Chemosphere 80 (2010) 1255–1260

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Application of a novel solid-phase-extraction sampler and ultra-performance liquid chromatography quadrupole-time-of-flight mass spectrometry for determination of pharmaceutical residues in surface sea water

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ARTICLE INFO

Article history:
Received 8 March 2010
Received in revised form 21 June 2010
Accepted 24 June 2010

Keywords:
Bag-solid phase extraction
Pharmaceuticals
Sea water
Ultra-performance liquid chromatography
Quadrupole time-of-flight mass-spectrometry

ABSTRACT

In the present study, a multi-residue method based on a bag-solid phase extraction (bag-SPE) technique was evaluated for determination of 10 pharmaceuticals in surface water close to the effluent of a sewage treatment plant (STP) and along a coastal gradient from a STP effluent.

The 10 compounds selected were caffeine, atenolol, metoprolol, oxazepam, carbamazepine, ketoprofen, naproxen, ibuprofen, diclofenac and gemfibrozil. All analyses were performed using ultra-performance liquid chromatography (UPLC) combined with quadrupole time-of-flight (QTOF) mass spectrometry. The detection limits (LOD) ranged from 1.0 to 13 ng L^{-1}. The method showed linear concentration ranges from 25 to 800 ng L^{-1} with regression coefficients (R^2) better than 0.9801. The recoveries of the selected analytes ranged from 11 to 65% with relative standard deviations (RSD) of <16% and inter-day variations of less than 18%. Isotopically labeled surrogate standards were used to compensate for sampling losses and matrix effects.

Four of the selected 10 pharmaceuticals (caffeine, metoprolol, oxazepam and carbamazepine) were quantified, at concentrations ranging from 4 to 210 ng L^{-1}.

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1. Introduction

It is well-established that sewage treatment plant (STP) effluents are the main sources of pharmaceutical residues in the aquatic environment (Kolpin et al., 2002; Buchberger, 2007). Although the persistence of pharmaceuticals in the environment is low, they are considered to be semi-persistent due to their continuous introduction to the environment (Bendz et al., 2005; Hernando et al., 2006).

Recent studies have shown that complex mixtures of pharmaceuticals of different therapeutic classes may have synergistic toxic effects on biota at environmental concentration levels (Fraker and Smith, 2004; Flaherty and Dodson, 2005; Hernando et al., 2005; Pomati et al., 2006). Therefore, it is of importance to develop analytical methods for multiple-classes of pharmaceuticals to establish their occurrence, behaviour, and fate in aquatic environments (Kolpin et al., 2002; Zhang and Zhou, 2007).

Solid phase extraction (SPE) is currently the most widely used enrichment technique for pharmaceutical residues in wastewater (Gros et al., 2006; Petrovic et al., 2006; Zhang and Zhou, 2007). One of the most commonly used polymeric sorbents in SPE-cartridges is Oasis HLB, due to its high efficiency in retaining multi-classes of pharmaceuticals (Santos et al., 2005; Buchberger, 2007; Fontanals et al., 2007).

However, the hydrophilic characteristics of Oasis HLB in combination with sampling of complex water samples results in co-extraction of humic substances (HS) present in natural water and wastewater, leading to poor recoveries and considerable ion-suppression (Renew and Huang, 2004; Chen et al., 2006). Previous studies have shown that interferences of HS may be lowered by using mixed-mode sorbents like Oasis MCX (cationic-exchanger) or Oasis MAX (anionic-exchanger), which are composed of the Oasis HLB skeleton with chargeable ionic-exchange groups present in its structure (Himmelsbach et al., 2006; Kasprzyk-Hordern et al., 2007). However, the drawback with the ion-exchange approach is that the analysis of one water sample typically results in several fractions which need to be analyzed separately to determine the concentrations of acidic, basic and neutral pharmaceuticals (Lavén et al., 2009). Another approach which limits the number of fractions for analysis to one, is to use a less selective sorbent like polystyrene–divinylbenzene (PS–DVB) without polar functional groups in its structure (Pichon et al., 1996; Filippov et al., 2003). The disadvantage of a more general sorbent is that it imposes a compromise on the selection of experimental conditions, often resulting in suboptimal detection for most of the analytes. However, a recent study has shown that although the recovery was lower for the selected pharmaceuticals on a PS–DVB sorbent compared to a more

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doi:10.1016/j.chemosphere.2010.06.065

polar sorbent, Oasis HLB, the two techniques showed similar detection limits due to less ion-suppression from the PS–DVB sorbent (Magnér et al., 2009a).

