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Serial mixed-mode cation- and anion-exchange solid-phase extraction for separation of basic, neutral and acidic pharmaceuticals in wastewater and analysis by high-performance liquid chromatography–quadrupole time-of-flight mass spectrometry

Martin Lavén[a], Tomas Alsberg[a,*], Yong Yu[b], Margaretha Adolfsson-Erici[a], Hongwen Sun[b]

[a] Department of Applied Environmental Science, Stockholm University, SE-106 91 Stockholm, Sweden
[b] Key Laboratory of Environmental Pollution Process and Standard of Ministry of Education, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China

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ABSTRACT

A novel solid-phase extraction (SPE) method is presented whereby 15 basic, neutral and acidic pharmaceuticals in wastewater were simultaneously extracted and subsequently separated into different fractions. This was achieved using mixed-mode cation- and anion-exchange SPE (Oasis MCX and MAX) in series. Analysis was performed by high-performance liquid chromatography–quadrupole time-of-flight mass spectrometry (HPLC/QTOF-MS). A fast separation was achieved, with all compounds eluting within 6 min, narrow chromatographic peaks, with a peak base width of 6 s on average, and a high mass accuracy of quantified wastewater sample ions, with average mass errors in absolute value of 0.7 mDa or 2.7 ppm. The recovery of the SPE method in the analysis of sewage treatment plant (STP) influent and effluent wastewater was on average 80% and the ion suppression 30%. For less demanding samples Oasis MCX used alone may be an alternative method, although for STP influent waters containing high loads of organic compounds the clean-up achieved using only Oasis MCX was insufficient, leading to unreliable quantitation. Furthermore, serial SPE separation according to molecular charge added an additional degree of analyte confirmation. For quantitation, an approach combining external standard calibration curves, isotopically labelled surrogate standards and single-point standard addition was used. The applicability of the method was demonstrated in the analysis of influent and effluent wastewater from an STP, using small sample volumes (25–50 mL). The effluent wastewater had been subjected to three different treatments; activated sludge, activated sludge followed by ozonation, and a membrane bioreactor (MBR). Ozone treatment proved superior in removal of the analysed pharmaceuticals, while the MBR provided higher removal efficiencies than the activated sludge process.

1. Introduction

The occurrence of pharmaceutical residues in the aquatic environment is an important environmental issue [1]. Since pharmaceuticals are designed to elicit biological responses these can pose a threat to organisms in the environment. Pharmaceuticals and their metabolites are introduced into the aquatic environment mainly via STPs and have been detected in effluents of these, in surface waters and even in ground- and drinking water [2–4]. Although these substances are generally detected at low concentrations in water (ng/L–μg/L), their continuous infusion may lead to adverse effects to aquatic life. Chronic toxic effects may occur, but have been difficult to assess due to lack of ecotoxicity data [5]. Unlike in the case of endocrine disrupting substances, no sensitive biomarkers are available to detect adverse effects of pharmaceuticals. However, it has for instance been shown that prolonged exposure to diclofenac [6] and gemfibrozil [7] causes toxic effects in fish and also that these pharmaceuticals tend to bioaccumulate.

In order to expand current knowledge on the environmental occurrence and effects of pharmaceutical residues there is a need for sensitive and accurate analytical methods for their determination. Due to the low concentrations of pharmaceuticals in the aquatic environment and to the complex nature of the sample matrix, e.g. wastewater, the sample needs to be processed by a clean-up and preconcentration step prior to further analysis. This step is often performed by SPE. Multi-residue SPE methods typi-

* Corresponding author. Tel.: +46 8 674 71 70; fax: +46 8 674 76 37.
E-mail address: tomas.alsberg@itm.su.se (T. Alsberg).

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cally employ polymeric phases, such as Isolute ENV+ and Waters Oasis HLB, and can achieve high recoveries for the simultaneous extraction of a great number of pharmaceuticals belonging to different therapeutic classes [8,34,35,36]. However, the lack of extraction specificity may result in large quantities of co-extracted compounds from the matrix. Other strategies include the use of mixed-mode phases. A mixed-mode cation-exchange sorbent (Oasis MCX) has for instance been used for analysis of β-blockers and β_2-agonists [9] and other pharmaceuticals [10] in wastewater. Acidic pharmaceuticals have been extracted from wastewater employing a mixed-mode anion-exchange sorbent (Oasis MAX) [11]. Furthermore, a mixed-mode weak cation-exchange sorbent (Oasis WCX) has been used for extraction of the amphoteric antibiotic ofloxacin from sewage water, followed by further purification using a mixed-mode anion-exchange sorbent [12]. The extraction/clean-up scheme presented here was designed to maximize the extraction of target analytes by the use of mixed-mode anion- and cation-exchange sorbents, and minimize matrix effects by separating the basic, acidic and neutral substances into different fractions, thus decreasing the extract complexity.

Methods designed for the determination of pharmaceuticals in wastewater have employed GC, LC and CE separation techniques coupled to MS [8,13,14]. LC/MS, using electrospray ionisation (ESI), has been frequently used in multi-residue methods, since sensitive detection, and fast and efficient separation, of a broad range of compounds can be achieved. However, matrix effects [15] have to be taken into consideration. In reversed phase LC, chromatographic separation is generally achieved by columns with particle size ranging from 3 to 5 μm. With the emergence of commercially available LC columns packed with sub 2 μm (d_p) particles and LC systems capable of handling pressures up to 15,000 psi, increased speed and efficiency can be attained [16].

Triple quadrupole mass analysers are the most commonly used MS instruments, providing sensitive detection in multiple reaction monitoring (MRM) mode of target analytes. Nevertheless, in recent years the application of hybrid mass spectrometric techniques, such as QTOF are being more widely employed due to their ability to provide accurate mass measurements [17–21]. An additional advantage of using QTOF instruments is that full scan data is obtained, providing structural information that is lost with QqQ equipments when operating in the MRM mode. Recently, studies have been published where QTOF instruments are employed for pharmaceutical residue analysis [17–21]. Besides providing exact mass measurements, high sensitivity is achieved, since all masses are sampled simultaneously. Additionally, fragmentation of selected ions can be obtained by isolation of precursor ions with the quadrupole mass filter. There are, however, also some disadvantages with using QTOF that need to be taken into consideration. The sensitivity is lower, and the dynamic range not as wide as that of triple-quadrupoles [18,19]. Therefore, the analytical scheme needs to be designed accordingly. However, with the development of technical improvements these shortcomings may be eliminated. In this work, instrument detection limits and dynamic range are reported for the target analytes.

The objective of this study was to develop an analytical method capable of extracting a broad range of pharmaceutical compounds (Table 1) from wastewater for subsequent analysis by HPLC/QTOF-MS. Combining mixed-mode cationic and anionic sorbents could increase the number of extractable pharmaceuticals from wastewater and enable separation of bases, acids and neutrals in the extraction step. The separation should result in fractions of lower complexity, and hence reduced matrix effects e.g. ion suppression. Furthermore, since these three groups are isolated in separate fractions, enrichment factors, i.e. end volumes, can be optimised for each compound group. To the authors' knowledge, this approach has not been previously published for multi-residue analysis of pharmaceuticals in wastewater and the idea was therefore explored in the present study. Precision, recovery and ion suppression of the method were studied, and also compared to using MCX alone, which resulted in only two fractions for analysis, and considered as a potential alternative to the more elaborate MCX/MAX method. The applicability of the latter method was demonstrated in the analysis of influent and three types of treated wastewater; activated sludge treated, activated sludge plus ozone treated and membrane bioreactor (MBR) wastewater obtained from a STP.

2. Experimental

2.1. Chemicals and materials

Acetonitrile (LiChrosolv), acetic acid (analytical reagent grade) and formic acid (analytical reagent grade) were obtained from Merck (Darmstadt, Germany) and methanol (Hipersolv) from BDH Chemicals (Poole, UK). Hydrochloric acid was purchased from J.T. Baker (Phillipsburg, NJ, USA) and ammonium hydroxide solution (25%) from Fluka (Steinheim, Germany).

Atenolol, carbamazepine and cyclophosphamide (monohydrate), enalapril (maleate salt), gemfibrozil, hydrochlorothiazide, ibuprofen and metoprolol (tartrate salt), paracetamol (acetaminophen), propranolol (hydrochloride), ranitidine (hydrochloride), sulfadimethoxine and terbutaline (hemisulfate salt) (Table 1) were purchased from Sigma (St. Louis, MO, USA). Ketoprofen was obtained from Riedel de Haen (Seelze, Germany) and naproxen from Fluka (Steinheim, Germany).

[$^2H_5$]Oxazepam (99% purity) was purchased from Isotec/Sigma–Aldrich (St. Louis, MO, USA). [$^2H_{10}$]Carbamazepine (98.2 atom% ^2H), [$^2H_3$]ibuprofen (99.4% atom% ^2H) and [$^2H_3$]paracetamol (99.1% atom% ^2H) were obtained from CDN Isotopes (Pointe-Claire, Quebec, Canada).

Glassmicrofibre filters (GF/D) were obtained from Whatman (Maidstone, UK). Oasis MCX and MAX SPE columns (60 mg, 30 μm, 3 mL) were purchased from Waters (Milford, MA, USA).

