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Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry

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Abstract

The paper presents the development and validation of a new multi-residue method for the determination of 28 basic/neutral pharmaceuticals (antiepileptics, antibacterial drugs, β-blockers, analgesics, lipid-regulating agents, bronchodilators, histamine-2-blockers, anti-inflammatory agents, calcium channel blockers, angiotensin-II antagonists and antidepressants) and illicit drugs in surface water with the usage of a new technique: ultra performance liquid chromatography–positive electrospray tandem mass spectrometry (UPLC–MS/MS). The usage of the novel UPLC system with 1.7 μm particle size and 1 mm internal diameter column allowed for low mobile phase flow rates (0.07 mL min^{-1}) and short retention times (from 1.3 to 15.5 min) for all compounds analysed. As a result, a fast and cost-effective method was developed. SPE with the usage of Oasis MCX strong cation-exchange mixed-mode polymeric sorbent was chosen for pharmaceuticals extraction from environmental samples. The influence of matrix-assisted ion suppression and low SPE recovery on the sensitivity of the method was studied. The instrumental limits of quantification varied from 0.2 to 10 μg L^{-1}. The method limits of quantification were at low nanogram per litre levels and ranged from 0.3 to 50 ng L^{-1}. The instrumental and method intra- and inter-day repeatabilities were on average less than 10%. The method was applied for the determination of pharmaceuticals in Rivers Taff (UK) and Warta (Poland). Fifteen compounds were determined in river water at levels ranging from single nanograms to single micrograms per litre.

Keywords: Pharmaceuticals; Illicit drugs; Ultra performance liquid chromatography–tandem mass spectrometry; Solid-phase extraction; Water contamination; Multi-residue method; Ion suppression

1. Introduction

Research on hazardous organic chemicals in the aqueous environment and their influence on humans and the environment has received much attention over recent years. As a result, a list of 33 priority pollutants in EU was created [1]. However, the widely recognised priority compounds which are included in the list constitute only a small percentage of hazardous compounds. Pharmaceuticals and personal care products (PPCPs) are a group of potentially hazardous compounds which have received minimal attention, although interest amongst researchers has been continuously increasing. Many of those investigated are biologically active compounds often of estrogenic activity, which potentially influence environmental and human health. Surprisingly, there is little or no data and minimal understanding of the environmental occurrence, transport, fate and exposure for many PPCPs, despite their frequently high annual usage [2–5]. One of the reasons has been, until recently, a lack of suitable analytical methods capable of detecting compounds at very low concentrations in a complicated matrix. However, due to increasing concern regarding the possible effect of PPCPs on humans and wildlife, an increase in interest in the presence of PPCPs, their fate and effects, is to be expected. The need for an extensive investigation in this field is continuously emphasised by environmental researchers [2–7].

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Pharmaceuticals represent a versatile group of compounds, which are found in surface and wastewaters at levels of up to a few $\mu g\,L^{-1}$ [2,3,7–21]. They can enter the environment as parent compounds, metabolites or conjugates of both. These compounds might also undergo transformation during wastewater/drinking water treatment to produce compounds of significant concern to humans and wildlife. Thousands of pharmaceuticals are approved for human or veterinary usage, but only a very small percentage of these compounds has been studied for presence in the environment (about 80–150 pharmaceuticals [3,7]), not to mention their active metabolites and wastewater treatment by-products.

Antibiotics, followed by steroid compounds, analgesics/non-steroidal and anti-inflammatory drugs, are the most widely studied pharmaceuticals. A high percentage of antibiotics such as doxycycline, oxytetracycline and levofloxacin is excreted by the human body unchanged [22]. Moreover, due to their direct influence on the natural microbiota and the formation of resistant strains, the risk concerning their usage is significant [2,5,7]. Antiepileptic drugs are ubiquitous, poorly removed in WWTP and toxic to bacteria and algae [2]. Carbamazepine has been widely detected in the environment, even if excreted at a low percentage as an unchanged drug (3%) [2,21,22].

Cocaine and amphetamine are the most common illicit drugs. The verification of the presence of illicit drugs in sewage and the aqueous environment is important due to two significant issues (from both an environmental and forensic perspective). Firstly, illicit drugs, as a result of their activity, might cause possible negative effects on wildlife. Secondly, more comprehensive knowledge of the concentrations of illicit drugs in raw sewage might enable more precise estimation of their illegal usage as proposed by Daughton and Ternes [2] and Castiglioni et al. [23].

In summary, there is a need for a fast and sensitive multi-residue method for the determination of PPCPs in the environment. Analysis of PPCPs in environmental samples at levels up to single ng per litre constitutes a significant analytical challenge. PPCPs are usually more polar than several widely recognised POPs (persistent organic pollutants). Due to both the very low concentrations of these compounds as well as a high demand for sensitive, fast and low cost analytical methods designed for the analysis of various compounds in complex matrices (e.g. environmental and biological samples), research into the generation of new analytical methods is of crucial importance. Traditional gas chromatography is of limited value as it requires time-consuming derivatization procedures resulting from the high polarity and low volatility of many PPCPs. The above requires the application of particular analytical methods such as LC (liquid chromatography) combined with necessary sample concentration/clean-up. Liquid chromatography–mass spectrometry (LC/MS) using mainly electrospray ionisation (ESI) is the method of choice for the analysis of polar compounds in complex matrices. So far, a few multi-residue analytical methods for the determination of pharmaceuticals from different therapeutic classes (mainly antibiotics, anti-inflammatory/analgesics, lipid regulators, histamine H_1 and H_2 inhibitors, antidepressants, psychiatric drugs and diuretics) in surface water and wastewater have been established [24–29]. These methods utilise solid-phase extraction as a sample preparation method and almost exclusively liquid chromatography coupled with electrospray ionisation tandem mass spectrometry for separation and quantification of up to 30 compounds on C18 column with up to 50 min elution gradient time and average mobile phase flow rate of $0.2\,mL\,min^{-1}$.

The main aim of the paper is to present a new, fast and sensitive method for the detection of a broad range of pharmaceuticals. The method uses a single SPE method and single LC/MS/MS method. In this work, the latest model of LC/MS, which is ultra performance liquid chromatography (UPLC™) coupled with triple quadrupole tandem mass spectrometry (Acquity UPLC System, Quattro Micro Spectrometer) was used for the analysis of PPCPs in surface water. UPLC is a novel solution designed for fast and cost-efficient separation of multiple compounds in bulk solution. A high speed of analysis, greater resolution, higher peak capacity and sensitivity are obtained due to the novel technology that utilises a new generation of LC columns with sub-2 μm hybrid material and high pressure fluidic modules [30].

2. Experimental

2.1. Chemicals and materials

Reference standards were purchased from Sigma–Aldrich (Gillingham, UK) and Sequoa Products Research Limited (Pangbourne, UK). All compounds were of >95% purity. Solvents used as mobile phases and solvent additives were of LC/MS quality. Ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2EDTA), toluene and acetone were of HPLC quality. Hydrochloric acid (31%) was of puriss quality (Sigma–Aldrich). 5% dimethylchlorosilane (DMDCS) in toluene was obtained from Sigma–Aldrich.

Surrogate/internal standards (IS): phenacetin-ethoxy-1-^{13}C (98.52 at.%^{13}C; CAS no. 72156-72-0) and caffeine-d9 (1,3,7-trimethyl-d9; CAS no. 72238-85-8) were purchased from Sigma–Aldrich and QMX Laboratories Limited (Essex, UK), respectively. Both standards, which were used as surrogate standards, were added to the samples before extraction and were also used for the quantification of the samples.

Stock solutions of pharmaceuticals ($0.5–1\,g\,L^{-1}$) were prepared in methanol and stored in the dark at 4 °C. Antibiotic stock solutions were stored for a maximum of 7 days. Working solutions were prepared fresh daily by diluting stock solution with methanol stored at 4 °C. Ultrapure water was obtained using Neptune, Purite (MJ Patterson Scientific Ltd., UK).

For method development and validation both HQ water (ultrapure water) and BB water (surface water collected from the source of the River Taff in Brecon Beacons National Park, which is not affected by anthropogenic contaminants such as pharmaceuticals), were used. The average dissolved organic carbon (DOC) of BB water was $4.5\,mg\,DOC\,L^{-1}$.

2.2. Glassware

Deactivation of the surface of glassware was applied to minimise sample loss through absorption of polar compounds onto –OH sites present on glass surfaces. The procedure included rinsing of the glass surface with the reagent (5%DMDCS in toluene) for 10–15 s, toluene (two times) and methanol (three times until the rinsing is neutral) and drying the surface with clean nitrogen.

2.3. Sample collection and preparation

All samples were collected in 1 L silanized bottles with Teflon faced phenolic caps (Wheaton, USA), acidified with 31%HCl to pH 2.5 and vacuum filtered through a 0.7 μm glass fibre filter GF/F (Whatman, UK). Samples were stored at 4 °C and extracted within 1 week. Two replicate grab samples were collected each time at each sampling point.