The development of general multi-residue analytical methods is important as it can simplify the preparation and analysis of samples in investigations comprising large numbers of samples.

Mass spectrometers (MS), like triple-quadruples (QqQ), operated in selected reaction monitoring (SRM) mode are often used for analysis of pharmaceuticals in environmental samples with high sensitivity. The weakness of the SRM technique is that only a limited number of pre-selected analytes can be recorded and full-scan mode with a triple-quadrupole MS lacks the selectivity needed, due to the low mass resolution (Petrovic et al., 2006; Ibáñez et al., 2008). However, when using time-of-flight (TOF) detection, with accurate mass determination errors of <5 ppm (Petrovic et al., 2006), full-scan analysis is a powerful alternative to analysis with QqQ using SRM. The high mass accuracy of the TOF detector enables the identification of the selected analytes and, additionally, allows for post-acquisition screening of non-target analytes (Ibáñez et al., 2009). Earlier, the applicability of QTOF instruments for trace analysis of environmental contaminants was hampered by the lower sensitivity, as compared with QqQs as shown by Hernández et al. (2005), and also by the limited dynamic range of QTOF instruments. However, as pointed out by Ibáñez et al. (2009) with the new generation of QTOF instruments introduced around 2005, instrumental detection limits (IDLs) were lowered, and method detection limits (MDLs) obtained with QTOF instruments for most compounds can be comparable to those obtained with QqQs (Farré et al., 2008). In addition, technical solutions for increasing the dynamic range were introduced, e.g. attenuation of the ion beam in order to avoid detector saturation. Also, by using UPLC in combination with either a QTOF, QqQ or other type of detector, the sensitivity is even further enhanced. The performance characteristics of the instrumental setup used in this study was reported earlier (Lavén et al., 2009). IDLs for the 15 studied pharmaceuticals ranged from 0.47 to 6.0 pg injected on column.

In the present study, a multi-residue method was applied, based on a previously described bag-solid phase extraction (bag-SPE) technique (Magnér et al., 2009a). In the previous study, the bag-SPE technique was applied to sewage treatment plant (STP) influent and effluent waters, and the bag-SPE method was compared to a conventional SPE method utilizing Oasis HLB cartridges. On the whole, the results obtained with the two methods were quite similar, both in terms of LOQs and determined concentrations. Hence, it was concluded that the analytical merits of the bag-SPE technique, in combination with the ease of handling, made it an attractive alternative to clean-up on conventional SPE columns. The aim of the present study was to investigate the applicability of the bag-SPE technique to recipient waters, i.e. in surface water close to the effluent of a sewage treatment plant (STP) and along a coastal gradient from a STP effluent. In order to increase the uptake of pharmaceuticals the bag-SPE method was scaled up from 20 mg to 100 mg adsorbent, and the sample volume was increased from 20 mL to 500 mL, as compared to the protocol used for sampling STP water. The method was evaluated based on its extraction efficiency of 10 pharmaceuticals (Table 1) at environmental levels (ng L^{-1}).

The 10 pharmaceuticals chosen for the validation were selected as model substances based on their occurrence in wastewater, and their distribution along the logarithmic octanol/water partitioning coefficient (Log K_{OW}) scale, from Log K_{OW} −0.13 to 4.39. The analytes were selected from different therapeutic classes, e.g. cardiovascular drugs (β-blockers) (atenolol and metoprolol), a central nervous system stimulant (caffeine) and inhibitor (oxazepam), an anti-epileptic drug (carbamazepine) in addition to five nonsteroidal anti-inflammatory drugs (ketoprofen, naproxen, ibuprofen, diclofenac, gemfibrozil).

2. Materials and methods

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).

Carbamazepine, diclofenac, gemfibrozil, ibuprofen, metoprolol, sulfadimethoxine, caffeine and terbutaline (Table 1) were purchased from Sigma (St Louis, MO, USA), ketoprofen from Riedel de Haën (Texas), naproxen from Fluka (Steinheim, Germany) and oxazepam from Cerilliant (Round rock, Texas).