2.2. Sampling

24-Hour composite samples were collected during 4 days from Hammarby Sjöstad STP, Stockholm, Sweden. The influent water was treated by a conventional activated sludge process, using a sludge residence time of 5 days, followed by sand filtration. Additionally, a fraction of the biologically treated effluent water was passed through an ozone treatment step, using 15 g O_3/m^3 of wastewater. In a separate treatment process, influent water was transferred to a membrane bioreactor (MBR) (Kubota Submerged Membrane Unit), in parallel to the activated sludge treatment. Samples were collected from the influent stream, from wastewater treated by activated sludge and a final sand filter (denominated effluent water in this study), from ozone treated effluent and from the MBR. The samples, collected in plastic (polyethylene) bottles, were immediately frozen and stored at −20 °C until further analysis. When thawed, samples collected from four different days were combined into one pooled sample.

2.3. Sample extraction and clean-up

Wastewater samples were initially passed through a glassmicrofibre filter (GF/D, Whatman) to remove any particulate matter. An aliquot of 50 mL was used for the analysis of effluent, ozone and MBR treated wastewater, whereas 25 mL of wastewater was sufficient for the analysis of influent water. Additionally, 25 mL of H_2O was added to influent water samples. The sample was adjusted to

Table 1
Analysed pharmaceuticals.

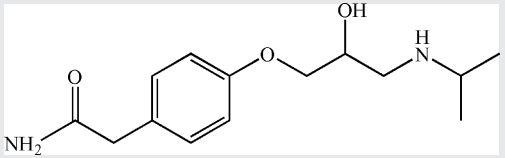
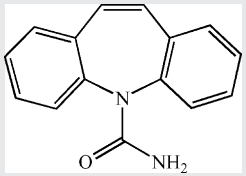
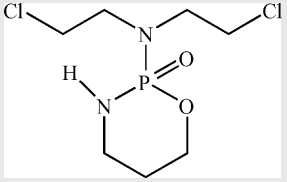
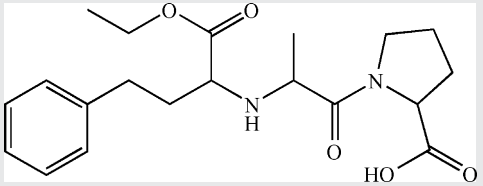
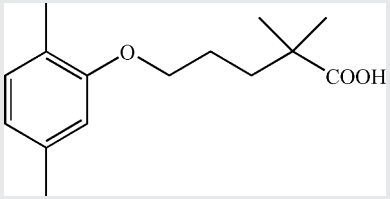
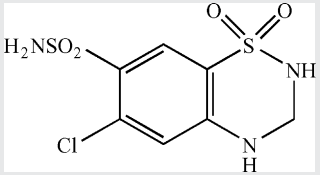
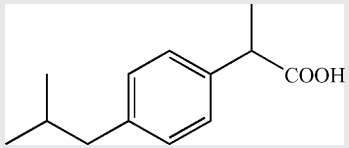
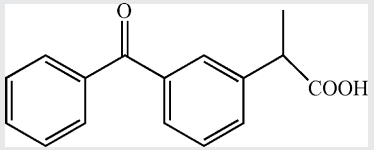
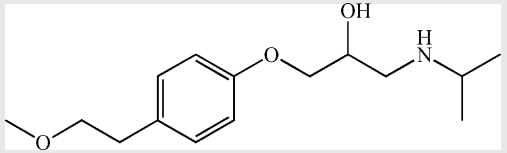
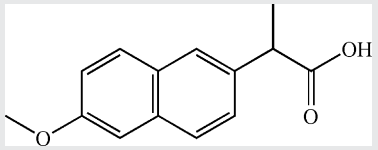
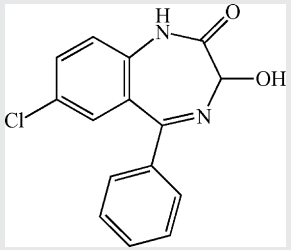
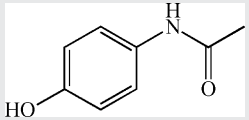
Compound	Type of compound	Structural formula	pK_a[a]	log D; pH 2[a]	log D; pH 7[a]	log D; pH 10[a]	Elemental composition (quasi-molecular and product ion)	Mass (m/z)[b]
Atenolol	β-Blocker		9.2	−3.00	−2.02	0.04	$[M+H]^+$	
							$C_{14}H_{23}N_2O_3$	267.1709
							$C_{10}H_9O$	145.0653
Carbamazepine	Anti-epileptic		13.9	2.67	2.67	2.67	$[M+H]^+$	
							$C_{15}H_{13}N_2O$	237.1028
							$C_{14}H_{12}N$	194.0970
Cyclophosphamide	Chemo-therapeutic		2.8	−0.64	0.23	0.23	$[M+H]^+$	
							$C_7H_{16}N_2O_2Cl_2P$	261.0326
							$C_4H_8NCl_2$	140.0034
Enalapril	Blood pressure regulator		3.2	−0.26	−0.72	−1.32	$[M+H]^+$	
			5.4				$C_{20}H_{29}N_2O_5$	377.2076
							$C_{14}H_{20}NO_2$	234.1494
Gemfibrozil	Lipid regulator		4.8	4.38	2.15	0.65	$[M-H]^-$	
							$C_{15}H_{21}O_3$	249.1491
							C_8H_9O	121.0653
Hydrochlorothiazide	Blood pressure regulator		9.0	−0.07	−0.08	−1.68	$[M-H]^-$	

Table 1 (*Continued*)

Compound	Type of compound	Structural formula	pK_a[a]	log *D*; pH 2[a]	log *D*; pH 7[a]	log *D*; pH 10[a]	Elemental composition (quasi-molecular and product ion)	Mass (*m*/*z*)[b]
							$C_7H_7N_3O_4S_2Cl$	295.9567
							$C_6H_6N_2O_2SCl$	204.9839
Ibuprofen	NSAID[c]		4.4	3.72	1.16	−0.02	$[M-H]^-$	
							$C_{13}H_{17}O_2$	205.1229
							$C_{12}H_{17}$	161.1330
Ketoprofen	NSAID[c]		4.2	2.81	0.09	−0.93	$[M+H]^+$	
							$C_{16}H_{15}O_3$	255.1021
							$C_{15}H_{13}O$	209.0966
Metoprolol	β-blocker		9.2	−1.31	−0.33	1.73	$[M+H]^+$	
							$C_{15}H_{26}NO_3$	268.1913
							$C_6H_{14}NO$	116.1075
Naproxen	NSAID[c]		4.8	3.00	0.85	−0.73	$[M-H]^-$	
							$C_{14}H_{13}O_3$	229.0865
							$C_{13}H_{13}O$	185.0966
Oxazepam	Sedative		1.7	2.14	2.31	2.25	$[M+H]^+$	
			10.9				$C_{15}H_{12}N_2O_2Cl$	287.0587
							$C_{14}H_{10}N_2Cl$	241.0533
Paracetamol	Analgesic		1.7	0.16	0.34	−0.04	$[M+H]^+$	

Propranolol	β-blocker	9.9				$C_8H_{10}NO_2$	[M+H]$^+$	152.0712	
		9.1	0.00	1.00	3.04	C_6H_8NO		110.0606	
Ranitidine	Anti-ulcer	8.4	−2.34	−0.18	1.22	$C_{16}H_{22}NO_2$	[M+H]$^+$	260.1651	
						$C_6H_{14}NO$		116.1075	
						$C_{13}H_{23}N_4O_3S$	[M+H]$^+$	315.1491	
						$C_5H_{10}N_3O_2S$		176.0494	
Terbutaline	Broncho-dilator	9.3	−2.62	−1.67	−0.47	$C_{12}H_{20}NO_3$	[M+H]$^+$	226.1443	
						$C_8H_{10}NO_2$		152.0712	

[a] Data extracted from Scifinder Scholar Version 2006.
[b] Masses (m/z) calculated using MassLynx v. 4.1.
[c] Non-steroidal anti-inflammatory drug.

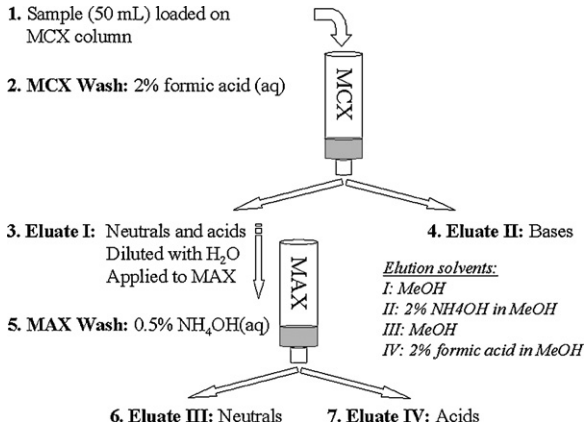


Fig. 1. Outline of the SPE procedure following an initial conditioning step.

pH 2 with HCl (37%), and surrogate standard and spiking solutions were added prior to the SPE step.

An outline of the SPE procedure is given in Fig. 1. For liquid handling, a vacuum manifold (Argonaut Technologies/Biotage, Hengoed, UK) was used. An MCX column was initially conditioned with 2 mL of methanol and 2 mL of 2% formic acid (aq.), followed by loading of the sample (50 mL). A wash solution of 2 mL 2% formic acid (aq.) was thereafter applied, in order to rinse the column free of the last remaining sample solution prior to elution. Neutrals and acids were eluted with 2 mL methanol (eluate I) and bases with 2 mL 2% ammonium hydroxide in methanol (eluate II). Eluate I was diluted with 40 mL H_2O and applied to a MAX column which had previously been conditioned with 2 mL of methanol followed by 2 mL H_2O. A wash solution of 2 mL 0.5% ammonium hydroxide (aq.) was thereafter applied. The column was dried for approximately 5 min, after which neutrals were eluted with 2 mL methanol (eluate III) and acids with 2 mL 2% formic acid in methanol (eluate IV).

