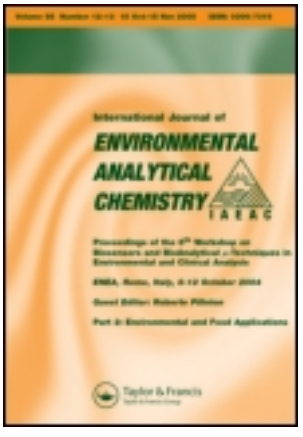

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Extraction and analysis of pharmaceuticals in polluted sediment using liquid chromatography mass spectrometry

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Extraction and analysis of pharmaceuticals in polluted sediment using liquid chromatography mass spectrometry

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A multi-residue method for the extraction and clean-up of sediment samples was developed for the analysis of pharmaceutical residues. Sediment samples were collected in the proximity of sewage water plant in Stockholm, Sweden. Target analytes were the basic β-blocker propranolol, the neutral neuroleptic carbamazepine and the acidic anticoagulant warfarin, the painkiller diclofenac and the lipid regulator gemfibrozil. The extraction solvent was optimised with regard to pH and organic modifer. Extraction and clean up were performed with liquid-liquid extraction and ultra-sonication followed by solid-phase extraction. One extraction solvent, containing acetone/McIlvaine buffer pH4, provided satisfactory extraction for all substances. LC/MSMS in the MRM mode was used for determination. The recoveries of the extraction and clean-up steps were 60–75% (±2–8%) and LOQs were in the range 0.4–8 ng/g sediment (dry weight). The pharmaceuticals found in the sediment samples were propranolol and carbamazepine, representing substances with basic and neutral properties. Additionally, the samples were analysed with LC/QTOF for verification with the use of accurate mass measurement in the full-scan mode. Pharmaceuticals not represented in the original method were looked for. Non-target pharmaceuticals found using the LC/QTOF system were the basic β-blocker metoprolol and the acidic painkiller naproxen.

Keywords: solid-phase extraction; multiple reaction monitoring; triple quadrupole; time-of-flight; matrix effects

1. Introduction

In recent years the occurrence of pharmaceuticals in the environment has been a matter of growing concern. Methods for the analysis of pharmaceuticals in water, in particular effluents from sewage treatment plants (STP), have been extensively reported [1–3]. In contrast, analytical methods for pharmaceuticals in solid matrices like sludge and sediment are not as well developed and especially methods for the analysis of sediments from polluted areas are few. Due to the high sulphur content of anoxic sediment and due to the heavy pollution caused by proximity to an STP, the matrix differs from oxic and unpolluted sediments, which causes matrix problems in the analytical procedure.

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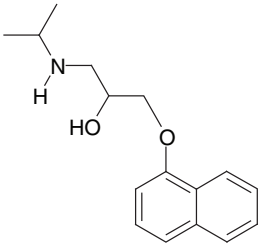
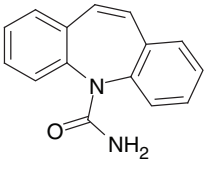
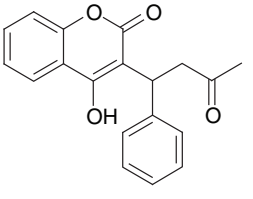
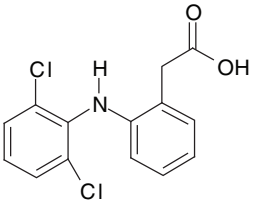
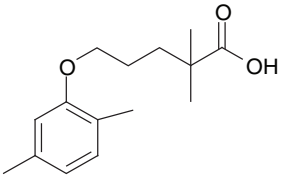
In developing an effective strategy for the extraction and the analysis of environmental contaminants, an understanding of the physico-chemical properties of the analytes and their interaction with environmental matrices is required. Many pharmaceuticals contain polar and ionisable functional groups. This means that pharmaceuticals have a greater affinity to the polar part of the sediment in comparison with hydrophobic environmental contaminants with non-polar characteristics. Sediment that consists of clay minerals has a surface layer with a net negative charge. This net negative charge leads to the accumulation of positively charged counter ions at the surface of sediment particles [4]. Cationic drugs in particular have an affinity for the negatively charged sites, and are therefore liable to be found in environmental sediment samples [5]. Cation exchange, cation bridging, surface complexation, hydrogen bonding as well as hydrophobic bonding are possible interactions between pharmaceuticals and sediment [6]. The solvents used for extraction need to be adapted to these circumstances.

Löffler and Ternes [7] extracted aerobic river sediment samples spiked with 11 acidic pharmaceuticals. The extraction was performed with acetone/acetic acid (20:1) followed by three times with ethyl acetate by shaking, and then ultrasonic extraction. For a spiking level of 20 ng/g they achieved a recovery of 56–206%. Antonic and Heath [8] compared ultrasonic extraction, soxhlet extraction, pressurised liquid extraction (PLE), supercritical fluid extraction (SFE) and microwave assisted extraction (MAE) for four NSAID (non-steroidal anti inflammatory drugs) in river sediment. The recovery with the most efficient extraction technique PLE was high (74–105%), whereas a recovery of only 5–18% was obtained with ultrasonic extraction. Cueva-Mestanza *et al.* [9] extracted eight pharmaceutical compounds with microwave assisted micellar extraction (MAME) in sediment, prior to determination with ultraviolet diode-array-detection (UV-DAD). Sediment samples from the Island of Gran Canaria were analysed and recoveries were high (80–100%). Sung-Chul and Carlson [10] extracted veterinary pharmaceuticals (e.g. tetracyclins) from sediment. They performed extraction by shaking sediment with McIlvaine buffer pH 4, without sophisticated instrumentation such as PLE or MAE, and obtained recoveries of 30–105%.