Naproxen-d_3, diclofenac-d_4, gemfibrozil-d_6, terbutaline-d_9, metoprolol-d_7, and ketoprofen-^{13}C-d_3 were purchased from Toronto Research Chemicals Inc. (North York, Canada), carbamazepine-d_{10} and ibuprofen-d_3 from CDN Isotopes (Pointe-Claire, Quebec, Canada), and oxazepam-d_5 from Isotec/Sigma–Aldrich (St Louis, MO, USA).

Amberlite XAD-2 resin (particle size 297–840 μm) was obtained from FlukaChemie (Buchs, Schweiz). The paper-towel was obtained from Kimberly-Clark (Reigate, UK) and the woven polyester fabric (pore size <120 μm) from Ohlssons Tyger & Stuvar AB (Stockholm, Sweden).

Table 1
Pseudo-molecular ion (m/z), Log K_{OW}, pKa_1, pKa_2, recovery, relative standard deviation (RSD), limit of detection (LOD), limit of quantification (LOQ), linear concentration range, linear regression coefficient (R^2) and inter-day variation for the selected pharmaceuticals sampled with the bag-SPE method.

Compound	$[M+H]^{1+}$ m/z	$[M-H]^{1-}$ m/z	Log K_{OW}[a]	pKa_1[a]	pKa_2[a]	Recovery (%)	RSD (%)	LOD ($S/N = 3$) (ng L^{-1})	LOQ ($S/N = 10$) (ng L^{-1})	Concentration range (ng L^{-1})	Regression coefficient R^2	Inter-day variation ($n = 6$) (%)
Caffeine	195.0882		−0.13	0.73	–	10.6	16.4	8	26	10–800	0.9965	14.2
Atenolol	267.1709		0.097	9.16	13.88	22.2	15.2	13	43	13–800	0.9895	13.8
Metoprolol	268.1913		1.79	9.17	13.89	28.5	13.2	2	7	10–800	0.9819	14.4
Oxazepam	287.0587		2.31	1.68	10.94	42.5	9.5	4	13	10–800	0.9985	12.2
Carbamazepine	237.1028		2.67	–	13.94	53.9	11.5	1	3	10–800	0.9908	9.5
Ketoprofen	255.1021		2.81	–	4.23	42.8	11.6	7	23	10–800	0.9801	17.7
Naproxen		229.0865	3.00	–	4.84	37.9	5.1	12	40	12–800	0.9874	13.4
Ibuprofen		205.1229	3.72	–	4.41	63.0	3.2	7	23	10–800	0.9939	9.2
Diclofenac		294.0089	4.06	–	4.18	37.6	7.2	4	13	10–800	0.9919	14.4
Gemfibrozil		249.1491	4.39	–	4.75	64.5	12.0	6	20	10–800	0.9872	12.5

[a] Values from SciFinderScholar™ 2006, calculated using Advanced Chemistry Development (ACD/Labs, Toronto, ON, Canada) Software V8.14 for solaris (© 1994–2007 ACD/Labs).

2.2. Preparation of the bag-SPE sampler

The bag-SPE sampler consisted of two pieces of woven polyester fabrics (25 × 25 mm). The two parts of the fabrics were placed on top of each other and three of the four sides were welded with a heat-sealer (Möllerström, Gothenburg, Sweden). The bag was filled with 100 mg XAD-2 resin (polystyrene–divinylbenzene [PS–DVB]) and the opening was sealed.

2.3. Optimization and validation of the bag-SPE method

To determine the extraction efficiency of the bag-SPE technique eighteen samplers, each containing 100 mg of XAD-2 resin, were wetted in methanol (MeOH) (Magnér et al., 2009a) and thereafter placed in 1 L glass-bottles filled with 500 mL of tap-water spiked with unlabeled analytes at a concentration of 1.0 μg L^{-1}. The final solution was adjusted to pH 7 with ammonium bicarbonate. The samplers equilibrated with its surrounding for 0, 1, 2, 4, 8 or 16 h, respectively in triplicates, under mixing at 550 rpm with a stir-bar (2.5 cm long). After removal of the samplers from the spiked water, they were left to dry on a paper-towel for 1 min before extracting them for 30 min in 3.0 mL of MeOH at room temperature (Magnér et al., 2009a). The extract was evaporated to dryness under nitrogen at 40 °C and redissolved in 500 μL of ACN:H_2O (1:4), prior to analysis.