Influent and effluent samples were additionally processed using Oasis MCX alone. Two fractions were collected from the MCX column following the same scheme that was developed for the serial MCX/MAX setup, but omitting the fractionation on MAX.

SPE eluates were evaporated to dryness under a gentle stream of nitrogen, in a heating block kept at 35 °C, and redissolved in 500 μL of 20% acetonitrile, 0.1% formic acid, prior to LC–MS analysis.

2.4. HPLC/QTOF-MS

A Waters ultra-performance liquid chromatography (UPLC) system was used for injection of samples and pumping of the mobile phase. The column, Waters Acquity HSS T3 (100 mm × 2.1 mm, d_p 1.8 μm), was kept at ambient temperature, while sample vials were kept at 20 °C. An injection volume of 5 μL was used. The mobile phase consisted of 10 mM acetic acid in water (A) and 10 mM acetic acid in acetonitrile (B). The system was programmed to deliver a linear gradient with an initial composition of 5% B in A to 90% B in A, during 5 min. This composition was held for 2 min and thereafter returned to 5% B in A. The total run-time was 10 min and the flow rate 0.3 mL/min.

A Waters Micromass QTOF Premier mass spectrometer was coupled to the LC system. It was operated in ESI positive and negative ion mode with the TOF detector in 'V-mode'. The quadrupole was set to a wide pass mode and the collision energy was alternated between 2 and 20 eV, using one MS scan function for each collision energy. The scan time of each MS scan function was 0.2 s and the

inter-scan time 0.01 s. The following settings were used in positive and negative ion mode, respectively: capillary voltage 3.0/2.0 kV; sampling cone voltage 25 V; extraction cone voltage 4.0/2.9 V; source temperature 100 °C, desolvation temperature 300/250 °C; cone gas (nitrogen) flow 50/25 L/h and desolvation gas (nitrogen) flow 700 L/h. Argon was used as collision gas, at a pressure of 3.5×10^{-3} mbar. External mass calibration was performed in the mass range m/z 100–1000, using 0.05 M NaOH and 0.5% formic acid dissolved in 2-propanol/H_2O (90:10). Sulfadimethoxine (m/z 309.0658 and 311.0814) in methanol (0.1 ng/μL) was used as a lockspray solution in both positive and negative ion mode. The lockspray frequency was 5 s, with 5 scans to average. In calculations of the theoretical mass of ions, the software (MassLynx v. 4.1) did not take into consideration the mass of the additional, or deficient, electron (0.55 mDa) giving rise to the charge of the ion (Table 1) [22]. Thus, the mass of an electron was not added to negatively charged species, nor was it subtracted from positively charged ions. However, this did not affect the mass accuracy, since the masses of ions used in the external and lockspray calibration were calculated in the same manner.

2.5. Quantitation

The quantitation protocols differs for the different analytes, depending on whether matching deuterated surrogate standards were available. Hence, the analytes are divided into two groups.

Group 1: Analytes for which deuterated analogues were available, i.e. carbamazepine ([$^2H_{10}$]carbamazepine), ibuprofen ([$^2H_3$] ibuprofen), oxazepam ([$^2H_5$]oxazepam) and paracetamol ([$^2H_3$]paracetamol).

Group 2: Analytes for which deuterated analogues were lacking, i.e. atenolol, enalapril, cyclophosphamide, gemfibrozil, hydrochlorothiazide, ketoprofen, metoprolol, naproxen, propranolol, ranitidine and terbutaline.

For determination of *Group 1* analytes, the deuterated surrogates were utilized. Known amounts of deuterated surrogate standards were thus added to the samples, prior to SPE extraction. Additionally, constant concentrations of deuterated surogates were added to the calibration standards, which also contained different concentrations of the non-deuterated analogues. Using the QuanLynx software of MassLynx 4.1 (Waters, Manchester, UK), calibration curves were constructed, where the area ratios between the non-deuterated and the deuterated standards were plotted versus the concentration of the non-deuterated analyte. The calibration curves were subsequently used by the software to calculate the analyte concentration in the sample extracts, from the area ratio between non-deuterated analyte and the respective deuterated surrogate and the concentration of the respective surrogate, in the extracts.

Concentrations of deuterated surrogate standards, in calibration solutions, ranged from 30–100 μg/L. The choice of surrogate concentrations in non-extracted samples were both dependent on type of analyte and wastewater analysed, and ranged from 0.3 to 50 μg/L.

Quantitation of *Group 2* analytes was performed in two steps. In the first step, the "apparent concentration" of each analyte was determined. This was done by external calibration, using standard curves made from analysis of standards dissolved in pure solvent. The term "apparent concentration" is used to emphasize that neither recovery nor matrix effects, e.g. ion suppression, was accounted for in the first step of the quantitation.

Separate stock solutions of each analyte were prepared in g/L quantities, dissolved in methanol and stored at −20 °C. Calibration standards, dissolved in 20% acetonitrile, 0.1% formic acid, were prepared for each day of analysis from stock solutions. Concentrations of external standard calibration curves ranged from 0.48 to 250 μg/L, depending on the analyte, and 4–6 calibration standard concentrations were used. External calibrations were performed using the QuanLynx software, employing weighted ($1/x$) linear regression. A 30 mDa mass window was employed to construct chromatograms of $[M+H]^+$ or $[M-H]^-$ ions (Table 1). In the case of naproxen, the fragment $C_{13}H_{13}O$ (m/z 185.0966), resulting from a CO_2 loss, was used for quantification.

The second step of the quantitation of the *Group 2* analytes involved the use of a one-point standard addition calibrator sample, in order to compensate for losses during clean-up as well as for matrix effects. For each wastewater sample, two aliquots were analysed, one of which (the calibrator sample) was spiked prior to the SPE step with a standard solution containing a known amount of each analyte. The "true" analyte concentration in the non-spiked sample was calculated by dividing the "apparent concentration" with the method efficiency (Eq. (1)). The method efficiency was determined according to Eq. (2)., as the ratio between the difference between "apparent concentrations" in the pre-spiked and the non-spiked sample aliquots, and the known concentration added to the calibrator sample.

$$\text{"true" concentration} = \frac{C_{\text{Non-Spiked}}}{\text{method efficiency}} \quad (1)$$

$$\text{method efficiency} = \frac{C_{\text{Pre-Extr}} - C_{\text{Non-Spiked}}}{C_{\text{Spike}}} \quad (2)$$

where $C_{\text{Non-Spiked}}$ and $C_{\text{Pre-Extr}}$, denote "apparent concentration" in the non-spiked and the calibrator samples, respectively, and C_{Spike} denote the known concentration added to the calibrator sample (as determined from the measured concentration of the spiking solution).

2.6. Method performance

The recovery and ion suppression of the method were studied by an experimental set-up using the following samples, in triplicates: Non-spiked wastewater (Non-Spiked), Wastewater spiked prior to extraction (Pre-Extr), Wastewater spiked after extraction in the reconstitution step (Post-Extr) and Spiking solution (Sp-Sol). The recovery was calculated from the "apparent" analyte concentration of samples spiked prior to and after extraction, using the following equation:

$$\text{recovery} = \frac{C_{\text{Pre-Extr}} - C_{\text{Non-Spiked}}}{C_{\text{Post-Extr}} - C_{\text{Non-Spiked}}} \times 100 \quad (3)$$

where C denotes the "apparent concentration". Since the samples already contained a number of analytes, the native contribution to the "apparent concentration" of spiked samples was subtracted. In the case of group 1 analytes, the deuterated analogues were used as spikes. Ion suppression was calculated using the following equation:

$$\text{ion suppression}\,(\%) = 1 - \frac{C_{\text{Post-Extr}} - C_{\text{Non-Spiked}}}{C_{\text{Sp-Sol}}} \times 100 \quad (4)$$

The instrumental detection limit (IDL) was set as a signal-to-noise (S/N) ratio of 3, obtained from serial dilution of standards. The lower limit of quantitation (LLOQ) of the method was determined by calculating S/N ratios of each compound from authentic wastewater samples. By extrapolation to a S/N ratio of 10, LLOQ concentrations were determined. The method detection limit (MDL) was determined in the same way as the LLOQ, but with a S/N ratio of 3 instead of 10.

Table 2
Recovery, ion suppression and precision using Oasis MCX/MAX columns in analysis of spiked influent wastewater (25 mL).

Compound	SPE fraction	Concentration of spike (μg/L)	Recovery[a] (%)	Ion suppression[a] (%)	Intra-day precision of determination (RSD%)[b]	Intra-day precision of recovery (RSD%)[b]	Dilution[c] (number)
Atenolol	Basic	2.5	94	43	1.1	1.1	3
Carbamazepine	Neutral	0.73	71	5	3.3	4.8	5
Cyclophosphamide	Neutral	0.39	87	30	2.0	1.7	0
Enalapril	Basic	0.29	87	29	3.6	3.6	0
Gemfibrozil	Acidic	1.2	79	74	6.8	8.4	0
Hydrochlorothiazide	Acidic	1.1	85	85	5.7	6.8	0
Ibuprofen	Acidic	6.0	88	66	1.8	3.5	0
Ketoprofen	Acidic	4.0	85	21	3.4	3.7	5
Metoprolol	Basic	1.1	101	20	1.2	1.3	3
Naproxen	Acidic	10	87	77	3.5	5.1	0
Oxazepam	Basic	0.50	89	7	7.8	5.4	0
Paracetamol	Neutral	51	11	−4	4.2	2.7	5
Propranolol	Basic	0.31	102	35	2.7	0.85	0
Ranitidine	Basic	0.69	98	49	5.8	8.3	0
Terbutaline	Basic	0.50	92	53	4.1	2.5	0

[a] See Section 2.6 for calculations.
[b] Determined using Oasis MCX/MAX in the extraction of three different spiked influent samples.
[c] Number of times that the final redissolved extract was diluted from 0.5 mL to fit the calibration curve.