2.4. Solid-phase extraction

Solid-phase extraction was utilised for sample preparation. A SPE Gilson, Aspec XL4 was used for all SPE steps. TurboVap LV concentration workstation (Caliper, UK) was used for evaporation of extracts to dryness. The method was optimised through several preliminary experiments involving the following variables: type of adsorbent, pH value of the sample, elution conditions. Preliminary experiments were carried out for HQ pure water spiked with pharmaceuticals to verify the extraction efficiency of several cartridges. The recoveries of pharmaceuticals were measured by extracting analytes from 100 to 1000 mL of deionised and surface water spiked with 0.05–5 $\mu g\,L^{-1}$ of compounds. The cartridges used were:

- Oasis HLB, 60 mg (Waters, UK): built of two monomers, hydrophilic *N*-vinylpyrrolidone and lipophilic divinylbenzene; retention, reversed-phase and polar interactions; application, acidic, neutral and basic compounds.
- Oasis MCX, 60 mg (Waters): strong cation-exchange mixed-mode polymeric sorbent built upon HLB copolymer; retention, reversed phase and cation-exchange (sulfonic acid content, 1.0 $meq\,g^{-1}$); application, bases.
- Oasis MAX, 60 mg (Waters): strong anion-exchange mixed-mode polymeric sorbent built upon HLB copolymer; retention, reversed phase and anion exchange (anion exchange (quaternary amine) capacity, 0.24 $meq\,g^{-1}$); application, acids.
- Oasis WCX, 60 mg (Waters): weak cation-exchange mixed-mode polymeric sorbent built upon HLB copolymer; retention, reversed phase and cation exchange (COOH content, 0.72 $meq\,g^{-1}$); application, strong bases.
- Oasis WAX, 60 mg (Waters, UK): weak anion-exchange mixed-mode polymeric sorbent built upon HLB copolymer; retention, reversed phase and anion exchange (amine (piperazine) content, 0.48 $meq\,g^{-1}$); application, strong acids.
- Chromabond C18ec, 200 mg (Anachem, UK): silica-based endcapped sorbent; retention, reversed-phase; application, non-polar compounds.
- Isolute, ENV+, 100 mg (Kinesis, UK): resin-based non-polar sorbent built of hydroxylated polysterene divinylbenzene; retention, reversed-phase (primary); application, wide polarity range analytes.
- Isolute, HCX, 200 mg (Kinesis, UK): silica-based mixed-mode sorbent containing octyl chains (C8, non-endcapped) and strong cation-exchange sites ($–SO_3^-$); retention, non-polar and strong cation exchange; application, non-polar and basic analytes.

The initial results allowed for the choice of Oasis MCX sorbent, which was used for further analysis. The final SPE extraction procedure is as follows. One litre of acidified and filtered water sample (see Section 2.3) spiked with 200 ng of surrogate/internal standards (phenacetin-ethoxy-1-^{13}C and caffeine-d9 (1,3,7-trimethyl-d9)) was passed through the MCX cartridge at a rate of 4 $mL\,min^{-1}$. Five hundred milligrams of Na_2EDTA was added to the sample prior to extraction to prevent tetracyclines complexing with Ca^{2+} and Mg^{+} ion and residual metals on the SPE cartridges [25]. The cartridges were conditioned with 2 mL of MeOH and equilibrated with 2 mL of water acidified with HCOOH (2%HCOOH; pH, 2.1) at a rate of 3 $mL\,min^{-1}$. After passage of the samples, the cartridges were washed with acidified water (2 mL 2%HCOOH/H_2O; flow rate, 3 $mL\,min^{-1}$). After drying, SPE cartridges were wrapped in aluminium foil and stored in a freezer until eluted. Pharmaceuticals were extracted with 1 mL of MeOH and 2 mL of 5%NH_4OH in MeOH at a rate of 1 $mL\,min^{-1}$. The extracts were directly collected into a 6 mL collection tube and were evaporated to dryness with TurboVap evaporator (40 °C, N_2, 5–15 psi) and finally reconstituted in 0.5 mL of HQ water acidified with CH_3COOH (mobile phase, 100%A: 94.5%H_2O, 5%MeOH, 0.5%CH_3COOH). All samples were transferred to maximum recovery deactivated vials with PTFE septa (Waters, UK). In order to remove possible solid particles from reconstituted SPE extract before UPLC/MS/MS 0.2 μm PTFE filters (Whatman, Puradisc, 13 mm) were used. It was established that out of all compounds analysed only losses of simvastatin due to sorption to PTFE filter were observed.

2.5. Ultra performance liquid chromatography–tandem mass spectrometry

2.5.1. Ultra performance liquid chromatography

Analyses were carried out with the usage of Waters ACQUITY UPLC™ system (Waters, Manchester, UK) consisting of ACQUITY UPLC™ binary solvent manager and ACQUITY UPLC™ sample manager. Separation of compounds was obtained with ACQUITY UPLC BEH C18 column (1.7 μm; 1 mm × 100 mm) (Waters, UK). Preliminary separation of analytes was made with the usage of UV detector set at 230 nm (ACQUITY UPLC™ UV detector). Several mobile phases (H_2O, MeOH, acetonitrile) and their additives were studied for an improvement of compounds separation in LC

and an improvement of ESI performance in positive ionisation mode. Among the mobile phase additives studied were basic additives: ammonia, ammonium formate and actetate; primary amines (methyl-, ethyl- and butylamine); secondary amines (dimethyl-, diethyl-, dibutylamine); tertiary amines (trimethyl-, triethyl-, tributylamine); acidic compounds: formic and acetic acid. Water and methanol acidified with acetic acid were chosen as mobile phases. Mobile phase A (pH, 2.8) was composed of 94.5%H_2O, 5%MeOH, 0.5%CH_3COOH and mobile phase B (pH, 3.2) was composed of 99.5%MeOH and 0.5%CH_3COOH. The gradient program was as follows: 0 min, 100%A–0.2 min, 100%A–1 min, 95%A–5 min, 90%A–8 min, 80%A–10 min, 55%A–11 min, 55%A–13 min, 0%A–15 min, 0%A–16 min, 100%A–20 min, 100%A. Ten microlitres of the sample was injected into the system. The column was kept at 22 °C and the temperature in the sample manager was kept at 6 °C. The flow rate of mobile phase was 0.07 mL min^{-1}, which gave an average initial pressure of 6500 psi.

2.5.2. Mass spectrometry

A Quatro Micro triple-quadrupole mass spectrometer (Micromass, Manchester, UK), equipped with an electrospray ionisation (ESI) source was used. The analyses were performed in positive mode with a capillary voltage of 3 kV, a source temperature of 120 °C and a desolvation temperature of 350 °C. A cone gas flow of 20 L h^{-1} and desolvation gas flow of 400 L h^{-1} were used. Nitrogen, used as a nebulising and desolvation gas, was provided by a high-purity nitrogen generator NM 30LA 230VOC (Peak Scientific Instrument Ltd., Scotland, UK). Argon (99.999%) was used as a collision gas. Argon pressure in the collision cell was kept at 2.5e^{-3} mbar. MassLynx 4.1 software was used to collect and analyse the obtained data.

The mobile phase at a flow rate of 0.07 mL min^{-1} was directly introduced into the ion source from LC, without splitting. Mass spectrometry analyses were performed in the multiple reaction monitoring (MRM) mode, measuring the fragmentation of the protonated pseudo-molecular ions of each pharmaceutical. A dwell time of 200 ms per ion pair was used.

The choice of fragmentation products for each substance based on the most intense signal and the optimisation of cone voltages, energy collisions and other instrument parameters was made individually for each compound in a continuous-flow mode through a direct infusion of standard solutions at concentrations of 1 mg L^{-1} into the stream of the mobile phase. Composition of mobile phase was set according to the retention time of each compound. Syringe pump flow was 10 μL min^{-1} and mobile phase flow was 0.07 mL min^{-1}. All standards were prepared by addition of a proper volume of stock solution into water acidified with 0.5%CH_3COOH and containing 5%MeOH.

For optimisation of precursor ion/product ion transitions QuanOptimise software was used.

2.5.3. Matrix effects and signal suppression

Signal suppression was evaluated for each pharmaceutical as a percentage decrease in signal intensity in a sample matrix versus in deionised water. The following equation was used for signal suppression calculation:

$$\text{Signal suppression}\,(\%) = \left(1 - \frac{I_{BB}}{I_{HQ}}\right) 100 \quad (1)$$

where I_{BB} is the pharmaceutical peak area in BB water extract spiked after extraction with 200 ng L^{-1} of each pharmaceutical, I_{HQ} the pharmaceutical peak area in HQ water extract spiked after extraction with 200 ng L^{-1} of each pharmaceutical. No pharmaceuticals were present in extracts of both HQ and BB water before their enrichment with pharmaceuticals.