The absolute recovery of the selected analytes, in the above experiment, was established from the ratio of the peak area of an analyte extracted by the sampler with the peak area of the analyte initially available in the water phase. Prior to calculating this ratio the peak areas were adjusted with respect to the sample volumes. The initial concentration of the analytes in the water was established by evaporating 5.0 mL of water to dryness under nitrogen at 40 °C and redissolving it in 500 μL of ACN:H_2O (1:4) , prior to analysis.

The woven polyester fabric bags were weighed before and after sampling in order to monitor possible losses of XAD-2 sorbent during sampling.

The linearity of the uptake of analytes on the bag-SPE sampler was estimated by analysing a series of six 500 mL tap-water samples with 0, 10, 50, 100, 200, 400 and 800 ng L^{-1} of unlabeled standards, and adjusted to pH 7 with ammonium bicarbonate.

LODs were determined at a signal to noise ratio of 3 ($S/N = 3$) and the limit of quantification (LOQ) was determined at a signal to noise ratio of 10 ($S/N = 10$). For compounds whose LOD and LOQ were below the tested concentration range, the LOD and the LOQ were extrapolated from the regression line.

None of the analytes was present in detectable concentrations in the tap-water.

The inter-day variability of the method was obtained by determining the concentration of deuterated surrogate standards in six identical samples, sampled with bag-SPE on six different days.

2.4. Sample collection

Eight 5 L surface water samples were collected in the central bay of Stockholm, receiving waters from the Henriksdal STP (Fig. 1A). Furthermore, four 5 L surface water samples were collected along a costal gradient of 3.7, 8.7, 18.9 and 31.5 km downstream of Himmerfjärdens STP (Södertälje). The gradient was sampled to assess migration patterns of the selected pharmaceuticals in sea water (Fig. 1B). An additional 5 L surface water sample from the lake Flaten in suburban Stockholm was collected, and used as a reference sample.

Samples were collected in, 5 L polyethylene bottles, rinsed with methanol, Milli-Q water, and sea water prior to sampling. In order to minimize manipulation of the samples, no preservative was added. However, the bottles were filled to the top in order to minimize the contact between water and air, and the samples were protected from light. Following sampling, the bottles were transported to the laboratory where they were immediately cooled and stored at +8 °C until extraction and analysis. The personnel who performed the sampling did not consume either coffee or any of the analysed drugs.

2.5. Quantification

Known amounts of deuterated surrogate standards were added to the samples, prior to the bag-SPE extraction, at a final

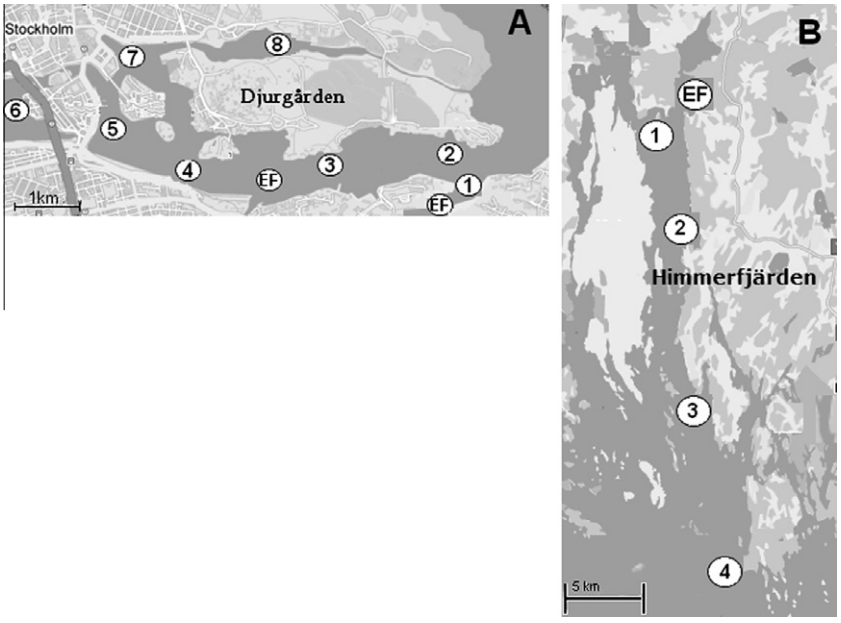

Fig. 1. (A) Sampling sites and wastewater effluents (EF) from the Henriksdals sewage treatment plant in the central bay of Stockholm. (B) Sampling sites along the coastal gradient from the wastewater effluent (EF) of Himmerfjärden sewage treatment plant (Södertälje).