The aim of this work was to develop a time and cost-effective multi-residue method (acidic, basic and neutral analytes) to extract and analyse pharmaceuticals in polluted sediment. The identification of efficient extraction solvents, without the need for costly instrumentation like MAE, SFE or PLE, was emphasised in the study. Five model substances with basic (propranolol), neutral (carbamazepine) and acidic (warfarin, diclofenac and gemfibrozil) properties were utilised for method development (Table 1). The quantification was performed with liquid chromatography triple-quadrupole mass spectrometry (LC/MSMS) in the multiple-reaction-monitoring (MRM) mode. Deuterated surrogate standards were used for accurate quantification and to correct for ion suppression. The samples were also analysed by liquid chromatography hyphenated with quadrupole time-of-flight mass spectrometry (LC/QTOF). The ability of accurate mass measurement provided by this technique was used in the full-scan mode to screen for pharmaceuticals not originally included in the method. This was done in order to broaden the information on which type of pharmaceuticals, in terms of chemical properties, are likely to be found in the sediment phase. However, it was considered to be beyond the scope of this study to distinguish between pharmaceutical residues sorbed to the sediment and those dissolved in the porewater.

Table 1. The five model substances. Data extracted from Scifinder Scholar 2007 (predicted values).

Substance	Structure	pKa (Log D)[(1)] log P[(2)]	MLD[(3)] MLQ[(4)]	Amount found[(5)] recovery[(6)] (n = 3)
Propranolol		9.14 (1.93) 3.10	0.2 ng/g 0.7 ng/g	4.1 (±0.4)ng/g 58 (±8) %
Carbamazepine		– (2.67) 2.67	0.1 ng/g 0.4 ng/g	0.9 (±0.1)ng/g 75 (±4)%
Warfarin		4.50 (0.22) 3.42	0.2 ng/g 0.7 ng/g	n.d. 66 (±4)%
Diclofenac		4.18 (0.57) 4.06	3 ng/g 8 ng/g	n.d. 63 (±4)%
Gemfibrozil		4.75 (1.26) 4.39	0.2 ng/g 0.5 ng/g	n.d. 60 (±2)%

Notes: (1): Log D at pH8, 25°C; (2): Log P: The octanol-water coefficient; (3): Highest method limit of detection, not corrected for recovery; (4): Highest method limit of quantification, not corrected for recovery; (5): Quantified amount in dry sediment, n.d.: not detected; (6): Recovery of the proposed method.

2. Experimental

2.1 *Materials*

Propranolol hydrochloride, carbamazepine, gemfibrozil, warfarin and diclofenac sodium salt were purchased from Sigma (St Louis, USA). Propranolol-d7 (99% ^{2}H), warfarin-d5 (99% ^{2}H), diclofenac-d4 (91% ^{2}H) and gemfibrozil-d6 (97% ^{2}H) were purchased from Toronto Research Chemicals (North York, Canada). Carbamazepine-d10 (98.2% ^{2}H) was purchased from CDN Isotopes, (Point-Claire, Canada). Acetone (SupraSolve), methanol (LiChrosolv), glacial acetic acid (p.a.) and formic acid (p.a.) were purchased from Merck (Darmstadt, Germany). Citric acid monohydrate (Ph. Eur.) was from VWR (Leuven, Belgium) and sodium phosphate dibasic heptahydrate (ACS-grade) from Solon Ind. Pkwy (Solon, USA). The water used was of milli-Q grade from a milli-Q ultrapure water system MilliQ PLUS 185 from Millipore (Stockholm, Sweden).

The standards used for LC/QTOF runs were atenolol, cyclophosphamide monohydrate, enalapril maleate salt, hydrochlorothiazide, ibuprofen, metoprolol tartrate salt, paracetamol, ranitidine hydrochloride, sulfadimethoxine (for lock spray calibration) and terbutaline hemisulfate salt purchased from Sigma (St Louis, USA). Ketoprofen was obtained from Riedel de Haen (Seelze, Germany) and naproxen from Fluka (Steinheim, Germany). Oxazepam-d5 (99% ^{2}H) was purchased from Isotec/Sigma–Aldrich (St Louis, USA).