3. Results and discussion

3.1. Stability of analytes

The stability of analyte stock solutions, with methanol as solvent, stored at −20 °C, was studied for two months (oxazepam), three months (carbamazepine, cyclophosphamide, gemfibrozil, hydrochlorothiazide, ibuprofen, ketoprofen, naproxen, paracetamol and propranolol) and four months (atenolol, enalapril, metoprolol, ranitidine and terbutaline). No analyte degradation was detected during this time period (<4%).

3.2. SPE set-up

Mixed-mode cation- and anion-exchange SPE columns were employed in conjunction in the extraction method (Fig. 1). In this set-up, all analytes were initially extracted on the mixed-mode cation-exchange sorbent Oasis MCX. The pH of the sample was adjusted to approximately 2, in order to protonate basic analytes for maximum ionic interaction with sulfonic acid moieties of the Oasis MCX sorbent. In an initial experiment, the pH was unadjusted, which lead to poor recoveries of bases, particularly for ranitidine. It has previously been reported to be difficult to achieve a high recovery of ranitidine by extraction using Oasis HLB at pH 7 [23]. Additionally, acidic analytes were mainly protonated at pH 2, hereby increasing retention by reversed phase interactions, while neutral pharmaceuticals were retained by the same mechanism. Acidic and neutral pharmaceuticals were subsequently eluted from the Oasis MCX sorbent using methanol (eluate I). Basic analytes were deprotonated using ammonium hydroxide in methanol and eluted into a separate fraction (eluate II). As a next step, the eluate containing acidic and neutral analytes (eluate I) was diluted with H_2O and loaded onto an Oasis MAX column. A wash solution of ammonium hydroxide (aq.) was applied to deprotonate acids, thereby increasing ionic interactions with quaternary amine groups of the Oasis MAX sorbent. Neutral compounds were thereafter eluted with methanol (eluate III). Acidic substances were protonated using formic acid in methanol and eluted into a separate fraction (eluate IV).

An advantage of employing dual mixed-mode columns, which enabled separation of acids, bases and neutrals, was an additional degree of analyte confirmation. Analyte confirmation was thus achieved by: (1) SPE separation, relying on ion-exchange and reversed-phase mechanisms, and (2) LC separation, using reversed-phase mechanisms, and the subsequent matching of fraction and retention times with standards, followed by (3) MS separation,

Table 3
Recovery, ion suppression and precision using Oasis MCX/MAX columns in analysis of spiked effluent wastewater (50 mL).

Compound	SPE fraction	Concentration of spike (μg/L)	Recovery[a] (%)	Ion suppression[a] (%)	Intra-day precision of determination (RSD%)[b]	Intra-day precision of recovery (RSD%)[b]
Atenolol	Basic	0.68	100	44	0.6	1.5
Carbamazepine	Neutral	0.31	93	18	1.0	2.3
Cyclophosphamide	Neutral	0.30	92	2	2.0	1.8
Enalapril	Basic	0.29	98	−3	2.8	2.8
Gemfibrozil	Acidic	0.30	61	14	0.7	0.6
Hydrochlorothiazide	Acidic	0.80	141	75	11	36
Ibuprofen	Acidic	1.0	53	13	7.2	8.1
Ketoprofen	Acidic	0.30	45	35	14	0.33
Metoprolol[c]	Basic	0.80	82	−7	3.6	8.7
Naproxen	Acidic	0.25	44	41	6.5	6.4
Oxazepam	Basic	0.50	89	16	3.2	2.9
Paracetamol	Neutral	1.2	26	10	9.6	2.5
Propranolol	Basic	0.15	98	19	1.2	1.8
Ranitidine	Basic	0.30	84	43	9.0	14
Terbutaline	Basic	0.25	96	29	2.7	2.5

[a] See Section 2.6 for calculations.
[b] Determined using Oasis MCX/MAX in the extraction of three different spiked effluent samples (n = 2 for gemfibrozil and ketoprofen).
[c] Sample extract diluted 5 times.

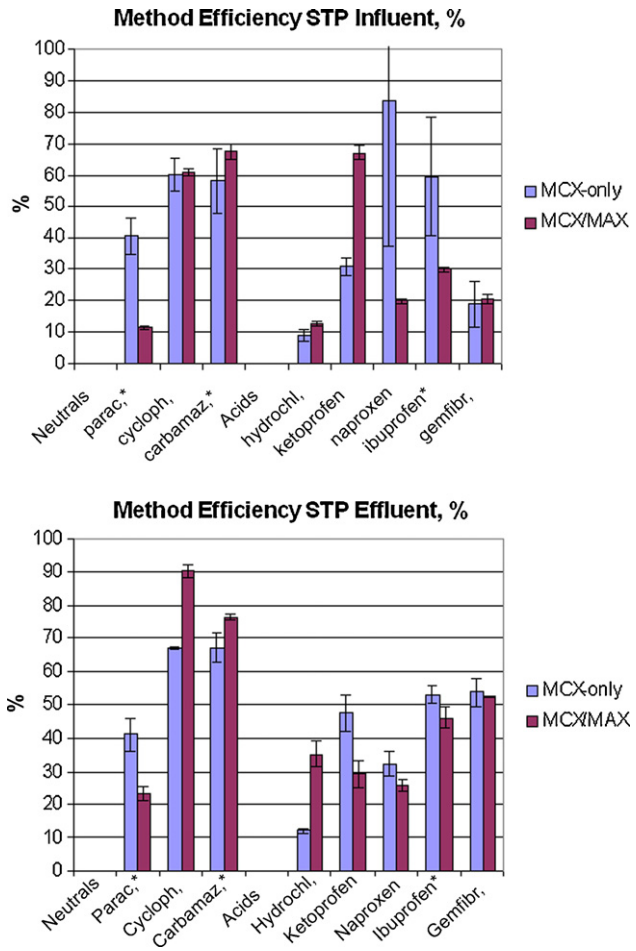


Fig. 2. Method efficiencies obtained from the MCX-alone (Eluate I) and the MCX/MAX method (Eluates III and IV), respectively, for STP influent (upper) and effluent water (lower), respectively. Error bars represent ±1 SD.

according to *m*/*z* ratios and accurate mass determination of quasi-molecular ions and fragments. The employed SPE separation can also be very useful in the characterisation of unknown water contaminants. Additionally, by using Oasis MCX and MAX columns in series, rather than in parallel, no additional sample volume was needed.

3.3. SPE recovery and ion suppression

In this work, an experimental set-up was used to determine both the recovery and ion suppression of the method. The recovery was defined as the recovery of the isolated SPE step and the ion suppression as the relative signal decrease of a wastewater sample spiked after extraction, compared to a standard solution (for calculations see Section 2.6). The occurrence of matrix effects when using ESI-MS in the analysis of wastewater is well documented [23–25]. It is thus not sufficient to determine only the recovery in the development of a method, since ion suppression may reduce the signal and increase detection limits. One important factor in this respect is the concentration factor. The concentration factor should be high enough to enable quantitation of low concentrations, but kept as low as possible in order to minimize matrix effects. In this work we used concentrations factors of 50 and 100, for influent and effluent wastewaters, respectively. If needed, the concentration factor can be easily increased by a factor of five by choosing an end-volume of 100 μL instead of 500 μL, which was used here. An advantage of the serial MCX/MAX method is that the concentration factor can be tailored to fit the different substance classes bases, neutrals, and acids, respectively.

The recovery and ion suppression of the method, when applied to the analysis of pre-spiked influent and effluent wastewater are listed in Tables 2 and 3 The recovery was on average 80% and the ion suppression 30%. The precision of the determination and also of the recovery was satisfactory, with relative standard deviations (RSD) below 10%, with exception for the determination of ketoprofen in influent water, which showed an RSD of 14%, and the recovery of ranitidine from effluent water, for which the recovery showed an RSD of 14%. For some substances, e.g. hydrochlorothiazide, the ion suppression was considerable, i.e. 75 and 85%, for influent and effluent water, respectively. It was therefore important to use a quantitation method that compensated for ion suppression effects (cf. Section 3.6).

3.4. Alternative clean-up schemes

Initially, Oasis MAX and MCX were evaluated on their own with the aim to design a method using a single SPE column for clean-up. However, it was found that recoveries for basic analytes were unsatisfactorily low in the case of Oasis MAX. Oasis MCX, on the other hand, showed acceptable recoveries for all analytes when used with pure standards. Therefore, MCX used alone was also tested as a candidate for clean-up of STP water.

Table 4
Recovery and ion suppression of neutral and acidic analytes, obtained from STP effluent samples subjected to the MCX-only and MCX/MAX methods, respectively.