concentration of 100 ng L^{-1}. Additionally, deuterated surrogate standards were added to the calibration standards, also containing the non-deuterated analogues. Using the QuanLynx software of MassLynx 4.1 (Waters, Manchester, UK), calibration curves were constructed, where the peak-area ratios of 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. For caffeine, for which no deuterated surrogate standard was available, a relative response factor was determined using metoprolol-d_7. The lower recovery of caffeine compared to metoprolol was compensated for by multiplying the calculated concentrations of caffeine by the ratio between the recoveries for metoprolol and caffeine. Since caffeine lacked its own deuterated surrogate standard, the results regarding concentrations of caffeine should be regarded as semi-quantitative.

Concentrations in calibration solutions, ranged from 10 to 200 pg μL^{-1}. Each sample was extracted and analysed in triplicates.

2.6. Instrumentation

A UPLC system (Waters, Milford, USA) was used for injection of samples and delivering of the mobile phase. The column, Acquity HSS T3 (2.1 × 100 mm, d_p 1.8 μm) (Waters, Milford, USA), was kept at 65 °C and the sample vials were kept at 20 °C. Injection volume was 5 μL. The mobile phase consisted of 10 mM acetic acid in water (A) and 10 mM acetic acid in acetonitrile (B). A linear gradient, at a flow rate of 0.6 mL min^{-1}, was used from 5% B to 90% B in 2.5 min, which was held for 2.5 min and thereafter returned to 5% B. The total cycle-time was 8.5 min.

For detection, a Micromass QTOF Premier (Waters, Manchester, UK) operated in ESI positive and negative ion mode with the TOF detector in 'V-mode', was used. The quadrupole was set to a wide pass mode and the collision energy was alternated between 2 and 20 eV, by using two full scan MS functions. The scan time of each MS scan function was 0.08 s and the inter-scan time 0.002 s.

The following settings were used in positive and negative ion mode, respectively: capillary voltage 3.0 kV; sampling cone voltage 25 V; extraction cone voltage 4.0/2.9 V; source temperature 100 °C, desolvation temperature 350 °C; cone gas (nitrogen) flow 50 L h^{-1} and desolvation gas (nitrogen) flow 700 L h^{-1}. Argon was used as a collision gas, at a flow rate of 0.5 mL min^{-1}giving a pressure of 3.5×10^{-3} mbar in the collision cell. External mass calibration was performed in the mass range m/z 100–1000 Th, using a series of cluster ions formed from 0.05 M NaOH and 0.5% formic acid dissolved in 2-propanol/H_2O (90:10). Sulfadimethoxine in methanol (0.1 ng μL^{-1}) was used as a lockspray solution in both positive (m/z 311.0814) and negative (m/z 309.0658) ion mode. The lockspray frequency was 5 s, with 5 scans to average.

3. Results and discussion

3.1. Validation of the method

The majority of the selected pharmaceuticals reached distribution equilibrium between the water and the bag-SPE sampler within 8 h (Fig. 2), hence 8 h was subsequently used as the extraction time. The time to reach distribution equilibrium between water and the sampler was highly dependent on the agitation speed of the stir-bar, i.e. a higher rotational speed on the stir-bar resulted in faster extraction of the analytes in the sample. However, when the rotational speed exceeded 550 rpm the bag-SPE sampler pushed the stir-bar out of its rotational path bringing the agitation of the water sample to an end. Hence, 550 rpm was subsequently used throughout the study.

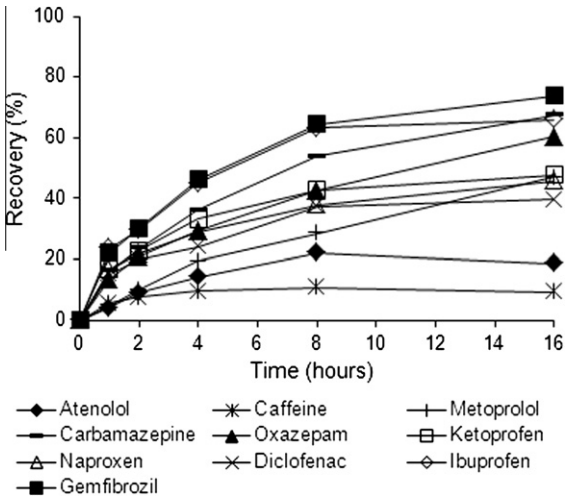

Fig. 2. The recovery of the selected analytes from the sample as a function of extraction time.