2.2 *Sampling*

The sediment samples were collected on 7 December 2007 from an accumulation bottom in the inner Stockholm archipelago (N 59° 19′ 06 E 18° 06′ 12) at a depth of 30 m. The site is situated close to the discharge points of two STPs, Henriksdal and Bromma. The effluents from Henriksdal and Bromma correspond to 690,000 person equivalents and 290,000 person equivalents, respectively [11]. Laminated surface sediments were sampled using a Ponar grab sampler and stored in plastic containers at −20°C. An aliquot of the sediment was removed for characterisation of sediment properties. The dry weight was determined by heating accurately weighed samples to 105°C for 24 hours. Loss of ignition (LOI) was determined by heating the dry sediment to 550°C for two hours. The water content in the sediment was 84% and the weight lost from the dry residue by LOI was 15%. The total organic carbon (TOC) content was estimated to be 5.9% by recalculating the LOI-value according to Persson *et al.* [12]. Prior to pharmaceutical analysis the sediment was freeze dried using a Hetosicc freeze dryer (Rødeby, Denmark). In order to remove larger particles, the freeze dried sediment was sieved (1 mm mesh) and the sediment was thereafter stored in a dark, dry place.

2.3 *Preparation of solutions*

The non-deuterated (native) standards, propranolol hydrochloride, carbamazepine, gemfibrozil, warfarin and diclofenac sodium salt, were accurately weighed and dissolved in methanol to yield 1.00 g/L stock solutions. Working solutions were made from the stock solutions and were further diluted with methanol into a mixture containing 350 µg/L of each substance for the spiking of sediment samples. The surrogate standard contained propranolol-d7, warfarin-d5, diclofenac-d4 and gemfibrozil-d6 at concentrations of 175 µg/L dissolved in methanol/water 1:1. Standard solutions for calibration curves

Table 2. Substances screened for in Q-TOF runs.

Substance	Ion-mode	Mass (m/z)[1]		Found in sediment[2]	
		Quasi molecular ion	Product ion	Quasi molecular ion	Product ion
Atenolol	+	267.1709	145.0653	–	
Carbamazepine	+	237.1028	194.0970	16 ppm	7 ppm
Cyclophosphamide	+	261.0326	140.0034	–	
Diclofenac	−	294.0089	250.0190	–	
Enalapril	+	377.2076	234.1494	–	
Gemfibrozil	−	249.1491	121.0653	–	
Hydrochlorothiazide	−	295.9567	204.9839	–	
Ibuprofen	−	205.1229	161.1330	–	
Ketoprofen	+	255.1021	209.0966	–	
Metoprolol	+	268.1913	116.1075	3 ppm	n.d.
Naproxen	−	229.0865	185.0966	13 ppm	
Oxazepam	+	287.0587	241.0533	–	
Paracetamol	+	152.0712	110.0606	–	
Propranolol	+	260.1651	116.1075	6 ppm	2 ppm
Ranitidine	+	315.1491	176.0494	–	
Terbutaline	+	226.1443	152.0712	–	
Warfarin	+	309.1127	250.063	–	

Notes: (1): Calculated accurate mass for the quasi-molecular ion and product ion used for identification of non-target pharmaceuticals. Calculations were done using MassLynx v. 4.1. (2): Mass difference (average of 3 scans) for each ion found.

were prepared by mixing of the non-deuterated mixture and the surrogate standard mixture. Stock, working and spiking solutions as well as the surrogate standard mixture were kept at −20°C, and were thawed on a daily basis when needed. The freshly made solutions were not used for more than three months. Standard solutions for LC/QTOF (Table 2) were mixed in a solution with concentrations providing peak areas within the linear range of the detector.

2.4 *Spiking of sediments*

The freeze dried sediment was weighed to 2.0 g in 50 mL glass centrifuge tubes which were covered with aluminium foil to prevent photo degradation. The samples were spiked with 200 µL of the surrogate standard. Three mL of methanol was added to each wet sediment sample in centrifuge tubes. The sediment was shaken for three minutes by hand and vortex mixed for two minutes. The tubes (without lids) were put in a fume hood to let the methanol completely evaporate overnight.

2.5 *Extraction and clean up*

Extraction was performed by adding 40 mL of acetone/McIlvaine buffer with a pH of 4.0 to the centrifuge tubes containing the sediment samples. The tubes were fitted to a rotational mixer and rotated at a speed of 25–30 rpm for 15 minutes. Additional extraction was performed by ultra-sonication of the samples for 15 minutes, followed by

centrifugation for 5 minutes at 2000 rpm. The extraction solvent was decanted into amber glass beakers. The extraction procedure was repeated once and the extracts were combined. The acetone content was allowed to evaporate overnight, after which the extracts were filtrated through GF/F glass microfibre filters from Whatman Int. (Maidstone, UK) by suction into Erlenmeyer flasks. The amber glass beakers were washed with 2 mL methanol. Solid-phase extraction (SPE) was performed in two steps. In the first step, the column used was Evolute ABN 50 µm 100 mg/10 cc from Biotage AB (Stockholm, Sweden). In the second step, Oasis HLB 30 µm 60 mg/3 cc from Waters (Milford, USA) was used. The columns were fitted into a vacuum manifold (Supelco Visiprep^{TM}, UK) which allowed for parallel extraction of 20 samples. The Evolute ABN columns were conditioned with 4 mL methanol and 4 mL of 0.1% formic acid prior to loading of the sample. Thereafter the columns were washed with 4 mL 20% methanol, and the sample was eluted with 4 mL methanol. The eluate was diluted with 80 mL milli-Q water. The Oasis HLB columns were conditioned with 2 mL methanol and 2 mL milli-Q water. The diluted eluate from the Evolute column was loaded on the HLB column and washed with 2 mL 20% methanol, prior to elution with 2 mL methanol. The final eluate was gently blown to dryness under a stream of nitrogen at a temperature of 30°C and the residue was redissolved in 200 µL methanol/water. The samples were stored in a freezer at −20°C for a minimum of 48 hours before analysis.