	Influent				Effluent			
	Recovery (RSD,%)		Ion suppression		Recovery (RSD,%)		Ion Suppression	
	MCX-only	MCX/MAX	MCX-only	MCX/MAX	MCX-only	MCX/MAX	MCX-only	MCX/MAX
Neutrals								
Paracetamol[a]		11 (2.7)		−4		26 (2.5)		10
Cyclophosphamide	89 (4.9)	87 (1.7)	36	30	78 (4.4)	92 (1.8)	23	2
Carbamazepine[a]		71 (4.8)		5		93 (2.3)		18
Acids								
Hydrochlorothiazide	105 (26)	85 (6.8)	91	85	163 (4.3)	141 (36)	93	75
Ketoprofen	76 (10)	85 (3.7)	59	21	79 (7.8)	45 (0.33)	39	35
Naproxen	60 (29)	87 (5.1)	−34	77	78 (33)	44 (6.4)	57	41
Ibuprofen[a]		88 (3.5)		66		53 (8.1)		13
Gemfibrozil	54 (48)	79 (8.4)	62	74	70 (36)	61 (0.6)	22	14
Mean[b]	77	85	62[c]	53	94	77	47	33

[a] For MCX-only, paracetamol, carbamazepine, and ibuprofen, the deuterated analogues were added to all samples. Thus, for these analytes, method efficiency was obtained (Fig. 2), but not recovery or ion suppression separately.
[b] Paracetamol, carbamazepine, and ibuprofen were not included in the mean values.
[c] Naproxen was not included in the mean value.

Hence, influent and effluent STP water samples, in triplicates, were subjected to clean-up by "MCX-only" in order to check the recovery and matrix effects from this less elaborate, compared to the serial MCX/MAX, clean-up scheme. The water samples and sample extracts were spiked with the group 2 analytes, following the spiking procedure used for the serial MCX/MAX method (Section 2.6). Deuterated surrogate standards for determination of group 1 analytes were added to all samples prior to extraction. Thus, only results for method efficiency, and not for recovery and ion suppression separately were obtained for group 1 analytes in this experiment. Two fractions were collected from the MCX column following the same scheme that was developed for the serial MCX/MAX setup, i.e. eluate I containing neutral and acidic compounds and eluate II containing basic compounds.

The results on recovery and ion suppression from the influent and effluent STP waters are seen in Table 4. Since the fraction containing the basic compounds was produced identically in the two methods, only results for the neutral and acidic analytes are shown. The results show that for most analytes in the effluent water, the recovery was higher using MCX-only (mean recovery: 94%) compared to serial MCX/MAX (mean recovery: 77%), whereas the opposite was seen for the influent water (mean recovery MCX-only: 77%; MCX/MAX: 85%). However, except for naproxen and gemfibrozil in the influent water, the ion suppression was more severe for all neutral and acidic analytes when using MCX-only in both influent and effluent. The crucial question was whether the net result of recovery and matrix effects, i.e. the method efficiency, as determined by Eq. (4), was acceptable.

$$\text{method efficiency} = 100 \times [\text{recovery} \times (1 - \text{ion suppression})] \quad (4)$$

In Fig. 2a and b, respectively, the method efficiencies for the neutral and acidic analytes resulting from the MCX-only method and the MCX/MAX method are shown. In the effluent water, for two of the neutral analytes, cyclophosphamide and carbamazepine, the method efficiency was higher when using MCX/MAX, while it was higher for paracetamol using MCX-only. The same trend, although not as pronounced could be seen for the influent water. For the acidic analytes the results were more diversified. Firstly, precision was poor for naproxen, ibuprofen, and gemfibrozil when using MCX-only on influent water, 56, 32 and 39% RSD, respectively, compared to 3.5, 1.8, and 6.8% RSD with MCX/MAX. Secondly, for naproxen and ibuprofen, MCX-only showed higher method efficiencies than MCX/MAX when applied to influent water. In the case of naproxen, the high method efficiency is explained by a negative ion suppression (Table 4), i.e. ion enhancement, which may be due to coeluting substances that promote the ionization efficiency for naproxen.

In conclusion, for less complex samples e.g. the active-sludge-treated effluent water described here, the MCX-alone method may be an alternative to the more elaborate MCX/MAX method. However, it should be emphasized that this is only valid for less complex water samples. In the case of STP influent water, clean-up by "MCX-only" is not recommended, due to the unreliable quantitative results, i.e. the large variations, Fig. 2 and Table 4.

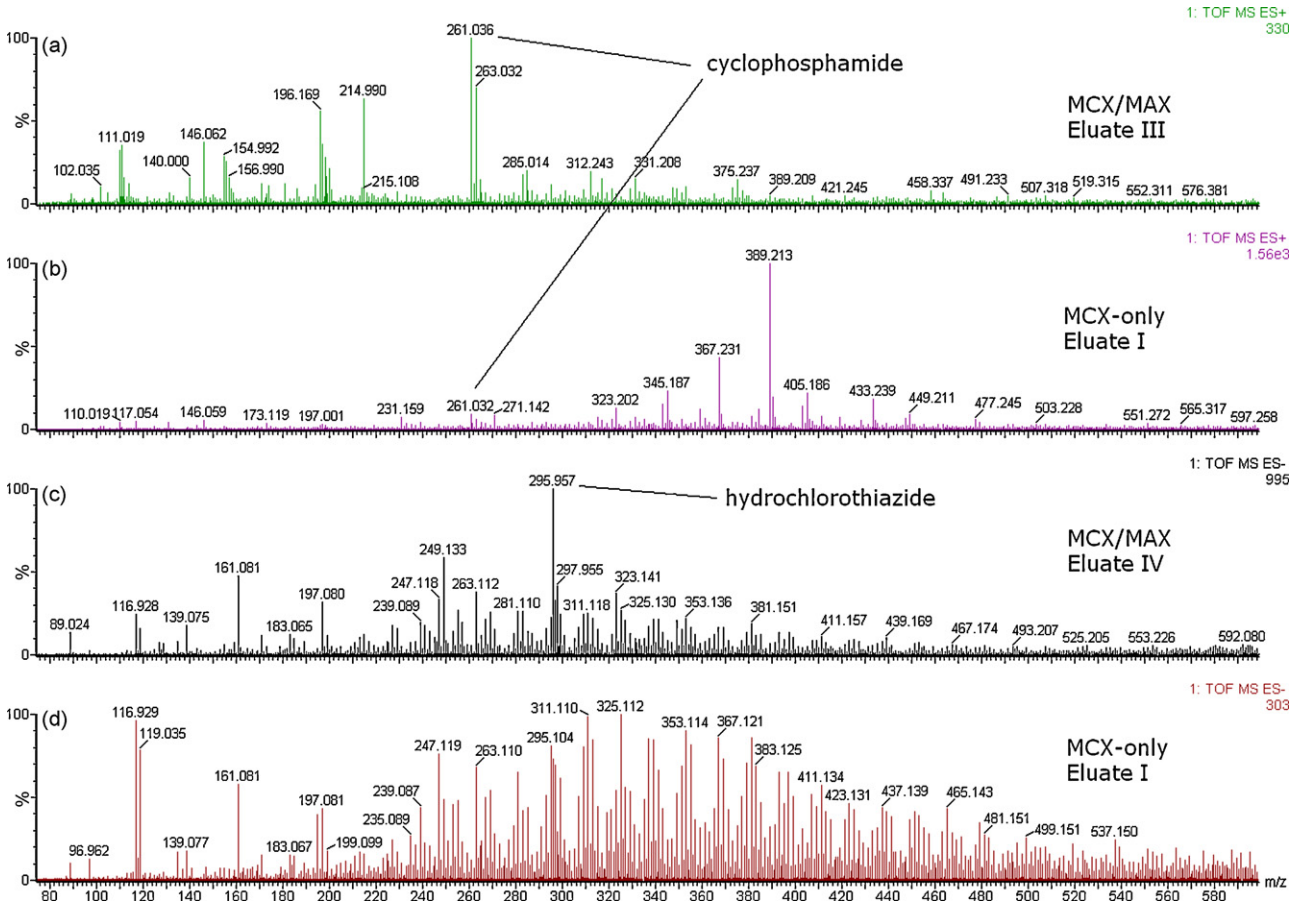


Fig. 3. Low collision energy (2 eV) mass spectra obtained from pre-spiked extracted effluent water from the cyclophosphamide peak, using (a) MCX/MAX Eluate III and (b) MCX-only Eluate I, and from the hydrochlorothiazide peak using (c) MCX/MAX Eluate IV, and (d) MCX-only Eluate I. Spectra were not background-subtracted.