The extraction efficiency of the bag-SPE sampler ranged from 10.6% to 64.5%, with relative standard deviations (RSD) of < 16.4%, and the linear concentration ranges showed regression coefficients (R^2) better than 0.9801 (Table 1). The low recovery of caffeine is most likely due to the polar/water-soluble properties of this compound. It has the lowest Log K_{ow}, −0.13, of all of the analytes in this investigation, Table 1.

The inter-day variation of the six identical samples, sampled with the bag-SPE sampler on six different days, was less than 17.7%, which was considered acceptable (Table 1).

Finally, LODs of the 10 analytes in the bag-SPE extract were below 13 ng L^{-1} (Table 1), demonstrating that the method is suitable for detection of trace levels (ng L^{-1}) of pharmaceuticals in natural sea waters.

3.2. Application to real samples

Four of the selected 10 pharmaceuticals (caffeine, metoprolol, oxazepam and carbamazepine) were quantified in the surface water samples from the central bay of Stockholm (Fig. 3 and Table S1). The concentrations were within earlier reported concentration ranges, e.g. <LOQ–36 ng L^{-1} of metoprolol in Glatt river, Switzerland (Alder et al., 2010), <LOD–129 ng L^{-1} of oxazepam and <LOD–1160 ng L^{-1} of carbamazepine in rivers of Madrid metropolitan area, Spain (Alonzo et al., 2010), and 7–87 ng L^{-1} of caffeine in seawater from Tromsö Sound, Norway (Weigel et al., 2004). The eight sampling sites in the Stockholm area showed similar concentrations of respective compound (Fig. 3), probably due to the continuous introduction of pharmaceutical residues from the two STPs into the relatively closed bay (Fig. 1A).

Also the water samples collected along the coastal gradient at Södertälje contained detectable levels only of caffeine, metoprolol, oxazepam and carbamazepine (Fig. 4). For both sample series, mass errors were generally <5 ppm (Table S2).

The four pharmaceuticals revealed different migration patterns along the gradient. For example, the concentration of metoprolol decreased more rapidly than carbamazepine with increasing distance from the STP effluent (Fig. 4), despite that the reported half-lives upon solar irradiation were longer for metoprolol (Liu and Williams, 2007) than for carbamazepine (Andreozzi et al., 2002). One explanation for the faster decline in metoprolol concentrations along the gradient. could be that metoprolol, in contrast to carbamazepine, is mainly protonated and positively charged at

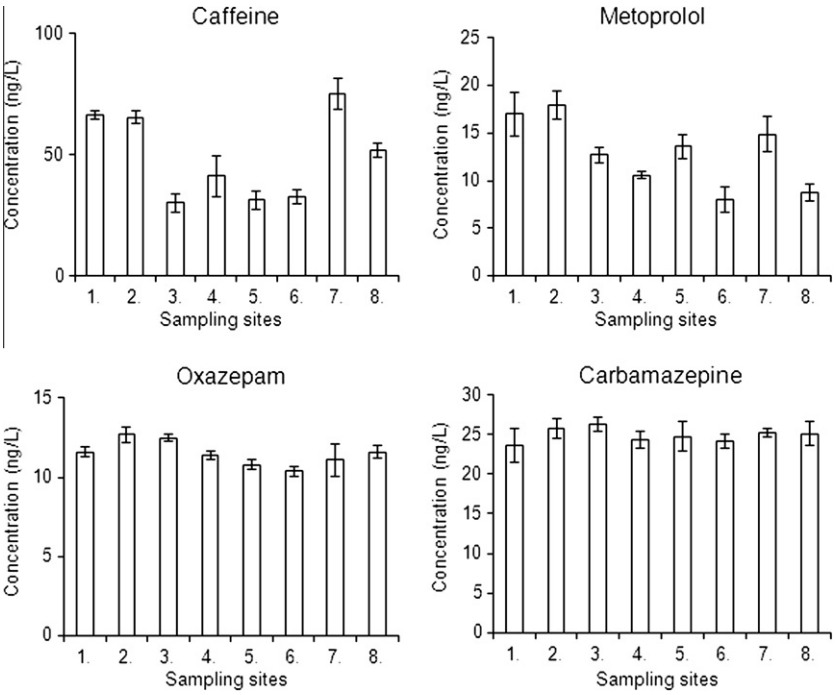

Fig. 3. The concentration of the detected pharmaceuticals in the central bay of Stockholm sampled with bag-SPE.

