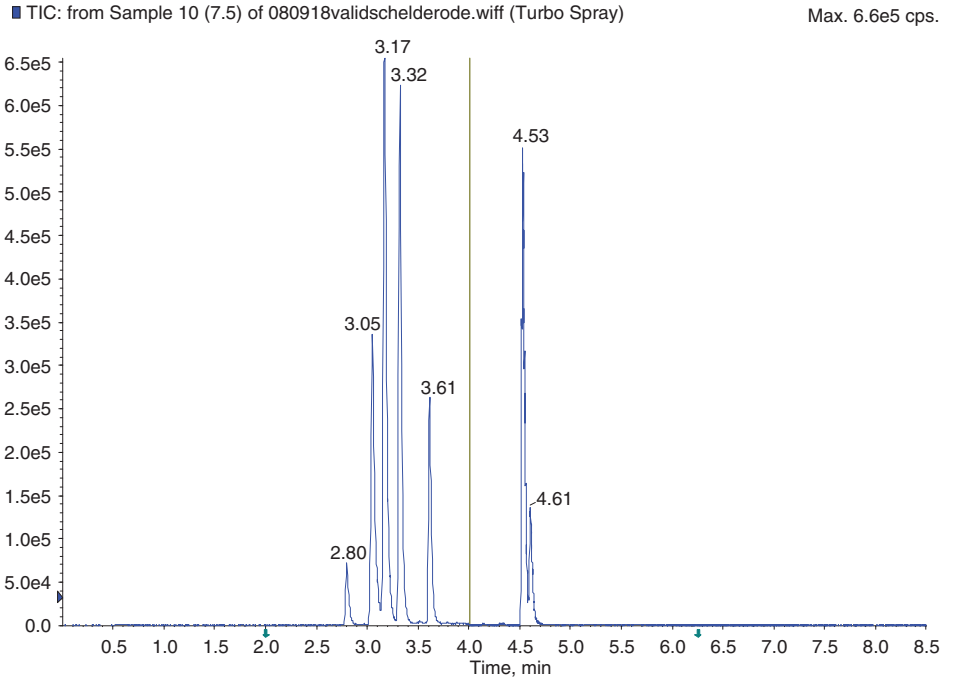


Figure 1. Total ion chromatogram of a spiked surface water sample (7.5 ng/l). From left to right: domperidone ($R_t = 2.80$ min), pipamperone ($R_t = 3.05$ min), IS 1 (cambendazole, $R_t = 3.17$ min), rabeprazole ($R_t = 3.32$ min), flubendazole ($R_t = 3.61$ min), IS 2 (hexaconazole; $R_t = 4.53$ min), propiconazole($R_t = 4.61$ min).

to 0.5 ng/l. For some analytes the LOD and LOQ are identical due to very different intensities of the SRM-transitions. Seven point calibration curves were successfully established in surface water ranging from 0.5 to 250 ng/l (Table 5). These were quadratic calibration lines with a 1/x weighting factor. Weighting is applied to improve accuracy. Correlation coefficients were all equal or higher than 0.998. Precision and accuracy data are also shown in Table 5. Variation is always below 15%, while accuracy is within 85–120%. Stability data for the analytes have been already discussed previously [12]. A representative chromatogram is shown in Figure 1. The results for a surface water sample ranged between 10 and 29 ng/l, except for propiconazole and rabeprazole, which were below the detection limit (Table 5).

4. Conclusion

Simplification of a previously published HPLC-MS/MS method was performed with this UPLC-method. Matrix effects are substantially reduced, demonstrating that internal standards can be used for quantification instead of the time-consuming and labour-intensive standard addition method. The extraction procedure is reduced to one SPE-step, eliminating one extra tedious sample clean-up step. The UPLC-MS/MS method was successfully validated in surface water. It is demonstrated that this offline single

SPE-UPLC-MS/MS method is accurate, precise, sensitive and free of substantial matrix effects.

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