2.6 *Extraction efficiency and recovery*

Different extraction methods were tested in order to find appropriate extraction solvent mixtures and for comparing the influence of pH and acetone content on the extraction yield. Sediment samples ($n = 2$) were extracted with McIlvaine buffer pH 4, acetone/McIlvaine buffer pH4 1 : 1, McIlvaine buffer pH 7, acetone/McIlvaine buffer pH 7 1 : 1, 0.2 M dibasic sodium phosphate (pH 10, checked with universal pH-paper) and acetone/0.2 M dibasic sodium phosphate 1 : 1. Prior to extraction, the sediment was spiked with 100 µL of a mixture of non-deuterated standards with a concentration of 350 µg/L. The samples were handled in the same manner as described under 'Spiking of sediments'. After the extraction and clean-up procedure, the samples were redissolved in 200 µL of the surrogate standard mixture used for quantification.

In order to distinguish between the extraction efficiency of pharmaceuticals sorbed to the sediment and the recovery of the clean-up method, the following experiment was performed: First, three sediment samples were spiked with 100 µL of the non-deuterated standard solution. Second, three sample extracts were spiked prior to the clean-up step. Finally, three samples were spiked after the clean-up step. The differences in mean recoveries between these three sets of samples were then used for calculation of the recoveries for each step. After evaporation to dryness under nitrogen, the nine samples were redissolved in 200 µL of the surrogate standard mixture. The recovery for two of the extraction solvents, the acidic acetone/McIlvaine buffer pH 4 1 : 1 and the basic acetone/0.2 M dibasic sodium phosphate (pH 10) 1 : 1 were evaluated in this way.

2.7 *High-performance liquid chromatography with tandem mass spectrometry*

The mass-spectrometric analysis of the sediment samples was carried out using a Micromass Quattro II tandem mass spectrometer (Manchester, UK), with an electrospray

Table 3. LC-MS/MS tune settings and retention times for model substances and deuterated standards.

Substance	MRM-transitions (m/z)	Ion-mode	Collision -voltage (V)	Collision -energy (eV)	Retention -time (min)
Propranolol	260 → 116	+	45	25	6.0
	260 → 183	+	45	20	6.0
Propranolol-d7	267 → 123	+	45	25	6.0
Carbamazepine	237 → 194	+	45	20	8.9
	237 → 192	+	45	20	8.9
Carbamazepine-d10	247 → 204	+	45	20	8.9
Warfarin	309 → 251	+	45	23	12.8
	309 → 163	+	45	15	12.8
Warfarin-d5	314 → 256	+	45	23	12.8
Diclofenac	294 → 250	−	30	15	16.1
	294 → 214	−	30	20	16.1
Diclofenac-d4	298 → 254	−	30	15	16.1
Gemfibrozil	249 → 121	−	35	15	17.9
	249 → 127	−	35	15	17.9
Gemfibrozil-d6	255 → 121	−	35	15	17.9

interface, operated in both positive and negative mode. The capillary voltage was set to 3.5 kV, the cone voltage and collision energy were tuned for optimal sensitivity using a syringe pump connected with a T-split into the LC-flow for each MRM transition (Table 3). Different time windows were used for each pharmaceutical using a dwell time of 0.30 s for the MRM transitions. Secondary ions and isotope labelled surrogate standards were given a dwell time of 0.10 seconds. The ion source was set to a temperature of 100°C and the desolvation temperature to 150°C. Nitrogen was used as both the drying gas and nebulising gas at flow rates of 400 L/h and 20 L/h, respectively. Argon was used as collision gas at a pressure of 2.3×10^{-3}mbar.

The liquid chromatography pump used was a Waters Alliance separations module 2695 equipped with an in-built auto sampler (Milford, USA). The column was a Fortis C8 HPLC column from Fortis Technologies (Neston, UK) with dimensions 2.1 × 100 mm and a particle size of 3 µm. To the analytical column, a 2.1 × 10 mm precolumn containing the same stationary phase was fitted. The mobile phase used in a gradient run consisted of (A) 5 mM acetic acid and (B) Methanol with the same amount of acetic acid added. The gradient, with a flow of 0.2 mL/min started with 60% A in 40% B followed by a linear change to 26% A in 74% B over a period of 10 minutes, and then followed by a linear change to 5% A in 95% B over 13 minutes. The latter composition was maintained for an additional 7 minutes. A pre-column volume setting of 550 µL was applied. The column was conditioned with the initial composition for 15 minutes between runs. MassLynx software was used for controlling system parameters and for acquiring and evaluating data.