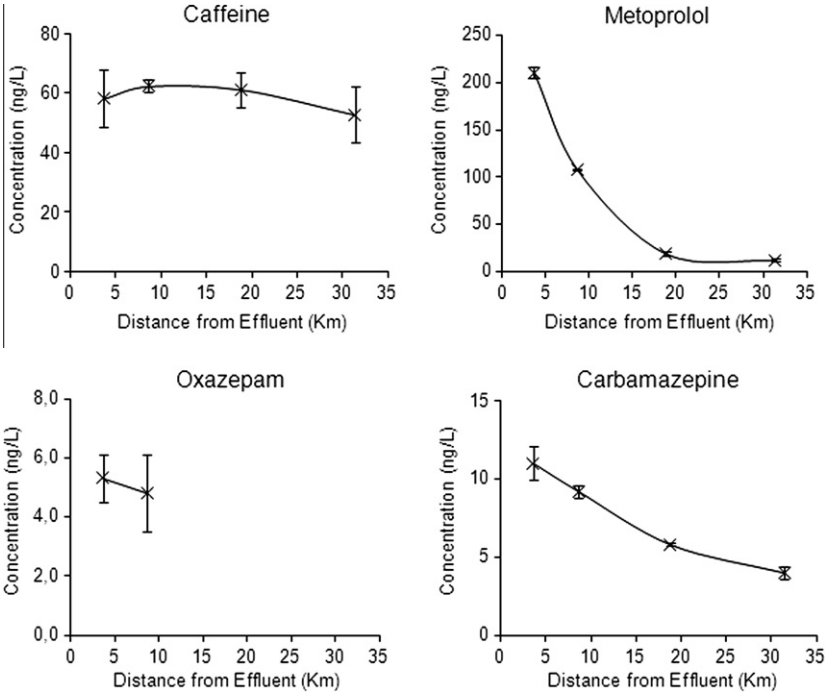

Fig. 4. The concentration of the detected pharmaceuticals along the coastal gradient from the effluent of Himmerfjärden sewage treatment plant (STP) sampled with bag-SPE.

natural pH, and therefore will associate with negatively charged carboxyl groups in dissolved organic matter (DOM) and particulate organic matter present in environmental waters (Lützhøft et al., 2000; Magnér et al., 2009b). The interaction with DOM and POM would result in a faster elimination of metoprolol from the water column, due to sedimentation.

Caffeine showed similar concentration levels throughout the gradient (Fig. 4), while similar measurements have reported a decrease in caffeine concentration with an increased distance from the STP (Weigel et al., 2004). However, caffeine is not only a constituent in pharmaceuticals but also an ingredient in beverages, e.g. coffee. One possible explanation to the even distribution of caffeine along the gradient could be that private homes and summer cottages along the coast in Sweden have their own septic tank system for their wastewater with a weeping bed that will contribute to the overall concentration of caffeine in the sea. This explanation could also be valid for the samples from the reference site, lake Flaten, which also showed detectable levels of caffeine (Table S1).

Additionally, the migration of oxazepam along the coastal gradient was impossible to interpret because the concentrations decreased below the LOD at the third and the fourth sampling sites (Fig. 4).

4. Conclusions

The primary aim of the work presented here was to explore the potential of the novel bag-SPE sampler for the sampling of pharmaceutical residues in natural waters. We conclude that the strength of the bag-SPE sampler over conventional SPE methods mainly is the ease of handling, i.e. no sample filtration required, unattended water extraction, and simple extraction of the analytes from the sampler, Taken together, the technique has great potential of being both time-effective and reliable. The use of labeled surrogates compensates for differences in extraction yields between different analytes, and for variations between water samples with regard to physico-chemical properties. Additionally the surrogates compensate for any degradation taking place during extraction, as well as for matrix effects e.g. ion suppression. Also, when samples are collected at locations far away from the analysing laboratory the bag-SPE samplers are easy to send by mail, as opposed to sending bulky water samples.

Acknowledgements

This research was financially supported by European Union (European Commission, FP6 Contract No. 003956) and by the Swedish research council Formas.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2010.06.065.

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