2.6. Quantification and method validation parameters

Compounds were quantified by MRM, using the highest characteristic precursor ion/product ion transitions and recording one to four transitions simultaneously. The following surrogate/internal standards (IS) were used: phenacetin-ethoxy-1-^{13}C and caffeine-d9 (1,3,7-trimethyl-d9) for the quantification of compounds analysed. Phenacetin-ethoxy-1-^{13}C was the main standard used for the analysis of most of pharmaceuticals. Caffeine-d9 was applied as the surrogate/internal standard for the analysis of chloramphenicol and diltiazem. The usage of only two internal standards is a limitation of the method due to the variability of chemical structure/properties between pharmaceuticals studied and the chosen internal standards. The choice of only two IS resulted from both the very high cost of isotope labelled compounds and difficulty with their purchase.

All instrumental validation parameters such as: linearity and range, accuracy, instrumental precision, instrumental detection and instrumental quantification limits (IDL and IQL, respectively) and calibration curve were determined for HQ water (high-quality pure water; Neptune, Purite, MJ Patterson Scientific Ltd.) spiked with known concentrations of pharmaceuticals.

Method quantification and detection parameters such as: linearity and range, accuracy, precision of analytical method, method detection and method quantification limits (MDL and MQL, respectively), and calibration curve were determined for BB water (surface water collected from the source of River Taff) spiked with known concentrations of pharmaceuticals and extracted according to the procedure described in Section 2.4.

For quantification purposes QuanLynx software was used.

2.6.1. Linearity and range

Linearity and range of the analytical procedure were performed by serial dilution of a stock solution of pharmaceuticals (10 mg L^{-1}). Several concentration levels (that are typically measured in surface and wastewater) were used: 0–1.2 mg L^{-1} of each pharmaceutical.

2.6.2. Accuracy

Accuracy of the method was evaluated as the percentage of deviation from the known added amount of analyte in the sample.

2.6.3. Precision

Precision was evaluated as the relative standard deviation (RSD) of replicate measurements. Both intra- and inter-day reproducibilities of the analytical method were assessed.

Instrumental intra-day precision was verified under the same operating conditions over a short interval of time. Nine determinations covered three concentrations (10, 100 and 1000 $\mu g\,L^{-1}$) of acidified HQ standards, three replicates of each. Instrumental inter-day precision was verified by determinations that covered three concentrations (10, 100 and 1000 $\mu g\,L^{-1}$) of acidified HQ standards, three replicates each undertaken on three different days.

Intra-day precision of the analytical method was verified under the same operating conditions over a short interval of time. Nine determinations covered three concentrations (10, 100 and 1000 $ng\,L^{-1}$) of BB surface water spiked before extraction, three replicates of each. Inter-day precision of the analytical method was verified by determinations that covered three concentrations (10, 100 and 1000 $ng\,L^{-1}$) of BB surface water spiked before extraction, three replicates each undertaken on three different days.

2.6.4. Limit of detection and limit of quantification

Quantification and detection limits were determined using a signal-to-noise approach. Standard solutions which were diluted with acidified HQ water were used for instrumental detection and instrumental quantification limits determinations (IDL and IQL, respectively). BB surface water spiked before extraction was used for method detection and method quantification limits determination (MDL and MQL, respectively).

The quantification limit (QL) was estimated for the concentration of compound that gave a signal-to-noise ratio of 10:1. The detection limit (DL) corresponded to the concentration that gave a signal-to-noise ratio of 3:1.

Solutions of different concentrations (diluted serially to lower concentrations) were prepared by spiking known amounts of related substances into matrix solution (HQ water and surface water). Each solution was analysed repeatedly to determine the S/N ratio. The concentration level that gives a S/N value of about 10 was assumed to be the QL.

2.6.5. Calibration curve/quantitative analysis

12-point multi-component internal standard calibration curves for the HQ water and BB surface water spiked before extraction used as the matrix were applied for quantification of pharmaceuticals. Calibration curve was performed by calculating the ratios between the peak area of each substance and the peak area of the relative internal standard. All concentrations that were above the highest point in the calibration curve were diluted and reanalysed.

3. Results and discussion

3.1. Choice of pharmaceuticals

The list of 28 pharmaceuticals studied is presented in Table 1. The choice of pharmaceuticals was mainly based on the list of 2004 and 2005 prescription data in Wales and England [31,32] and the metabolism routes of pharmaceuticals, mainly excretion as parent compounds and active main metabolites. As can be observed from Table 1, human excretion rates of the studied pharmaceuticals as parent compounds often exceed 50%. Among them are: gabapentin, trimethoprim, amoxicillin, doxycycline, ciprofloxacin, cimetidine and valsartan. Additionally, some pharmaceuticals are excreted in the form of metabolites, e.g. conjugates with glucuronic acid, which subsequently might be transformed in the environment into a parent compound and as a result add to the level of pharmaceuticals concentration in the environment. These are, for example, chloramphenicol, paracetamol and codeine.

3.2. Liquid chromatography and mass spectrometry

3.2.1. Mobile phase and additives

In order to optimise chromatographic separation (reduction of peak tailing and better resolution) and ESI ionisation, different mobile phases (H_2O, MeOH, CH_3CN) and several mobile phase additives (basic and acidic compounds) were tested.

Basic additives such as ammonia, ammonium acetate, ammonium formate and alkylamines (primary, secondary and tertiary amines) are known to suppress the signal in ESI+ interface, which was confirmed by the present research. Acidic additives, on the other hand, are known to promote protonation of basic molecules and as a result an increase of signal in ESI source operating in positive mode takes place [29]. Both formic and acetic acid applied into mobile phase at varying concentrations (0.01–0.5%) were found to provide both good separation and sensitivity of ESI source. Acetic acid at the concentration of 0.5% was chosen as a mobile phase additive for the discussed method. The suitability of acetic acid as a mobile phase additive in pharmaceuticals analysis was also reported by other research groups [16,42].

3.2.2. UPLC/MS/MS—the method

A sufficient chromatographic separation, which is crucial for high sensitivity and low signal suppression, was obtained with ACQUITY UPLC BEH C18 column (1.7 μm; 1 mm × 100 mm) at 22 °C and simple gradient (Section 2.5.1). Chromatograms of SPE extract of BB water spiked with pharmaceuticals before extraction are presented in Fig. 1. Chromatograms of HQ water spiked with pharmaceuticals are presented in Fig. 2 in Supplementary Material. The usage of the novel ultra performance liquid chromatography system with 1.7 μm particle size and 1 mm internal diameter column allowed for the establishment of low mobile phase flow rates (0.07 $mL\,min^{-1}$) and short retention times (from 1.3 to 15.5 min) for all 28 compounds analysed. As a result a fast and cost-effective method was developed. A high speed of analysis and low mobile phase flow rates enabling direct introduction of analytes into the ion source from LC without splitting are some of the main advantages of the method when compared to other multi-residue methods using high-performance liquid chromatography for analytes separation [24,27].

The ESI parameters were optimised as discussed in Section 2.5.2. All of the compounds showed maximum sensitivity in the positive ionisation mode. The degree of ionisation of the compounds varied significantly due to the different functionalities present in the molecule. The highest response was

Table 1
Chosen pharmaceuticals and their properties [22,31,33–41]