2.8 *Ultra-performance liquid chromatography with quadropol time-of-flight mass spectrometry (UPLC/QTOF)*

The samples extracted with the acetone/McIlvaine buffer pH 4 1 : 1 and acetone/ 0.2 M dibasic sodium phosphate 1 : 1 were also analysed using a Waters Aquity

ultra-performance liquid chromatography system connected to a Waters QTOF Premier mass spectrometer (Milford, USA). The mass spectrometer was operated in ESI positive and negative mode with the TOF detector in V-mode. The following settings were used (in the form positive mode/negative mode): capillary voltage 3.0/2.9 kV, sampling cone voltage 25/25 V, extraction cone voltage 1.5/2.8 V, source temperature 100°C; desolvation temperature 300°C/275°C, cone gas (nitrogen) flow 48/35 L/h and desolvation gas flow 676/687 L/h. Argon was used as the collision gas, at a pressure of $3.36 \times 10^{-3}/2.95 \times 10^{-3}$ mbar. External mass range calibration was performed in the mass range m/z 100–1000, using a series of cluster ions formed from infusion of a solution containing 0.05 M NaOH and 0.5% formic acid dissolved in 2-propanol/water 9:1. Sulfadimethoxine m/z 333.0633 $(M + Na)^+$ in positive ion mode and m/z 309.0658 $(M\text{-}H)^-$ in negative ion mode was used as internal mass calibrant, infused via the lock spray during chromatographic runs. The concentration of the sulfadimethoxine solution in both positive and negative ion mode was 0.1 ng/μL. The lock spray frequency was 5 s, with 10 scans to average.

The samples were divided into two 60 μL aliquots, to one of which was added 20 μL of methanol/water 1 : 1 and the other was spiked with 20 μl of 1.11 ng/μL of each of the five pharmaceuticals in methanol/water 1 : 1. A standard that contained the substances presented in Table 2 was analysed. The pharmaceuticals were separated using a HSS T3 column with dimensions 2.1 × 100 mm and a particle size of 1.8 μm from Waters (Milford, USA). To the analytical column a 2.1 × 5 mm precolumn containing the same stationary phase was fitted. The injection volume was 5 μL and the mobile phase solvents were (A) 95% 10 mM acetic acid and 5% acetonitrile and (B) 5% 10 mM acetic acid and 95% acetonitrile. Separation was achieved at a column temperature of 65°C using a flow rate of 0.6 mL/min with 100% A for 2.5 minutes followed by a linear change to 5% A in B over 3.5 minutes with this composition maintained for 0.25 minutes. The column was conditioned with the initial composition for 1.75 minutes between runs.

3. Results and discussion

3.1 *Extraction and clean up*

In the search for solvents that would break the bonds between pharmaceuticals and sediment McIlvaine buffer was tried. The two ingredients in McIlvaine buffer, 0.1 M citric acid and 0.2 M dibasic sodium phosphate, can be mixed to provide buffers ranging from pH 2.2 to 8.0. Thus, this buffer was used for the evaluation of which pH provided the best extraction efficiency. Acetone was used as organic modifier. Pure acetone in addition to buffers of pH 4, 7 and 10 with and without acetone were evaluated. The results from the extraction experiments revealed that the recovery was highest using acetone/McIllvaine buffer pH 4 1 : 1 (Figure 1). For the basic solvent containing acetone and 0.2 M dibasic sodium phosphate, the extraction from sediment was more efficient for the acidic pharmaceuticals. However, these analytes were lost during the clean-up step. In addition, the extraction yields of both propranolol and carbamazepine were low using this solvent.

The pH of the Baltic Sea is approximately 8 [13], which makes the acidic pharmaceuticals negatively charged and therefore more water soluble. Repelling forces could be expected to be acting between negatively charged pharmaceuticals and negatively charged sites on the surface of the sediment. Nevertheless, the presence of acidic

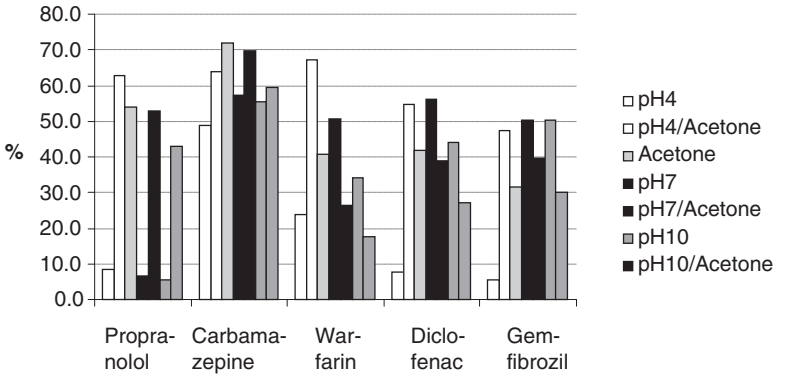


Figure 1. Extraction survey of spiked sediment (no compensation for propranolol and carbamazepine present in the sediment from the beginning).