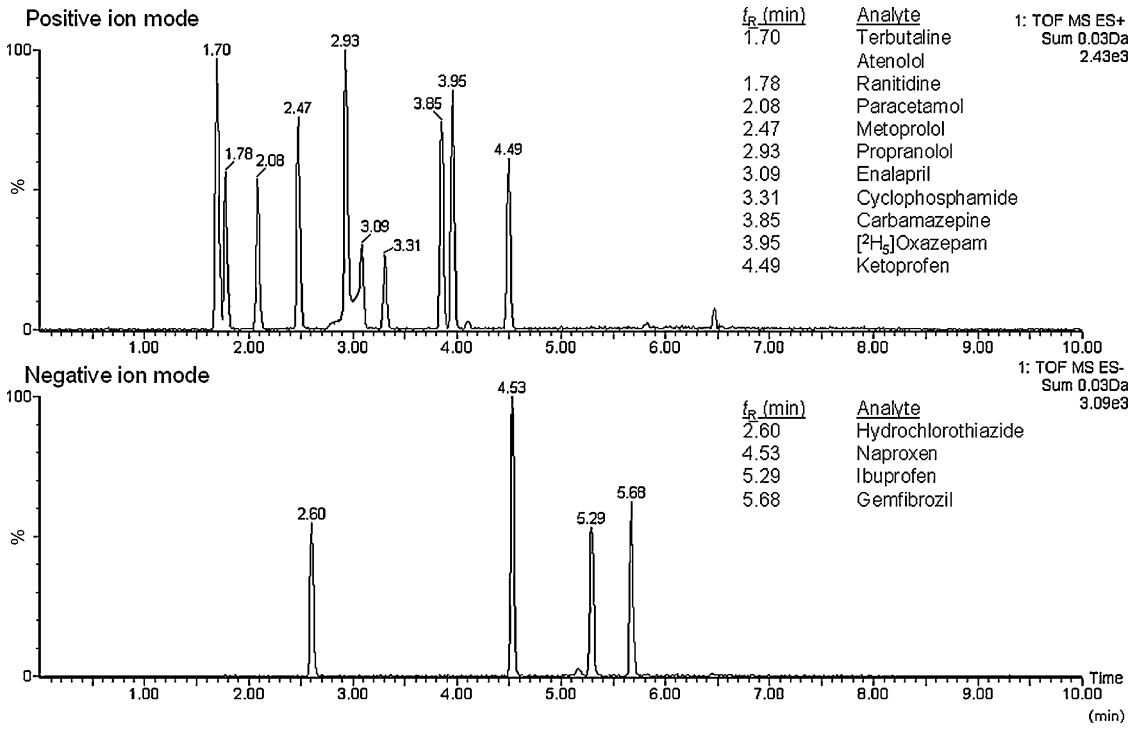


Fig. 4. Extracted, and summed, ion chromatograms of a standard solution obtained in positive and negative ion mode. Standard concentrations (μg/L): terbutaline (32), atenolol (55), ranitidine (81), paracetamol (125), metoprolol (31), propranolol (31), enalapril (37), cyclophosphamide (32), carbamazepine (31), [$^2H_5$]oxazepam (65), ketoprofen (55), hydrochlorothiazide (64), naproxen (111), ibuprofen (126) and gemfibrozil (62).

Additionally, the MCX/MAX method generated mass spectra with a reduced number of interfering ions compared to extraction by MCX-only. This can clearly be seen in Fig. 3, showing that the MCX/MAX method produced significantly "cleaner" mass spectra than that of the MCX-only extraction. The spectra in Fig. 3, taken from the apex of the cyclophosphamide and hydrochlorothiazide peaks, are not background-subtracted, hence, considerably more sample matrix ions, co-eluting with the analyte, are revealed from the MCX-only spectra as compared to the spectra originating from the two MCX/MAX fractions. It is likely that these matrix ions interfere with the ionization of the analytes, which is in accordance with the data presented in Table 4, thus demonstrating the advantage of the MCX/MAX method over the MCX-only method.

3.5. HPLC/QTOF-MS

A fast separation, with all compounds eluting within 6 min, was achieved with the HPLC-system (Fig. 4). Peak widths, measured at the base, were 6 s on average (with the exception of enalapril). The scan time was adjusted to accommodate for the narrow peaks, giving on average 12 scans (of each MS function) per peak, which was adequate for quantitation purposes. The substances were well separated, except atenolol and terbutaline, which were only moderately retained on the RP column in accordance with low log *D* values (Table 1). Enalapril eluted in a broad asymmetric peak, due to the existence of both *cis* and *trans* isomers [26]. Quantitation of this compound could, however, be performed with high precision (Tables 2 and 3).

The mobile phase was optimised to accommodate analysis in both positive and negative ion mode, in order to reduce equilibration and preparation time. Initially, formic acid (0.1%) was explored as a mobile phase modifier, but the response of ibuprofen was very low. Switching to acetic acid (10 mM) resulted in a signal increase for all compounds. However, it was accompanied by an increase in background signal. More importantly, the S/N ratio increased for the acids by on average four times, except for a 20% reduction for ketoprofen. The bases were on average unaffected, whereas the S/N ratio increased by on average 1.2 times for the neutrals.

The quadrupole of the QTOF instrument was set to a wide pass mode with alternating low and high collision energies in the T-wave collision cell, for analyte confirmation purposes, as has previously been described [27,28]. This approach enabled the generation of product ion spectra with a minimum loss of signal intensity. Product ions used for analyte confirmation are listed in Table 1. The spectra from low and high collision energies, respectively, were stored in different data sets by the software in such a way that separate low and high collision energy chromatograms were obtained. In addition, retroactive screening of previously acquired MS-data files of compounds not originally intended for analysis could be carried out. This was possible to perform since no precursor ions were selected and the instrument was operated in full scan mode (*m*/*z* 75–1000). The selectivity of the approach may, however, be reduced compared to a conventional MSMS method, since fragment ions may originate from a number of co-eluting precursor ions, although elution characteristics, i.e. peak shape, can be used to link precursor and product ions. An example of the confirmation approach is given in Fig. 5, with mass spectra from extracted effluent wastewater. In the low collision energy spectrum, the $[M+H]^+$ ions of oxazepam and its deuterated counterpart, [$^2H_5$]oxazepam, can be seen at *m*/*z* 287.0587 and 292.0899, respectively. In the high collision energy spectrum, ions produced by an initial loss of H_2O; *m*/*z* 269.0505 and 274.0813, followed by a loss of CO; *m*/*z* 241.0534 and 246.0854, are displayed [29]. The mass errors of the oxazepam ions in Fig. 5 were ≤1.5 mDa or ≤5.1 ppm. Throughout this study, analysed wastewater samples (non-spiked), with concentrations ranging from 38 ng/L to 84 μg/L, displayed average mass errors in absolute value of 0.7 mDa or 2.7 ppm for quantified ions, and 1.0 mDa or 5.6 ppm for confirmation product ions (Table 6). Detection limits for the target analytes using the HPLC/QTOF system are presented in Table 5.

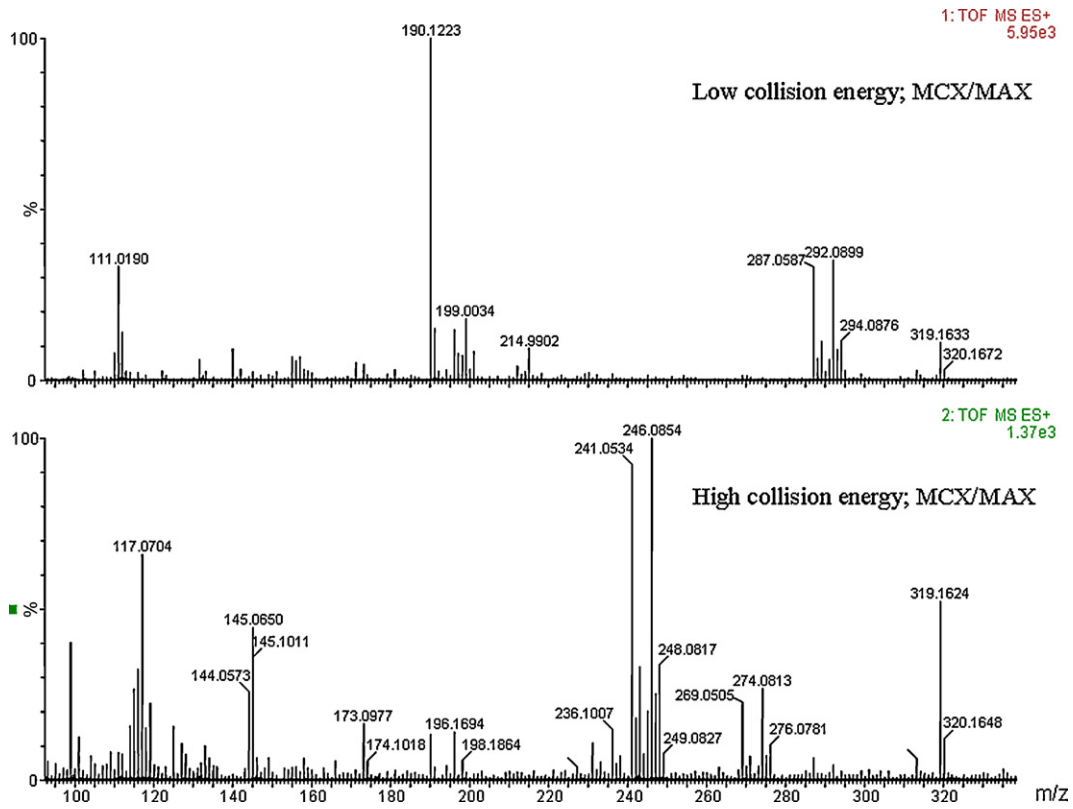


Fig. 5. Low collision energy (2 eV, upper) and high collision energy (20 eV, lower) mass spectra of oxazepam and [$^2H_5$]oxazepam in an effluent water extract. The effluent wastewater concentration of oxazepam was 0.50 μg/L. Both spectra were background-subtracted.