Group	Properties								
	Compound	CAS number	Molecular formula	MW	pK_a	log K_{ow}	Prescription [31] (kg)	Excretion	
								Unchanged (%)	Metabolites
Antiepileptic drugs	Carbamazepine	298-46-4	$C_{15}H_{12}N_2O$	236.27	13.9	2.4–2.9	2571.2	3	Hydroxylated (10,11-epoxide) (active) and conjugated metabolites
	Gabapentin	60142-96-3	$C_9H_{17}NO_2$	171.24	3.7, 10.7	(−)1.1–0.8	2280.1	100	No metabolites
Antibacterial drugs	Trimethoprim	738-70-5	$C_{14}H_{18}N_4O_3$	290.32	6.6–7.1	0.8–1.4	596.1	80	1,3-oxides; 3′,4-hydroxy derivatives
	Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253.28	5.8	0.9–2.5		30	N_4-Acetylated metabolite
	Amoxicillin	26787-78-0	$C_{16}H_{19}N_3O_5S$	365.40	2.8, 7.2	(−)0.6–0.9	9574.8	60–80	Penicilloic acid (10–25%)
	Chloramphenicol	56-75-7	$C_{11}H_{12}C_{l2}N_2O_5$	323.13	11.0	(−)0.2–1.5	–	8–12	Glucuronide conjugates
	Doxycycline	564-25-0	$C_{22}H_{24}N_2O_8$	444.44	4.5	(−)3.7-(−)0.02	86.4	Most	–
	Erythromycin	114-07-8	$C_{37}H_{67}NO_{13}$	733.93	8.9	3.1	3265	5%	Erythromycin-H_2O
	Ciprofloxacin	85721-33-1	$C_{17}H_{18}FN_3O_3$	331.34	5.9, 8.9	0.3–1.3	459.1	50–70	Oxociprofloxacin (3%, active), sulfociprofloxacin (8%, active)
	Metronidazole	443-48-1	$C_6H_9N_3O_3$	171.15	2.4	(−)0.3–0.02	639.1	20	1-(β-Hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole and 2-methyl-5-nitroimidazole-1-yl-acetic acid
Beta-adrenoceptor blocking drugs	Propranolol	525-66-6	$C_{16}H_{21}NO_2$	259.35	9.4	2.7–3.6	478.2	<0.5	4-Hydroxypropranolol (active); glucuronide conjugates (20%)
	Metoprolol	37350-58-6	$C_{15}H_{25}NO_3$	267.36	9.7	1.9–2.5	127.1	10–30	Not active metabolites
	Atenolol	29122-68-7	$C_{14}H_{22}N_2O_3$	266.34	9.2–9.6	0.2–0.5	2565.8	50	Hydroxylated metabolite (3%)
Non-Opioid Analgesics	Paracetamol	103-90-2	$C_8H_9NO_2$	151.16	9.4–9.9	0.5–0.9	110245.2	80% as conjugates	Sulfate conjugate (30%), paracetamol cysteinate and mercapturate (5%)
Opioid analgesics	Codeine	76-57-3	$C_{18}H_{21}NO_3$	299.36	8.2	1.2–2.0	2471.3	70 free or as conjugates	Codeine-6-glucuronide (main); free or conjugated morphine (10–15%), and norcodeine (10–20%)
	Tramadol	27203-92-5	$C_{16}H_{25}NO_2$	263.04	9.4	3.0	1622.5	15–35	Desmethyltramadol (active)
Lipid-regulating agents	Simvastatin	79902-63-9	$C_{25}H_{38}O_5$	418.57	13.5	4.4–4.9	933.6		β-Hydroxyacid metabolite
Bronchodilators	Salbutamol	18559-94-9	$C_{13}H_{21}NO_3$	239.31	–	1.0	28.9	30	Phenolic sulfate (45–60%), 4′-*o*-sulfate ester (inactive)
Histamine-2 blockers	Ranitidine	66357-35-5	$C_{13}H_{22}N_4O_3S$	314.41	8.2, 2.7	(−)1.1–1.9	2696.7	30	N-oxide (3–6%), S-oxide (1–2%) and desmethyl ranitidine (1–2%)
	Cimetidine	51481-61-9	$C_{10}H_{16}N_6S$	252.34	6.8	0.4–0.9	1019	48–75	Cimetidine *N*-glucuronide (24%), cimetidine suphoxide (7–14%), hydroxymethylcimetidine (4%)
Anti-inflammatory agents	Sulfapyridine	144-83-2	$C_{11}H_{11}N_3O_2S$	249.29	8.4–8.5	0.03–0.4	1.4	–	–
	5-Aminosalicylic acid	89-57-6	$C_7H_7NO_3$	153.14	1.9	0.4–1.0	2790.2	<12	*N*-Acetyl-5-aminosalicylic acid (8–77%)
Antidepressants	Amitriptyline	50-48-6	$C_{20}H_{23}N$	277.41	9.4	4.4–4.9	381.5	Little	Nortriptyline, 10-hydroxyamitriptyline (active), 10-hydroxynortriptyline (active)
Drugs of abuse, dopamine uptake inhibitors	Amphetamine	300-62-9	$C_9H_{13}N$	135.21	10.1	1.8	–	1–74	<25% Phenylacetone, benzoic acid, and hippuric acid; <10% 4-hydroxy-amphetamine, 4-hydroxy-norephedrine, and norephedrine
	Cocaine	50-36-2	$C_{17}H_{21}NO_4$	303.36	8.6	2.3	–	–	Benzoylecgonine, ecgonine methyl ester (main)
	Benzoylecgonine, cocaine metabolite	519-09-5	$C_{16}H_{19}NO_4$	289.32	–	(−)1.3	–	–	–
Calcium channel blockers	Diltiazem	42399-41-7	$C_{22}H_{26}N_2O_4S$	414.52	7.7	2.7–3.1	1744.3	2–4	Desacetyldiltiazem and *N*-monodemethyldiltiazem (active)
Angiotensin II antagonists	Valsartan	137862-53-4	$C_{24}H_{29}N_5O_3$	435.50	3.7–3.9	4.7–5.2	492.7	80	9% valeryl 4-hydroxy valsartan

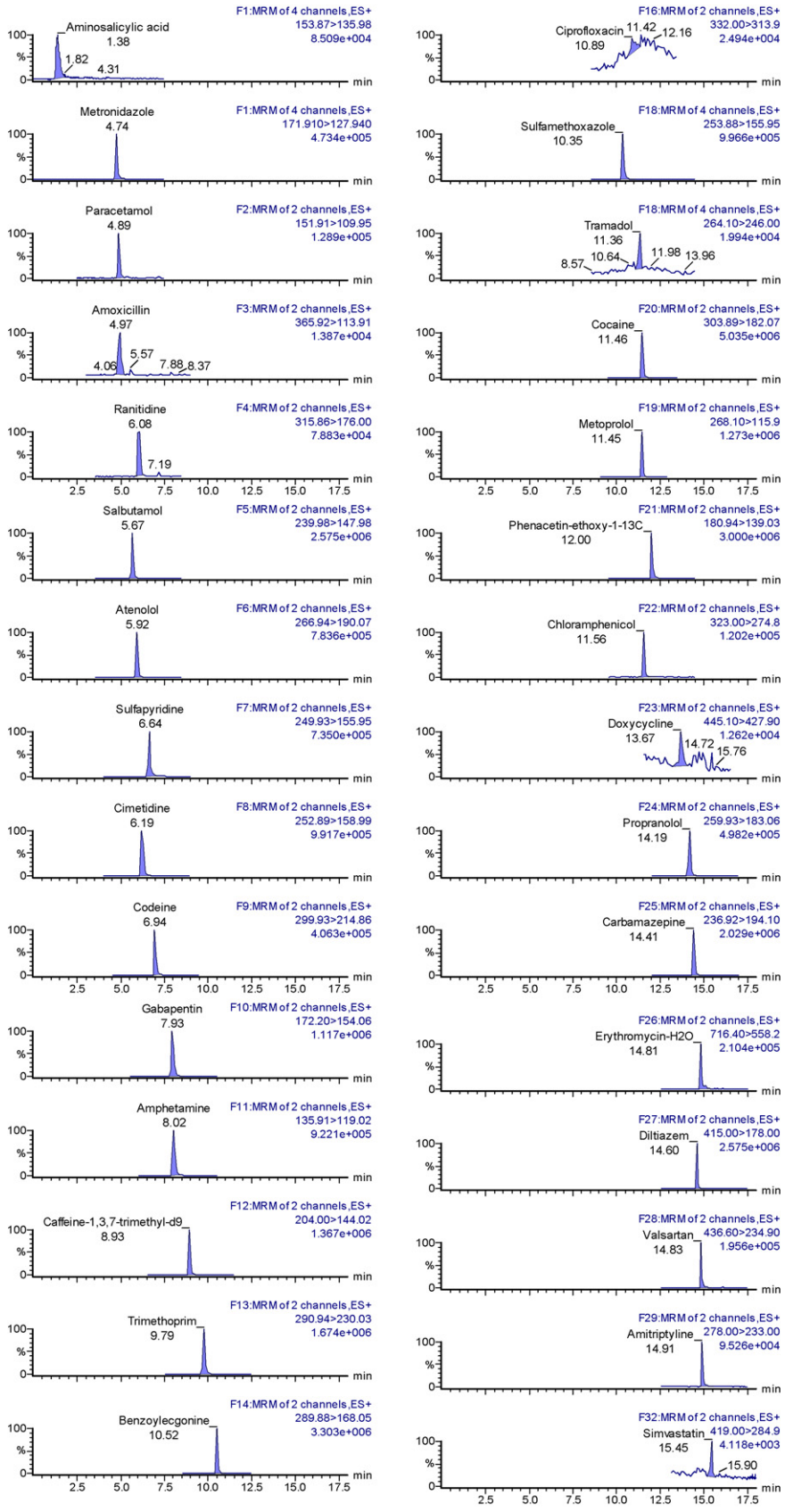

Fig. 1. UPLC/MS/MS separations for chosen pharmaceuticals spiked into BB water and extracted by SPE (concentration of pharmaceuticals, 100 ng L^{-1}; IS, 200 ng L^{-1}).

observed for: salbutamol, atenolol, cocaine, benzoylecgonine and carbamazepine. Amoxicillin, 5-aminosalicylic acid simvastatin and tramadol were characterised, apart from doxycycline and ciprofloxacin, with the lowest response. However, the response was sufficient enough to undertake environmental analysis (Fig. 1). Doxycycline and ciprofloxacin could not be efficiently extracted by SPE (see Fig. 1 and Fig. 2 in Supplementary Material) and therefore these compounds were not analysed with the proposed method as discussed below.