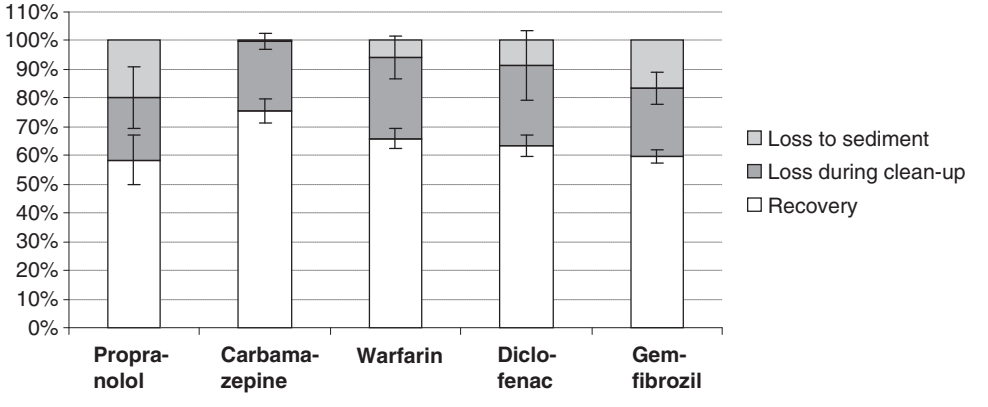


Figure 2. Recovery of model substances extracted with acetone/McIlvaine buffer pH 4. Error bars show the standard deviation ($n = 3$).

pharmaceuticals in river sediment has been reported [8,14]. On the other hand, basic pharmaceuticals like propranolol, containing amine groups with pKa values of approximately 9, are to a large extent positively charged at pH 8. These pharmaceuticals are expected to bind to sediment through electrostatic forces i.e. ionic bonding [5,15]. Table 1 shows the calculated log D values for pH 8. Since log D values take into account the fraction that is charged, it may be regarded as a better measure of the partitioning of a pharmaceutical between octanol and water than the log P value.

Figures 2 and 3 show the comparison of the extraction efficiency (loss to sediment) as well as the recovery from the clean-up process (loss to clean up) for two of the mixtures: acetone/ McIlvaine buffer pH 4 and acetone/ 0.2M dibasic sodium phosphate (pH 10). For the basic pharmaceutical propranolol, the acidic extraction solvent provided higher efficiency of extraction from the sediment as well as a higher recovery during the clean-up than the basic solvent. It is likely that electrostatic forces make extraction of propranolol from sediment difficult. As Figure 1 shows, pure acetone was the best choice for the neutral carbamazepine. The results obtained for the three acidic pharmaceuticals showed that the basic extraction solvent resulted in substantial losses during the clean-up

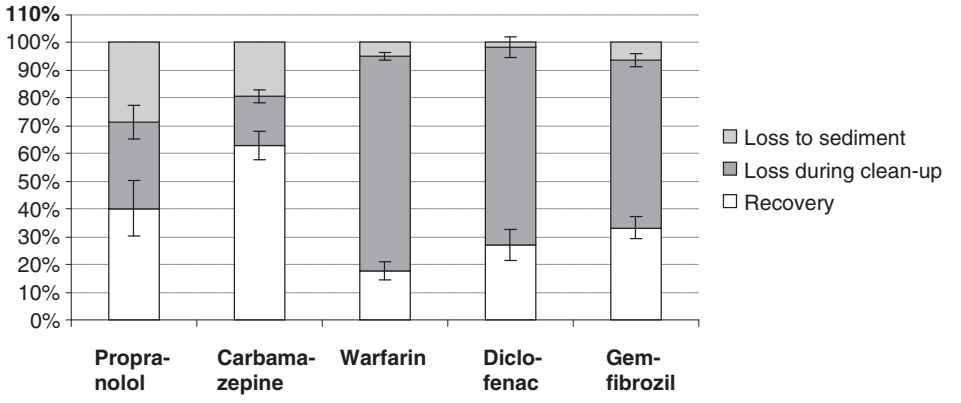


Figure 3. Recovery of model substances extracted with acetone/ 0.2 M dibasic sodium phosphate. Error bars show the standard deviation ($n = 3$).

process. Although the yield of extraction from the sediment was higher with the basic compared to the acidic solvent, probably due to repulsive forces between negative sites and negative analytes, the total recovery was lower. Acetone/McIlvaine buffer pH 4 was the preferred extraction solvent.

Initially, the SPE columns Oasis HLB (retention based upon mixed mode lipophilic-hydrophilic mechanisms) and Oasis MAX (a column with anion exchange characteristics) were tested and evaluated. The recovery obtained when using these SPE columns, however, was low. All five model substances were retained on the Evolute ABN columns (retention provided in small cavities, mixed mode lipophilic-hydrophilic interactions as well as size exclusion). Oasis HLB retained the pharmaceuticals in the resulting eluate from the ABN column. Thus, evidently, clean up of the extract on the Evolute ABN column removed matrix constituents, that otherwise would lower the extraction yield of the analytes from the Oasis HLB column, possibly by competing for adsorption sites. Hence, Evolute ABN and Oasis HLB were selected for sequential clean up, which was considered necessary, since no single SPE clean up provided sufficiently clean extracts for analysis of these polluted sediments.