3.6. Quantitation and method validation

Quantitative analysis of complex matrices such as wastewater is a challenge. This holds particularly true for analysis using ESI-MS, where the wastewater matrix can cause ion suppression [23–25]. Ideally, a representative matrix, free from analysed substances, is used for an external calibration curve. This approach compensates for variabilities in SPE recoveries and ion suppression and it is quite

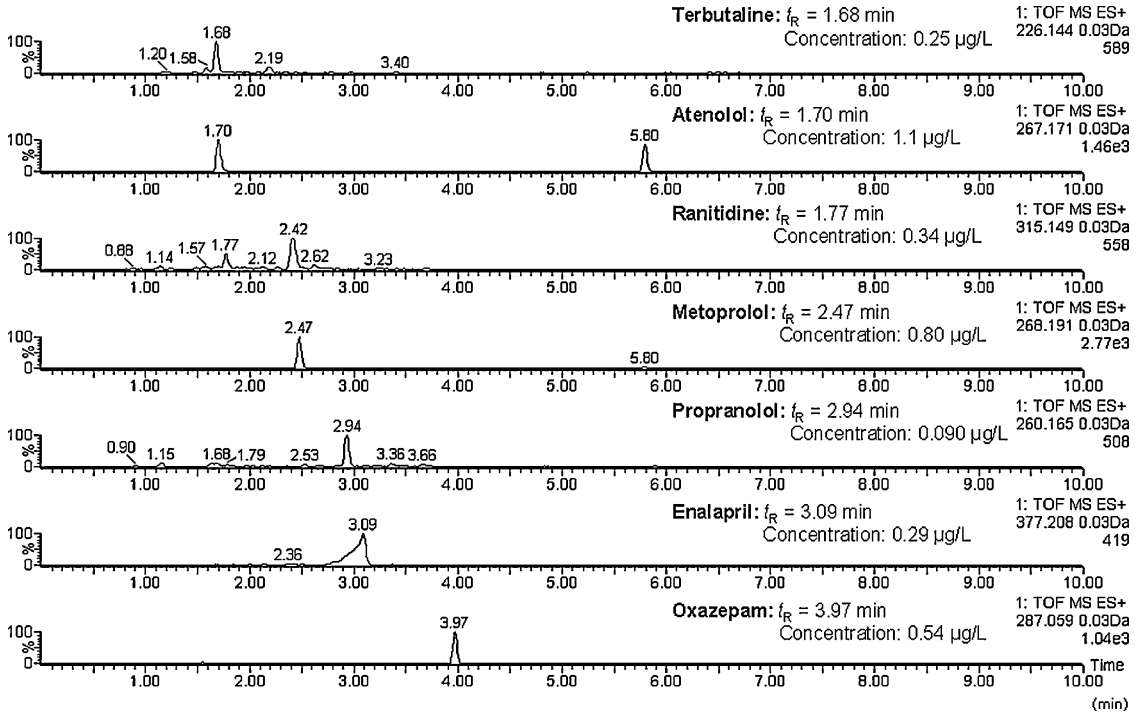


Fig. 6. Positive ion mode extracted ion chromatograms of the basic SPE fraction of an effluent water sample.

Table 5
Repeatability, reproducibility, linearity, instrumental detection limit (IDL), lower limit of quantification (LLOQ), and method detection limit (MDL) of determined pharmaceuticals in influent and effluent wastewaters.

Compound	Eluate	Ion. mode	Peak area repeatability (RSD%) ($n = 5$)[a]	Peak area reproducibility (RSD%) ($n = 3$)[b]	Instrumental linear range (pg injected)[c]	Instrumental linearity (R^2)[d]	Calibration linear range (pg injected)[e]	Calibration linearity (R^2)[b]	IDL (pg inj)[f]	LLOQ influent (ng/L)[g]	LLOQ effluent (ng/L)	MDL influent (ng/L)[h]	MDL effluent (ng/L)
Atenolol	II	pos	2.5	30	2.2–1400	0.996	35–560	0.9967	2.2	140	40	42	12
Carbamazepine	III	pos	0.94	11	0.47–480	0.993	39–310	0.9995	0.47	65	7	20	2.1
Cyclophosph-amide	III	pos	4.3	9.1	1.8–1900	0.994	20–320	0.9937	1.8	30	12.5	9.1	3.8
Enalapril	II	pos	3.2	11	1.8–1800	0.999	46–370	1.0000	1.8	60	16	18	4.8
Gemfibrozil	IV	neg	7.0	20	1.8–1870	0.996	19–630	0.9998	1.8	55	15	17	4.5
Hydrochloro-thiazide	IV	neg	2.5	10	6–6200	0.990	20–640	0.9964	6.0	88	41	27	13
Ibuprofen	IV	neg	2.8	25	1.5–1580	0.991	40–1270	0.9986	1.5	65	30	20	9.1
Ketoprofen	IV	pos	3.2	8.2	1.1–2900	0.986	17–550	0.9986	1.1	110	15	33	4.5
Metoprolol	II	pos	1.7	9.9	1.9–4980	0.991	39–310	1.0000	1.9	35	25	11	7.6
Naproxen	IV	neg	2.3	23	1.8–1900	0.989	35–1100	0.9980	1.8	90	30	27	9.1
Oxazepam	II	pos	3.0	7.1	0.76–780	0.998	40–320	0.9981	0.76	25	15	7.6	4.5
Paracetamol	III	pos	1.3	19	1.8–1860	0.987	39–1300	0.9959	1.8	940	110	285	33
Propranolol	II	pos	2.6	3.4	0.49–920	0.990	10–160	0.9985	0.49	15	8	4.5	2.4
Ranitidine	II	pos	3.0	10	2.5–2600	0.993	13–400	0.9998	1	130	140	39	42
Terbutaline	II	pos	3.1	12	1.5–1560	0.985	5–160	0.9990	1.5	280	20	85	6.1

[a] RSD of peak areas obtained from repeated injections of eluates II, III and IV a spiked effluent sample.
[b] RSD of peak areas from three spiked aliquots of an STP effluent water sample extracted and analysed on three different days.
[c] Obtained from serial dilutions of a standard solution.
[d] Correlation coefficient of instrumental linear range determinations.
[e] Range of calibration curve used in quantification.
[f] Instrument Detection Limit, pg injected on column.
[g] Lower Limit Of Quantification determined as a signal-to-noise ratio of 10 in 25 mL (influent) or 50 mL (effluent) wastewater samples.
[h] Method Detection Limit determined as a signal-to-noise ratio of 3 in 25 mL (influent) or 50 mL (effluent) wastewater samples.

time effective. Although drug free plasma is used as a calibration matrix in pharmaceutical bioanalysis, the equivalent matrix is difficult to obtain in drug analysis of wastewater. Consequently, a number of different approaches have been used for analysis of pharmaceutical residues in wastewater. Variability in recoveries and ion suppression that may arise in the analysis of large inhomogeneous sample sets can be compensated for by using a standard addition strategy [30], by the use of labelled surrogate standards [25], or by dilution [23]. A full scale standard addition method is, however, highly time-consuming and requires additional sample volumes. Labelled surrogate standards, on the other hand, can be difficult and expensive to obtain, particularly for a multi-residue method, and dilution requires that the analytes are present in high enough concentrations.

In the described method, a quantitation strategy combining all of the above mentioned approaches was used to obtain reliable data in a time effective manner. Quantitation was thus performed using external standard calibration curves, with standards dissolved in pure solvent, together with a one-point standard addition calibrator sample for each unknown sample analysed. Correction of matrix effects by single-point standard addition has previously been successfully applied in quantitative LC/MS analysis [31]. Furthermore, deuterium labelled surrogate standards were used for carbamazepine, ibuprofen, oxazepam and paracetamol. The calibrator sample and labelled surrogate standards compensated for the recovery and matrix effects. Care was taken to prepare analyte concentrations of the calibrator sample to be of the same magnitude, or somewhat higher, as those of analysed wastewater samples, in order to obtain accurate recovery and matrix effect compensation data.

The MCX/MAX method was validated with respect to recovery, ion suppression, and reproducibility (Tables 2 and 3), as well as the detection limits of the method (Table 5). The sensitivity, variability and dynamic range of the instrumental determination was also validated, as well as the combined inter-day variability of the extraction and instrumental determination, the results of which are seen in Table 5 (inter-day reproducibility). Measured concentrations of the calibration curve deviated less than 10% from theoretical values, while R^2 values were on average 0.9977 ± 0.0020 ($\pm$SD), ranging from 0.9937 to 1.0000.

LLOQs of influent and effluent water were calculated for the method (Table 5). Since small water sample volumes were used in the extraction (25 mL of influent water and 50 mL of effluent water), LLOQ values may be lowered by increasing sample volumes and/or decreasing the final volume. Additionally, sample extract dilutions (Tables 2 and 3) performed after the SPE step were taken into account when LLOQs were calculated. Omitting such dilutions may thus further lower LLOQs.

On the whole, the method performance was very good, as can be seen in Tables 2 and 3, and 5, although, the inter-day reproducibility of the atenolol, ibuprofen and naproxen determination, was lower than for the other target analytes. The reasons for these deviations are not known, but it seems reasonable to assume that even small variations in the relative concentrations of target analytes and matrix constituents can have negative effects on the precision of the method. Additionally, the unreasonably high recovery of hydrochlorothiazide (141%, Table 3) calls for further investigation.

3.7. Application of the method

The method developed in this work was applied in the analysis of influent and effluent wastewater (Table 6 and Figs. 6–8). Several substances were detected at >1 μg/L quantities in influent water, with for instance paracetamol at 84 μg/L. Paracetamol was, however, effectively removed in the STP, as were ibuprofen

Table 6
Concentrations and mass accuracy of determined pharmaceuticals in influent and treated wastewaters.