The protonated molecular ion ($[M+H]^+$) of molecule was chosen as a parent ion, with the exception of erythromycin. Erythromycin at pH < 7 is converted into erythromycin-H_2O, a degradation product with a loss of one molecule of H_2O. Therefore, the protonated ion of erythromycin-H_2O was analysed [43]. The mass spectrometry parameters are presented in Table 2. The transitions were in agreement with the literature data. The most intensive fragment ion from each precursor ion was selected for quantification. Retention time was the other primary criterion for identification of compound. A less sensitive secondary transition was used as the second criterion for confirmation purposes. In the case of simvastatin no secondary transition was observed.

3.3. Solid-phase extraction

The greatest difficulty with the multi-residue analysis of pharmaceuticals from different therapeutic classes concerns the choice of the best SPE adsorbent giving an acceptable recovery for all compounds characterised by different physicochemical properties. In this work, eight different adsorbents were studied. Among them were polymer and silica-based sorbents capable of non-polar or/and ion-exchange interactions (see Section 2.4). Oasis MCX was found to be the most effective for studied pharmaceuticals at acidic pH (pH 2.5). Oasis MCX is a strong cation-exchange mixed-mode polymeric sorbent, which provides both ion-exchange and reversed-phase retention. MCX sorbent is built upon HLB copolymer containing two monomers: hydrophilic *N*-vinylpyrrolidone and lipophilic divinylbenzene. The additional presence of sulfonic groups allows for cation-exchange interactions. Therefore, MCX adsorbent is the most suited for the extraction of basic and neutral compounds from aqueous solution. Acidic pH of the solution is required in order to ionise basic compounds.

High recovery and reproducibility for MCX adsorbent was obtained for many of the pharmaceuticals studied in this work both in HQ and BB water. The variety of chemical classes of pharmaceuticals studied resulted in different recoveries. The mean absolute and relative (relative to the recovery of surrogate/internal standard) recoveries and standard deviations for pharmaceuticals in both HQ and BB water are presented in Table 3. Surrogate/internal standard was added to the sample before the whole analytical procedure so as to compensate for losses of compounds during both the sam-

Table 2
Optimised MRM conditions for the analysis of chosen pharmaceuticals by UPLC/MS/MS

Compound	CV/CE	MRM1 (quantification)	CV/CE	MRM2 (confirmation)
5-Aminosalicylic acid	26/15	153.9 > 136.0	26/20	153.9 > 108.0
Metronidazole	26/15	171.9 > 127.9	26/23	171.9 > 81.9
Paracetamol	26/16	151.9 > 110.0	26/24	151.9 > 92.9
Amoxicillin	26/28	365.9 > 113.9	26/15	365.9 > 159.9
Ranitidine	26/17	315.9 > 176.0	26/24	315.9 > 123.9
Salbutamol	26/20	240.0 > 148.0	26/10	240.0 > 222.1
Atenolol	34/19	266.9 > 190.1	34/25	266.9 > 145.0
Sulfapyridine	26/16	249.9 > 156.0	26/16	249.9 > 184.0
Cimetidine	26/15	252.9 > 159.0	26/15	252.9 > 117.0
Codeine	45/25	299.9 > 214.9	45/4	299.9 > 224.9
Gabapentin	26/10	172.2 > 154.1	26/10	172.2 > 137.0
Amphetamine	18/10	135.9 > 119.0	18/16	135.9 > 90.9
Trimethoprim	42/22	290.9 > 230.0	42/22	290.9 > 123.0
Benzoylecgonine	30/25	289.9 > 168.1	30/18	289.9 > 104.9
Ciprofloxacin	35/17	332.0 > 313.9	35/17	332.0 > 288.0
Sulfamethoxazole	26/16	253.9 > 156.0	26/21	253.9 > 107.9
Tramadol	15/15	264.1 > 246.0	15/15	264.1 > 57.8
Cocaine	34/22	303.9 > 182.1	34/22	303.9 > 81.9
Metoprolol	35/17	268.1 > 115.9	35/20	268.1 > 97.9
Chloramphenicol	20/10	323.0 > 274.8	20/10	323.0 > 304.8
Doxycycline	30/17	445.1 > 427.9	30/25	445.1 > 409.9
Propranolol	34/18	259.9 > 183.1	34/16	259.9 > 116.0
Carbamazepine	26/19	236.9 > 194.1	26/19	236.9 > 192.1
Erythromycin-H_2O	26/15	716.4 > 558.2	26/34	716.4 > 158.1
Diltiazem	35/20	415.0 > 178.0	35/20	415.0 > 310.0
Valsartan	20/15	436.6 > 234.9	20/15	436.6 > 290.9
Amitriptyline	30/20	278.0 > 233.0	30/20	278.0 > 191.0
Simvastatin	25/10	419.0 > 284.9	25/10	419.0 > 199.0
Caffeine-d9 (1,3,7-trimethyl-d9)	34/16	204.0 > 144.0	–	–
Phenacetin-ethoxy-1-^{13}C	34/15	180.9 > 139.0	–	–

CV, cone voltage (V); CE, collision energy (eV).

Table 3
SPE recovery for studied pharmaceuticals (concentration, 200 ng L^{-1})

Compound	Recovery (%) ($n = 3$)				Literature recovery (%)
	HQ water		BB water		Mineral/tap/surface water
	Absolute	Relative[a]	Absolute	Relative[a]	Absolute
5-Aminosalicylic acid	22.7 ± 1.0	21.9 ± 1.5[1]	5.2 ± 0.3	7.3 ± 0.4[1]	–
Metronidazole	35.3 ± 1.4	34.0 ± 2.2[1]	34.0 ± 1.0	47.7 ± 2.5[1]	33–37[b(c)]
Paracetamol	8.5 ± 0.3	8.2 ± 0.8[1]	8.7 ± 0.1	12.2 ± 0.7[1]	10–29[c]; 62–85[d]; 61[e(a)]; 75[e(b)]; 60–71[f]
Amoxicillin	53.1 ± 1.6	51.9 ± 2.4[1]	40.6 ± 8.5	57 ± 10.2[1]	36[b(c)]
Ranitidine	63.4 ± 2.2	62.1 ± 1.7[1]	44.3 ± 3.3	62.2 ± 6.3[1]	27–54[d]; 51–94[f]; 95[g]
Salbutamol	71.5 ± 0.5	69.3 ± 2.8[1]	88.2 ± 1.9	119.2 ± 2.9[1]	76[g]; 66–80[b(a)]
Atenolol	75.3 ± 2.2	73.5 ± 0.9[1]	90.0 ± 3.2	119.6 ± 1.5[1]	87–96[f]; 106[g]; 67–86[b(a)]
Sulfapyridine	77.8 ± 1.2	76.6 ± 2.3[1]	68.8 ± 1.9	96.7 ± 5.9[1]	–
Cimetidine	53.6 ± 2.1	51.8 ± 1.8[1]	64.8 ± 5.6	91.1 ± 3.4[1]	21–52[d]
Codeine	88.3 ± 2.4	86.9 ± 2.9[1]	75.1 ± 5.7	101.7 ± 3.4[1]	–
Gabapentin	61.6 ± 2.9	75.5 ± 1.1[1]	85.9 ± 2.9	113.8 ± 3.1[1]	–
Amphetamine	107.4 ± 4.9	105.6 ± 1.3[1]	90.5 ± 1.2	121.4 ± 3.7[1]	–
Trimethoprim	80.5 ± 1.7	76.7 ± 3.8[1]	83.7 ± 5.6	107.8 ± 5.4[1]	28–66[c]; 71–124[d]; 39[e(a)]; 123[e(b)]; 93–96[f]; 50–55[b(c)]
Benzoylecgonine	95.9 ± 3.3	90.6 ± 2.7[1]	131.2 ± 2.8	175.9 ± 7.3[1]	–
Ciprofloxacin	82.5 ± 1.1	79.9 ± 2.1[1]	0.3 ± 0.2	0.5 ± 0.4[1]	107–112[c]; 32[g];
Sulfamethoxazole	67.4 ± 1.1	64.9 ± 1.9[1]	60.0 ± 1.3	84.3 ± 5.4[1]	13–35[c]; 43[e(a)]; 120[e(b)]; 90–108[f]; 65[g]; 21–23[b(c)]
Tramadol	109.0 ± 3.0	109.5 ± 5.4[1]	75.8 ± 1.6	101.0 ± 2.7[1]	
Cocaine	90.0 ± 2.1	87.5 ± 0.8[1]	70.1 ± 2.9	98.5 ± 1.2[1]	–
Metoprolol	85.4 ± 4.2	81.9 ± 5.4[1]	55.4 ± 7.7	77.9 ± 10.4[1]	60–103[f]; 54–96[b(a)]
Chloramphenicol	57.3 ± 2.9	55.0 ± 4.4[1] 55.9 ± 3.0[2]	9.4 ± 0.1	13.2 ± 0.7[1] 34.9 ± 6.5[2]	84[b(c)]
Doxycycline	77.5 ± 3.1	72.1 ± 4.6[1]	26.8 ± 9.5	37.7 ± 13.5[1]	–
Propranolol	68.7 ± 3.4	65.3 ± 1.7[1]	40.0 ± 4.5	60.2 ± 3.9[1]	41[e(a)]; 45[e(b)]; 81–102[f]; 48–84[b(a)]
Erythromycin-H_2O	61.6 ± 2.9	65.7 ± 9.4[1]	73.8 ± 14.1	92.5 ± 12.5[1]	27–55[c]; 0.9[e(a)]; 73[e(b)]; 70–81[f]; 50[g(b)]; 91–94[h]
Carbamazepine	107.1 ± 4.5	102.5 ± 2.9[1]	68.0 ± 2.2	96.3 ± 5.4[1]	54–60[c]; 67–93[f]; 98[g(b)]; 74–80[b(b)]
Diltiazem	72.2 ± 3.0	69.4 ± 0.6[1] 71.9 ± 2.0[2]	9.0 ± 1.5	18.2 ± 2.0[1] 38.4 ± 6.2[2]	70–99[d]
Valsartan	146.2 ± 16.5	134.1 ± 15.8[1]	47.7 ± 7.9	70.8 ± 12.5[1]	–
Amitriptyline	83.0 ± 7.9	95.2 ± 10.4[1]	37.0 ± 5.6	45.4 ± 8.6[1]	–
Simvastatin	103.8 ± 16.6	99.8 ± 14.4[1]	40.2 ± 6.5	53.4 ± 9.8[1]	53–70[b(a)]; 73[i(a)]; 78[i(b)]; 82[i(c)]
Caffeine-d9 (1,3,7-trimethyl-d9)	35.2 ± 0.9	–	42.3 ± 0.8	–	–
Phenacetin-ethoxy-1-^{13}C	103.4 ± 3.5	–	71.2 ± 3.1	–	–