3.2 *Optimisation of the LC/MSMS method*

A number of mobile phase additives e.g. formic acid, ammonium bicarbonate, ammonium acetate and acetic acid were evaluated, with the aim of obtaining chromatographic separation as well as high sensitivity for the pharmaceuticals. The intention was to find a single mobile phase providing sufficient separation and sensitivity for the five pharmaceuticals. Methanol and acetic acid provided separation of the peaks as well as high sensitivity for the basic (propranolol), the neutral (carbamazepine) and the acidic pharmaceuticals (warfarin, diclofenac and gemfibrozil).

Optimisation of cone voltage and collision energy was performed as follows. A 2.5 mL syringe, fitted to a syringe pump CMA/100 from Carnegie Medicin (Stockholm, Sweden), was filled with 100 µg/mL of each pharmaceutical dissolved in methanol. The standard solution from the syringe was pumped through a silica capillary via a T-connection

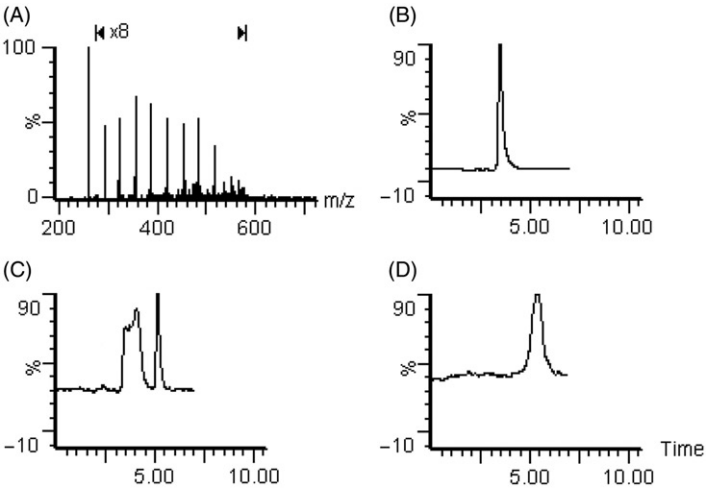


Figure 4. A: Parent-ion spectra of m/z 260 (propranolol) showing eight extra peaks with a mass difference of 32 Da, indicating an interaction with S_8. The spectrum originates from the left peak in C, but the same pattern can be seen in the peak to the right. B: MRM-chromatogram for the standard, m/z 260 → 116. C: MRM chromatogram for a sediment sample; and D: the same sample as in C after freezing to −20°C for 48 h.

and mixed with the flow from the HPLC. The flow of 0.01 mL/min from the syringe into the mobile-phase flow of 0.19 mL/min equalled the flow of 0.2 mL/min used in the resulting method. The settings for source temperature, capillary voltage and high and low mass resolution were tuned for optimal sensitivity and thereafter set and used on a regular basis throughout method development and analysis.

A severe problem was the peak of propranolol, which had a square shape (Figure 4C) and a different retention time in samples compared to the standard. Parent-ion spectra of propranolol revealed that interaction between propranolol and sulphur in the form of elementary sulphur (S_8) probably was the reason. Addition of copper powder to the sediment for the removal of sulphur was tried [16], but this had no significant effect on the bad peak shape or the retention time. Freezing the samples at −20° C for 48 hours prior to injection resulted in a better peak shape (Figure 4D). The reason for this is not fully understood, but since water solubility decreases with decreasing temperature, the sulphur may have precipitated on the glass walls and remained undissolved after thawing of the samples. Nevertheless, the retention time still differed by approximately one minute. However, quantification by the standard-addition method (spiking with two higher concentrations) confirmed the results obtained using the surrogate-standard method. Therefore the method was considered reliable in spite of this problem, and freezing of the samples prior to injection was performed regularly.

3.3 *Method validation and results*

In the early stages of method development, an issue concerning quantification of the sediment samples was discovered. A significant decrease in the signal intensity was

observed between consecutive samples analysed with the LC/MS (the triple quadrupole system). Loss of signal due to ion suppression caused by accumulating debris from the sample matrix in the pepper pot and sample cone (in the mass spectrometer interface) had previously been observed. It turned out that the signal was highest directly after cleaning of the interface and then decreased as samples were injected. However, the signal intensity levelled off to a steady level when the interface reached a certain level of contamination. To correct for erroneous results due to bias caused by this problem, a quantification standard (QS) sample was injected as every second run (injections <30), and later every third run (injections >30). The QS samples, containing known amounts of deuterated and non-deuterated standards, were used for one-point calibration of the samples injected in between. The area ratio between deuterated and non-deuterated standards was calculated in the two QS samples and the average was used for the quantification of the intervening samples. The difference between two QS samples was not more than 10%. This also compensated for ion suppression in the samples, why ion suppression is not reported separately. The results would differ depending on how clean the interface was at the particular time for injection. It is also not possible to find sediment samples that indisputably not will contain pharmaceuticals and at the same time provide an identical matrix. This is because cleaner sediments at a distance from the STP are more oxic and thus will not contain as high concentration of sulphur as anoxic sediments. Subsequent to every batch of samples, a seven-point calibration curve was injected with concentrations from 10 to 350 μg/L. This was to confirm that the measured areas were within the linear range of the detector. The value of the correlation coefficient (r^2) corresponded to >0.996 for all of the pharmaceuticals except diclofenac for which the r^2-value was 0.987 ($n = 3$). The method limit of quantification (MLQ) and the method limit of detection (MLD) defined as 10 and 3 times the signal-to-noise ratio (S/N) are shown in Table 1. Table 1 also shows the concentrations quantified in the Stockholm sediment per dry weight sample and the recoveries of extraction and clean up ($n = 3$). None of the acidic model substances were detected in the sediment.