Compound	Concentration (μg/L)				Average mass error[a]			
	Influent	Effluent	Ozone treated effluent	MBR	Quantified ions[b]		Confirmation product ions[b]	
					mDa	ppm	mDa	ppm
Atenolol	1.4	1.1	<0.110	0.22	0.4	1.4	1.3	9.0
Carbamazepine	0.28	0.41	<0.110	0.36	0.8	3.4	0.8	4.3
Cyclophosphamide	<0.025	<0.015	<0.015	<0.020	<LLOQ	–	–	–
Enalapril	<0.26	<0.055	<0.025	<0.040	<LLOQ	–	–	–
Gemfibrozil	0.30	0.14	<0.030	<0.050	0.6	2.2	0.05	0.4
Hydrochlorothiazide	1.2	1.2	<0.070	1.2	0.8	2.8	0.5	2.6
Ibuprofen	7.7	0.38	0.038	<0.030	0.4	2.1	1.5	9.3
Ketoprofen	1.2	0.66	0.042	0.042	0.1	0.4	1.2	5.5
Metoprolol	0.79	0.80	<0.030	0.34	0.6	2.4	0.3	2.6
Naproxen	3.3	0.35	<0.050	0.075	0.5	2.9	1.5	6.5
Oxazepam	0.40	0.54	0.051	0.45	0.6	2.0	0.7	3.0
Paracetamol	84	<0.60	<0.26	<0.54	1.2	7.9	1.0	9.1
Propranolol	0.087	0.090	<0.015	0.094	0.9	3.5	0.5	4.6
Ranitidine	0.50	0.34	<0.35	0.14	1.8	5.8	2.8	15.6
Terbutaline	<0.21	<0.085	<0.12	<0.080	<LLOQ	–	–	–

[a] Average mass errors in absolute value in the analysis of influent, effluent, ozone treated effluent and MBR wastewater.
[b] cf. Table 1.

and naproxen, with removal efficiencies of ≥90%. Atenolol, gemfibrozil, ketoprofen and ranitidine, on the other hand, were only partially removed and were detected at concentrations greater than half their influent concentrations. The most persistent compounds were carbamazepine, hydrochlorothiazide, metoprolol, oxazepam and propranolol, where no removal could be detected. In the case of carbamazepine and oxazepam, the concentrations were 1.5 and 1.4 times greater in effluent than influent water. Similar findings have previously been reported for carbamazepine, and it has been suggested that cleavage of glucuronide conjugates in the STP may contribute to higher effluent than influent concentrations [32]. Utilising the fact that all analyses in this study were performed in full scan mode with alternating low and high collision energies, a post-acquisition screening of glucuronide conjugates was carried out. No conjugates were however identified, neither in fractions of the MCX/MAX method nor in MCX-only extracts. In this case there must be another reason for the increase in carbamazepine and oxazepam concentrations in effluent water.

Effluent water treated with an additional ozone step was also analysed (Table 6). This proved to be a very effective treatment, as has previously been reported [33]. Only ibuprofen, ketoprofen and oxazepam were determined at levels above the LLOQ. The ozonation removal efficiencies of these pharmaceuticals were 90, 94 and 91%, respectively.

In a separate, parallel, stream, the same influent water was treated by an MBR, the effluent of which was additionally sampled and analysed (Table 6). The MBR provided higher removal efficiencies of most pharmaceuticals, compared with the activated sludge treatment process. However, carbamazepine, hydrochlorothiazide, oxazepam and propranolol resisted any degradation in the MBR. To

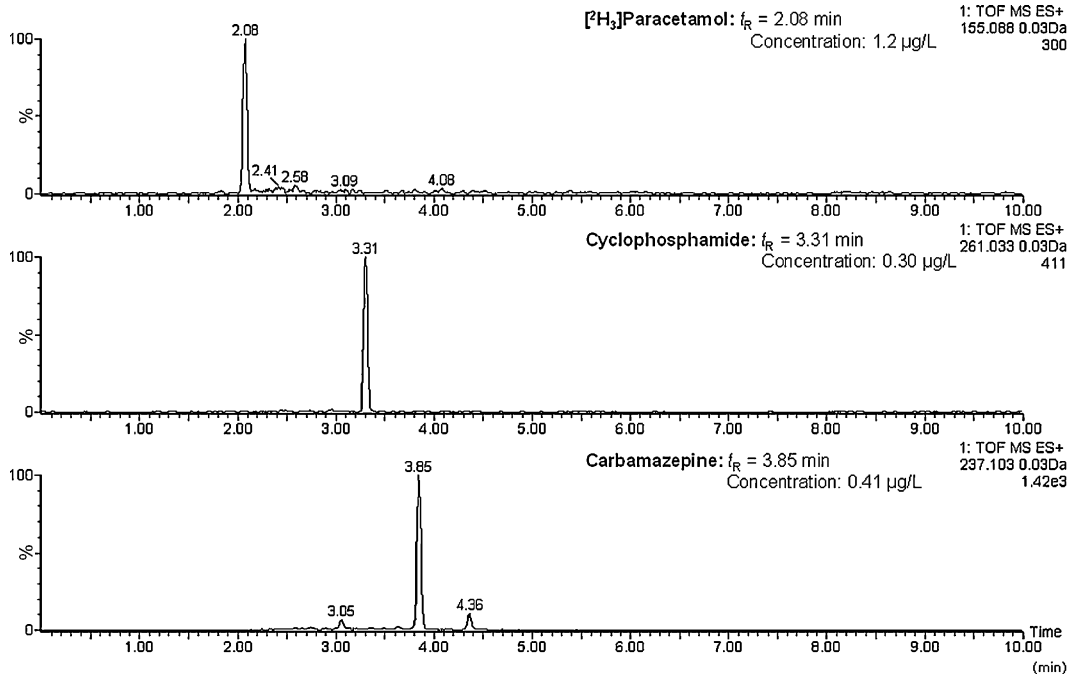


Fig. 7. Positive ion mode extracted ion chromatograms of the neutral SPE fraction of an effluent water sample.

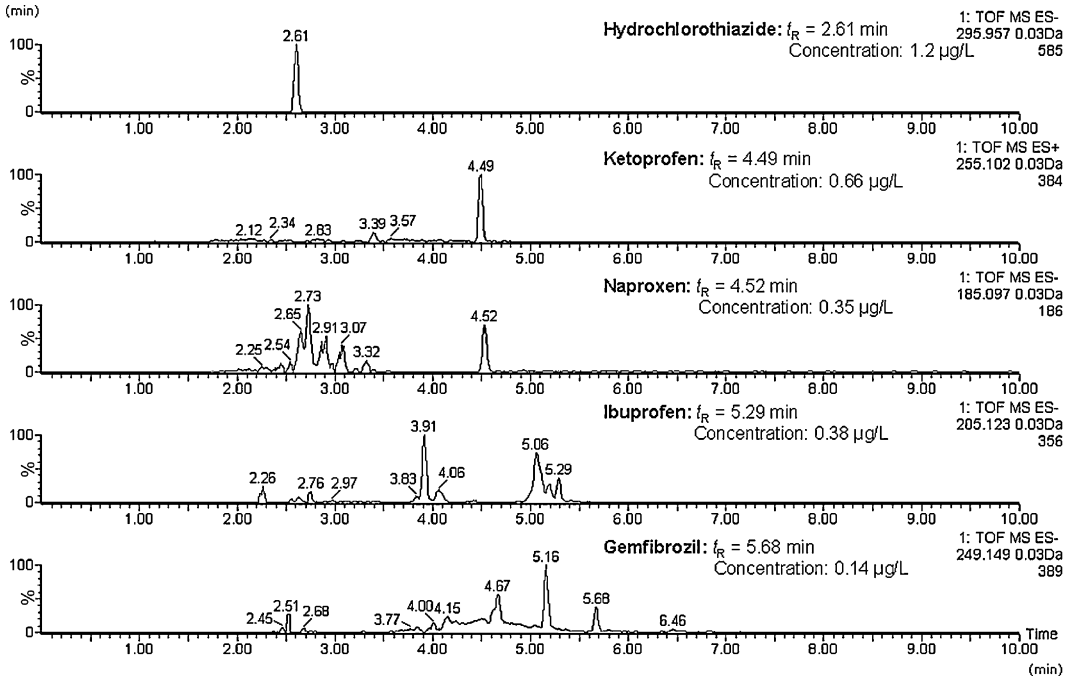


Fig. 8. Extracted ion chromatograms of the acidic SPE fraction of an effluent water sample. Ketoprofen was analysed in positive ion mode and the other compounds in negative ion mode.

reduce the levels of these compounds, more efficient techniques, e.g. ozone treatment, are necessary.

4. Conclusions

In this study, it was demonstrated that a simultaneous extraction and subsequent separation of basic, neutral and acidic pharmaceuticals in wastewater can be achieved using mixed-mode cation- and anion-exchange SPE sorbents in series. A considerable improvement in precision, and reduced ion suppression was achieved compared to extraction by the sorbent Oasis MCX-only. Furthermore, mass spectra containing fewer background ions, were obtained for a number of the pharmaceuticals analysed. Also, the SPE separation according to molecular charge added an additional degree of analyte confirmation. The described SPE set-up is not limited to analysis of the 15 pharmaceuticals described herein, but may be extended to cover other compounds. Utilising the ability to separate substances by mixed-mode SPE according to basic and acidic functionalities should for instance be very useful in the characterisation of unknown water contaminants.

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