[a] Recovery relative to surrogate/internal standard: [1]phenacetin-ethoxy-1-^{13}C, [2]Caffeine-d9 (1,3,7-trimethyl-d9).
[b] (a) Varian Bond Elut, 200 mg, (b) C18, 1 g, (c) Isolute ENV+, 100 mg; tap and surface water [44].
[c] Oasis HLB, 500 mg; surface water [26].
[d] Oasis HLB, 500 mg [24].
[e] (a) Varian Bond Elut C_{18}, (b) Phenomenex Strata X, 200 mg; tap water [46].
[f] Oasis, HLB, 60 mg; surface water [27].
[g] (a) Oasis MCX, 60 mg, mineral water; (b) Lichrolut EN, 200 mg; mineral water [25].
[h] Oasis, HLB, 60 mg; surface water [43].
[i] (a) HLB, (b) Bond Elute C8, (c) DSC-18; surface water [47].

ple preparation procedure and resulting from matrix assisted suppression.

A significant influence of the matrix components in BB water on the decrease of the recovery of pharmaceuticals (compared to recoveries in HQ water) was observed mainly in the case of the following compounds: 5-aminosalicylic acid, chloramphenicol, doxycycline, ciprofloxacin, 5-aminosalicylic acid, amitriptyline, diltiazem and valsartan. The decrease of the recovery of pharmaceuticals in BB water when compared to HQ water can result from both a reduction of sorption efficiency of SPE cartridges and also signal suppression in the electrospray interface due to the presence of matrix impurities. This, however, will be discussed in the next paragraph.

Among the compounds of low relative recovery (<60%) in BB water, the following: chloramphenicol, amoxicillin, metronidazole, paracetamol, 5-aminosalicylic acid, amitriptyline, simvastatin and diltiazem were reproducible enough to be used in environmental analysis, although their analysis should be regarded on semi-quantitative bases. As will be discussed in Section 3.5, the mean correlation coefficients of the calibration curves for these compounds were higher than 0.996 in BB water spiked before extraction. Doxycycline and ciprofloxacin could not be efficiently extracted from BB water and therefore no quantitative analysis was carried out for these compounds.

For most compounds compensation for losses of pharmaceuticals was obtained through the addition of surrogate/internal

standard. As can be observed from Table 3, the relative recoveries were calculated to be significantly higher than absolute recoveries, which is to be expected due to the suppression effects observed for some of the compounds studied, as will be discussed in the following paragraph. Phenacetin-13C was chosen as the surrogate/internal standard for most of pharmaceuticals studied. Caffeine-d9, due to low SPE recovery, was found to be an adequate standard for chloramphenicol and diltiazem only (Table 3).

The recoveries obtained in this work were found to be similar to those reported by other research groups (Table 3). Lower recoveries were observed only for paracetamol, ciprofloxacin and diltiazem. Higher recoveries were obtained for the following compounds: cimetidine, amoxicillin and salbutamol.

3.4. Matrix effects and signal suppression

The main disadvantage of electrospray mass spectrometry is the fact that it is susceptible to matrix components. As a result, signal suppression (rarely enhancement) of the analyte signal might take place. The decrease of method sensitivity can be caused by several factors. Among them are: reduction of ionisation efficiency of analytes by taking up excess charged sites on the surface of electrospray droplets, masking the analyte peaks by contaminants due to rising chromatogram baseline and sorption of analytes to the dissolved organic carbon [44,45].

The signal suppression observed for analysed pharmaceuticals dissolved in SPE extract of BB water is presented in Table 4. No or only a slight signal suppression was observed for salbutamol, atenolol, metoprolol, codeine, amphetamine, trimethoprim, sulfamethoxazole and carbamazepine. A low signal enhancement was observed in the case of metronidazole, amoxicillin, sulfapyridine, cimetidine, gabapentin and diltiazem. 5-Aminosalicylic acid, ciprofloxacin, chloramphenicol, doxycycline, erythromycin-H_2O, valsartan and amitriptyline are the most susceptible to matrix components. Additionally, in the case of ciprofloxacin and doxycycline, not only matrix assisted low SPE recovery and signal suppression but also a lack of linearity of calibration curve (see Section 3.5) made the establishment of a quantitative method impossible. Additional work will be carried out to establish a modified SPE procedure for the analysis of these compounds that allows for the extraction of pharmaceuticals without interfering matrix components.

It has to be also emphasised that BB water used for method establishment was characterised by approximately 5 mg L^{-1} dissolved organic carbon content. It is expected that the suppression effect of analysed pharmaceuticals might be much more significant for samples of much higher DOC concentration, e.g. wastewater. The influence of matrix components on signal suppression was also discussed by Renew and Huang [42] and Gómez et al. [45] and Gross et al. [27].

In summary, the effects of signal suppression and low SPE recovery, both resulting from the presence of matrix interferences, are the main factors affecting the sensitivity of the analytical method. Among the compounds characterised by the highest ion suppression in ESI source are: ranitidine, cocaine, chloramphenicol, doxycycline, erythromycin-H_2O, valsartan, simvastatin, amitriptyline and tramadol. Therefore, for these compounds, the lower absolute SPE recoveries (Table 3) are probably due to the suppression of the signal during electrospray ionisation. Low SPE recovery resulting from matrix interferences, as the main factor affecting sensitivity of the method, was observed for paracetamol, amoxicillin, sulfamethoxazole, metoprolol, diltiazem, cimetidine, metronidazole, sulfapyridine, codeine and carbamazepine. Both effects were observed for the following compounds: 5-aminosalicylic acid, ciprofloxacin and propranolol. Salbutamol, atenolol, trimethoprim, benzoylecgonine, gabapentin and amphetamine were not affected by any of the effects studied. The low SPE recovery and suppression effects of many of the pharmaceuticals studied were in this work corrected by the usage of the surrogate/internal standard (Table 3). However, an overestimation (>100% relative recovery) was observed for the relative recoveries of these pharmaceuticals, which were not affected by matrix assisted signal suppression/low recovery (e.g. amphetamine and benzoylecgonine). This phenomenon can be explained by the fact that signal suppression for the surrogate/internal standard is higher than for the analyte. Resulting from the above discussion, there is an

Table 4
Signal suppression of pharmaceuticals in BB water spiked after extraction (pharmaceuticals and IS concentration, 200 μg L^{-1})