3.4 *Screening for non-target analytes with time-of-flight (TOF) detection*

The unspiked and spiked sediment samples from extractions with acetone/McIlvaine buffer pH4 and acetone/ 0.2 M dibasic sodium phosphate, respectively, were analysed in the full-scan mode. A standard solution containing the five model substances and the 12 extra pharmaceuticals (Table 2) was analysed for comparison of retention times and spectra. Quantification was not considered to be an issue in this experiment, merely a qualitative search for the pharmaceutical in question. Analyses of unspiked samples confirmed the presence of propranolol and carbamazepine in the sediment. The analyses of spiked samples revealed that the acidic pharmaceuticals warfarin, diclofenac and gemfibrozil could not be detected. A possibility might be that the matrix effects, which were a greater problem in the full-scan mode compared to the MRM-mode from the LC/MSMS runs, were too high. This has also been observed in a study comparing a QTOF (full-scan mode) and an ion trap triple-quadrupole instrument (MRM-mode) for the analysis of oestrogens in sediment [17]. Another cause could be isobaric interferences, which may impair the accurate mass measurements [18]. Two non-target pharmaceuticals were found, i.e. the basic β-blocker metoprolol (Figure 5) and the acidic pain killer naproxen (Figure 6), both of which were verified by accurate mass (Table 2). The retention

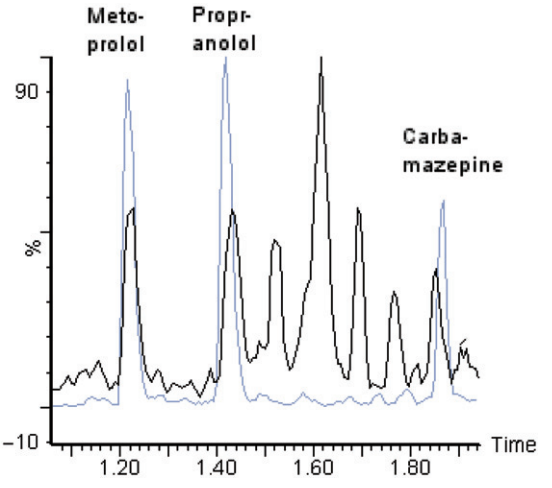


Figure 5. Full scan Q-TOF chromatogram, showing detected substances in positive mode. Mass window 30 mDa. Overlayed mass chromatogram in black shows a sediment sample and grey shows the standard run. Mass window 30 mDa, extracted ions m/z 268.1913, m/z 116.1075, m/z 260.1651, m/z 237.1028 and m/z 194.0970.

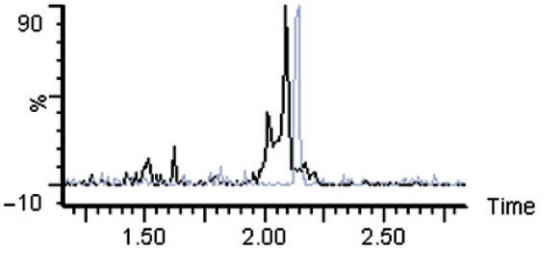


Figure 6. Full scan Q-TOF chromatogram, showing naproxen in negative mode. Mass window 30 mDa. Overlayed mass chromatogram in black shows a sediment sample and grey shows the standard run. Mass window 30 mDa, extracted ion m/z 229.0865.

time was as expected for metoprolol but differed for naproxen. Also the peak shape was distorted. This could possibly be an effect of interaction with substances in the matrix as in the case of propranolol. The parent-ion spectra of the distorted propranolol peak from the LC/MSMS runs, revealed an interaction with elementary sulphur, S_8. It is speculated that elementary sulphur, having properties of high polarisability, is a sorbent for charged compounds in the environment. Naproxen was also found in river sediment by Antonic *et al.* [8] and Mitra *et al.* [14]. In the present study, the presence of naproxen was additionally confirmed with LC/MSMS (MRM).

4. Conclusions

Acetone/McIlvaine buffer pH 4 was successful in desorbing acidic, basic and neutral pharmaceuticals from sediment. Also, the SPE columns of the clean-up method were able

to retain the analytes in this solvent and provided sufficient recovery for the model substances. It is therefore our belief that the extraction of polar compounds from sediment benefits from the use of polar solvents with high water content. In order to obtain reliable results, quantification using isotope labelled surrogate standards is needed. The matrix caused retention time drifts as well as ion suppression. The analytical method relying on the LC/QTOF instrument operated in the full-scan mode may require a more specific clean-up method due to high matrix effects caused by the complex matrix represented of the polluted sediment. The results confirmed the prediction that basic and neutral substances would have a greater affinity for the sediment than acidic compounds.

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