Compound	Signal suppression (%)	Compound	Signal suppression (%)
5-Aminosalicylic acid	34.1 ± 6.8	Sulfamethoxazole	6.3 ± 1.9
Metronidazole	−6.0 ± 1.7	Tramadol	17.2 ± 1.0
Paracetamol	13.0 ± 1.8	Cocaine	22.0 ± 0.8
Amoxicillin	−5.6 ± 1.9	Metoprolol	6.7 ± 1.5
Ranitidine	24.4 ± 3.3	Chloramphenicol	86.0 ± 0.1
Salbutamol	1.2 ± 2.4	Doxycycline	80.4 ± 0.4
Atenolol	6.4 ± 2.9	Propranolol	18.0 ± 0.7
Sulfapyridine	−3.4 ± 0.2	Carbamazepine	6.7 ± 1.8
Cimetidine	−9.0 ± 4.0	Erythromycin-H_2O	49.0 ± 0.7
Codeine	0.2 ± 1.0	Diltiazem	−26.4 ± 2.8
Gabapentin	−10.2 ± 4.1	Valsartan	59.8 ± 0.8
Amphetamine	2.6 ± 2.9	Amitriptyline	69.3 ± 1.3
Trimethoprim	4.8 ± 4.0	Simvastatin	43.5 ± 5.3
Benzoylecgonine	15.5 ± 1.0	Caffeine-d9 (1,3,7-trimethyl-d9)	42.9 ± 1.4
Ciprofloxacin	64.3 ± 0.8	Phenacetin-ethoxy-1-^{13}C	29.1 ± 2.0

Table 5
Performance data for pharmaceuticals (instrumental/method limits of detection and quantification; linearity, R^2)

Pharmaceuticals	Instrumental parameters[a]			Method parameters[b]		
	IDL ($\mu g\,L^{-1}$)	IQL ($\mu g\,L^{-1}$)	R^2	MDL ($ng\,L^{-1}$)	MQL ($ng\,L^{-1}$)	R^2
5-Aminosalicylic acid	2	5	0.998	5	15	0.998
Metronidazole	0.2	1	0.997	0.5	1.5	0.999
Paracetamol	0.5	2	0.997	0.5	1.5	0.998
Amoxicillin	2.5	10	0.996	2.5	10	0.998
Ranitidine	0.25	1	0.997	1	3	0.998
Salbutamol	0.1	0.5	0.998	0.1	0.5	0.997
Atenolol	0.15	0.5	0.999	0.2	1	0.999
Sulfapyridine	0.5	2	0.999	0.5	2	0.999
Cimetidine	0.15	0.5	0.999	0.1	0.5	1.000
Codeine	0.15	0.5	0.997	0.5	1.5	0.998
Gabapentin	0.3	1	0.998	0.2	0.6	0.998
Amphetamine	0.3	1	0.998	0.2	1	0.999
Trimethoprim	0.2	0.7	0.997	0.5	1.5	0.999
Benzoylecgonine	0.05	0.2	0.998	0.2	1	0.996
Ciprofloxacin	0.1	0.4	0.996	–	–	–
Sulfamethoxazole	0.1	0.4	0.999	0.1	0.5	0.998
Tramadol	2	5	0.998	10	30	0.999
Cocaine	0.05	0.2	0.995	0.1	0.3	0.998
Metoprolol	0.07	0.2	0.998	0.1	0.5	0.995
Chloramphenicol	0.5	1.5	0.997	2.5	10	0.999
Doxycycline	0.05	2	0.995	–	–	–
Propranolol	0.05	0.2	0.998	0.1	0.5	0.997
Carbamazepine	0.05	0.2	0.998	0.1	0.5	0.999
Erythromycin-H_2O	0.1	0.3	0.994	0.1	0.5	0.998
Diltiazem	0.1	0.5	0.998	0.5	1	0.998
Valsartan	0.5	1.5	0.996	0.2	1	0.998
Amitriptyline	0.1	0.3	0.997	0.1	0.5	0.995
Simvastatin	0.2	0.5	0.997	20	50	0.996

[a] HQ standards spiked with pharmaceuticals; concentration, 0–1200 $\mu g\,L^{-1}$.
[b] BB water spiked with pharmaceuticals before extraction; concentration, 0–1200 $ng\,L^{-1}$.

obvious need for the application of a higher number of surrogate/internal standards to more accurately compensate for matrix assisted signal suppression and the low SPE recovery of different groups of pharmaceuticals studied. This is, however, very often impossible due to the lack of suitable surrogate/internal standards or their high cost. The other possibilities that could eliminate matrix effect involve selective extraction/better sample clean-up, time consuming standard addition or dilution of sample extracts as proposed by Gross et al. [27] and Gómez et al. [45].

3.5. Quantification and method validation parameters

Concentrations of pharmaceuticals were calculated using the standard calibration curves for the HQ water spiked with pharmaceuticals and BB surface water spiked with pharmaceuticals before extraction, which were constructed using a detector response defined as the ratio of the peak ion (the specific product ion of the highest intensity) to the base peak ion of the internal standard. Phenacetin-ethoxy-1-^{13}C was chosen as a standard for most pharmaceuticals analysed. Caffeine-d9 was used as an adequate surrogate/internal standard for two analytes only: chloramphenicol and diltiazem.

The mean correlation coefficients (R^2) of the calibration curves, which are higher than 0.996 in both HQ water and BB surface water (Table 5) show good linearity of the method in the range of 0–1200 $ng\,L^{-1}$.

The instrumental and method limits of detection and quantification are presented in Table 5. The instrumental limits of quantification varied from 0.2 $\mu g\,L^{-1}$ for cocaine, benzoylecgonine, metoprolol, propanolol and carbamazepine to 10 $\mu g\,L^{-1}$ for amoxicillin. The method limits of quantification were at low nanogram per litre levels and ranged from 0.3 $ng\,L^{-1}$ for cocaine to 50 $ng\,L^{-1}$ for simvastatin, which makes the method useful for the determination of very low levels of pharmaceuticals in the aqueous environment such as surface waters.

The accuracy range was within the value of −30 to 20%. The instrumental intra-day repeatability as indicated by standard deviation calculated from the analysis of three replicates was below 10%. The method intra-day repeatability was on average less than 10% (Table 6). The instrumental and method inter-day repeatabilities were also less than 10%. Diltiazem was found to be the only compound that showed higher than average repeatabilities.

3.6. Environmental application

The new multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs was applied for

Table 6
Performance data for pharmaceuticals (inter- and intra-day repeatability)

Pharmaceuticals	Method parameters[a]											
	t_R (min)	Precision										
		Intra-day RSD% ($n=3$)									Inter-day RSD% ($n=3$)	
		10 (ng L^{-1})			100 (ng L^{-1})			1000 (ng L^{-1})			10 (ng L^{-1})	100 (ng L^{-1})	1000 (ng L^{-1})
		D1	D2	D3	D1	D2	D3	D1	D2	D3			
5-Aminosalicylic acid	1.38	6.2	8.7	9.9	6.8	12.7	2.2	4.1	7.9	0.9	10.4	9.5	7.3
Metronidazole	4.74	11.0	9.6	11.4	2.3	4.0	1.3	2.3	10.2	5.6	11.4	8.8	7.1
Paracetamol	4.89	7.0	5.9	8.9	5.7	5.4	10.9	9.9	5.1	4.1	13.4	7.1	6.0
Amoxicillin	4.97	6.0	14.3	5.6	1.6	12.9	13.8	0.2	12.0	6.2	10.3	11.2	8.7
Ranitidine	6.08	8.2	10.7	4.2	6.7	3.9	5.8	0.8	2.2	3.8	13.0	9.6	6.4
Salbutamol	5.67	5.4	8.6	4.3	4.9	4.4	0.8	6.3	7.5	6.5	8.1	7.4	7.2
Atenolol	5.92	8.9	12.5	16.6	6.2	9.0	3.1	1.0	9.0	2.8	12.4	6.1	7.3
Sulfapyridine	6.64	9.2	4.0	5.3	4.7	9.1	7.7	4.7	6.8	2.2	8.4	8.3	8.1
Cimetidine	6.19	6.3	1.9	5.7	8.1	3.8	5.4	5.7	8.3	3.2	8.0	7.2	7.3
Codeine	6.94	7.6	10.4	11.2	5.0	7.4	6.9	3.3	7.5	0.2	14.3	6.8	6.3
Gabapentin	7.93	2.6	5.2	4.5	2.4	8.8	1.4	5.2	7.1	0.5	6.5	5.2	5.5
Amphetamine	8.02	6.6	9.3	10.7	7.1	1.8	5.5	2.5	8.7	6.2	8.8	4.4	5.5
Trimethoprim	9.79	3.7	5.5	2.9	7.9	7.0	3.7	2.2	8.2	6.9	7.6	6.3	6.0
Benzoylecgonine	10.52	11.8	6.4	11.0	3.2	5.1	11.6	0.8	5.0	4.1	12.9	9.9	6.4
Sulfamethoxazole	10.35	5.8	6.8	7.4	5.4	5.4	1.7	4.8	12.8	4.9	7.3	5.5	7.0
Tramadol	11.36	11.0	12.6	11.4	8.0	14.4	11.2	5.1	1.9	1.2	11.7	16.7	4.2
Cocaine	11.46	5.2	7.9	3.9	2.3	10.2	0.4	2.6	7.1	2.0	7.1	6.5	6.6
Metoprolol	11.45	2.3	6.3	3.3	4.4	6.2	0.2	5.8	11.1	5.9	–	3.8	7.4
Chloramphenicol	11.56	4.3	3.9	3.1	6.4	4.9	3.6	2.3	11.6	3.5	7.2	6.0	8.6
Propranolol	14.19	5.7	14.3	5.4	1.5	8.5	8.4	0.3	8.9	0.3	10.1	6.7	5.9
Carbamazepine	14.41	5.8	7.3	5.3	2.8	13.2	4.7	2.2	9.9	2.8	9.6	12.8	8.0
Erythromycin-H_2O	14.81	4.2	3.8	8.2	7.8	17.5	6.1	2.9	0.6	1.0	–	12.4	6.4
Diltiazem	14.60	15.1	23.9	15.2	5.3	9.6	2.8	2.7	10.1	0.1	24.8	6.1	7.7
Valsartan	14.83	6.3	7.6	11.3	11.5	5.5	9.2	3.2	5.5	6.4	17.6	10.0	6.9
Amitriptyline	14.91	4.3	16.8	15.1	3.3	8.1	8.5	5.0	8.0	6.0	11.9	9.1	11.9
Simvastatin	15.45	–	–	–	9.3	23.5	3.8	2.8	8.2	0.5	–	17.7	16.8

[a] BB water spiked with pharmaceuticals before extraction; concentration, 0–1200 ng L^{-1}.

the verification of the occurrence of pharmaceuticals in river water in the UK and Poland.

UK samples were collected in December 2006 from River Taff after an extensive rainfall. The results are presented in Table 7. The first sampling point was positioned in Brecon Beacons National Park. The results confirmed the assumption that no contamination with pharmaceuticals occurs in that region. The second sampling point was located 23.5 km downstream from Brecon Beacons, just after Merthyr Tydfil (13 km upstream of a wastewater plant), a town with a population of about 55,000. The third sampling point was situated 22 km further in Trefforest Estate located after Pontypridd (population, approximately 33,000) and downstream (8.5 km) of a wastewater treatment plant. The influence of the presence of the wastewater plant treating mainly domestic sewage is clearly visible. An increase of all detected pharmaceuticals was observed. The last sampling point was located approximately 18 km downstream from Trefforest Estate, in Cardiff (population, approximately 320,000). The concentration of selected pharmaceuticals decreased slightly but still remained high. Pharmaceuticals were identified at the concentrations of a few ng L^{-1} to single μg L^{-1}. The highest concentration was observed for paracetamol, amoxicillin and tramadol. The comparison of the results presented in Table 7 and prescription data for Wales (Table 1) clearly indicates the correlation between pharmaceuticals usage and their presence in the environment.

Collection of samples from River Warta (Poznan, Poland) took place in January 2007, also after extensive rainfall. The first sampling point was located at Lechicka Street, which is approximately 1 km before Poznań Wastewater Treatment Plant in Koziegłowy, which serves approximately 400,000 inhabitants of Poznań. The next sampling point was located 3 km downstream of the WWTP Koziegłowy. Here again, wastewater effluent significantly influenced concentrations of identified pharmaceuticals in River Warta. Pharmaceuticals were identified at concentrations of a few ng L^{-1} to single μg L^{-1} depending on compound and sampling point. The highest concentration was observed in Koziegłowy sample for carbamazepine, metoprolol and tramadol.

The data presented in Table 7 show that the new multi-residue method is suitable for environmental monitoring of the presence of pharmaceuticals in surface waters. The compounds were found at the concentrations of a few ng L^{-1} to single μg L^{-1}.

Table 7
Concentration of basic/neutral pharmaceuticals in UK and Poland (two replicate samples)

Compound	Concentration (ng L^{-1})					
	UK, Wales, River Taff				Poland, River Warta	
	Brecon Beacons	Merthyr Tydfil	Trefforest Estate	Cardiff	Poznań, Lechicka Street	Koziegłowy
5-Aminosalicylic acid[a]	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Metronidazole[a]	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Paracetamol[a]	<MQL	216–376	1013–1388	551–572	11–15	24–58
Amoxicillin[a]	<MQL	39–49	198–245	56–60	<MQL	<MQL
Ranitidine	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Salbutamol	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Atenolol	<MQL	3–4	54–60	31–32	<MQL	3–22
Sulfapyridine	<MQL	<MQL	8–10	5	22–39	14–31
Cimetidine	<MQL	<MQL	9–11	5	<MQL	<MQL
Codeine	<MQL	<MQL	29–34	25–28	<MQL	9–15
Gabapentin	<MQL	19–21	87–98	57–59	42–64	58–75
Amphetamine	<MQL	<MQL	6	8–9	<MQL	<MQL
Trimethoprim	<MQL	<MQL	<MQL	<MQL	<MQL	8–27
Benzoylecgonine	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Sulfamethoxazole	<MQL	<MQL	<MQL	<MQL	26–30	30–60
Tramadol	<MQL	28–85	203–252	202–219	425–676	895–2108
Cocaine	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Metoprolol	<MQL	7	7–8	7	51–67	101–155
Chloramphenicol[a]	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Propranolol	<MQL	5	6–7	6	<MQL	<MQL
Carbamazepine	<MQL	<MQL	4–9	1–2	311–433	678–794
Erythromycin-H_2O	<MQL	<MQL	17–22	7–8	<MQL	<MQL
Diltiazem[a]	<MQL	<MQL	<MQL-1	<MQL	<MQL	<MQL
Valsartan	<MQL	1	12–14	5–6	20–27	34–133
Amitriptyline[a]	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Simvastatin[a]	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL

[a] Results semi-quantitative.

4. Conclusions

A new multi-residue method was developed for environmental monitoring of 26 basic/neutral pharmaceuticals (antiepileptics, antibacterial drugs, β-blockers, analgesics, lipid-regulating agents, bronchodilators, histamine-2-blockers, anti-inflammatory agents, calcium channel blockers, angiotensin-II antagonists and antidepressants), illicit drugs and their metabolites in the low nanogram per litre range. The method involved solid-phase extraction with the usage of strong cation-exchange mixed-mode polymeric sorbent (Oasis MCX, 60 mg) and subsequent ultra performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry. The usage of the novel ultra performance liquid chromatography system with 1.7 μm particle size and 1 mm internal diameter column allowed for the application of low mobile phase flow rates (0.07 mL min^{-1}) and short retention times for all compounds analysed (from 1.3 to 15.5 min). As a result, a fast and cost-effective method was developed. A high speed of analysis and low mobile phase flow rates enabling direct introduction of analytes into the ion source from LC without splitting, combined with a high sensitivity, are some of the main advantages of the method when compared to other multi-residue methods using high-performance liquid chromatography–tandem mass spectrometry.

High recovery and reproducibility for MCX adsorbent was obtained for many of the pharmaceuticals studied. The effects of signal suppression and low SPE recovery, both resulting from the presence of matrix interferences, were found to be the main factors affecting the sensitivity of the established analytical method. Surrogate/internal standard was therefore added to the sample so as to compensate for losses of compounds during both the sample preparation procedure and resulting from matrix-assisted suppression.

The mean correlation coefficients (R^2) of the calibration curves, which are higher than 0.996 in both HQ water and BB surface water showed good linearity of the method in the range of 0–1200 ng L^{-1}. The instrumental limits of quantification varied from 0.2 μg L^{-1} for cocaine, benzoylecgonine, metoprolol, propanolol and carbamazepine to 10 μg L^{-1} for amoxicillin. The method limits of quantification were at low nanogram per litre levels and ranged from 0.3 ng L^{-1} for cocaine to 50 ng L^{-1} for simvastatin, which makes the method useful for the determination of very low levels of pharmaceuticals in the aqueous environment such as surface water. The instrumental and method inter-day and intra-day repeatabilities were on average less than 10%.

The method was applied for the analysis of chosen pharmaceuticals and illicit drugs in surface water in the UK and Poland. The results confirmed its applicability in environmental moni-

toring. Fifteen compounds were determined in river water at the levels ranging from single nanograms to single micrograms per litre. The highest concentrations were determined in river water samples collected after wastewater plants for the following pharmaceuticals: paracetamol, amoxicillin and carbamazepine.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2007.05.074.

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