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Simultaneous solid-phase extraction of acidic, neutral and basic pharmaceuticals from aqueous samples at ambient (neutral) pH and their determination by gas chromatography–mass spectrometry

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Abstract

Seven polymeric solid-phase extraction (SPE) sorbents were evaluated with regard to their ability to extract acidic, neutral and basic pharmaceuticals and estrogens simultaneously from water at neutral pH. Highest recoveries (70–100%) for the majority of the analytes were obtained with styrene–methacrylate and styrene–*N*-vinylpyrrolidone co-polymers. The latter one (Oasis HLB) was chosen for further refinement of an extraction method for the quantitative determination of acidic and neutral drugs in surface water samples at detection limits below 1 ng/l. A sequential elution protocol was applied for clean-up and separation of the extracted analytes into fractions suitable for further compound specific processing. The neutral analytes as well as the acidic compounds after derivatisation were quantified by GC–MS. Caffeine, ibuprofen, its metabolites and diclofenac were detected in river water samples in the 1–100 ng/l range.

Keywords: Pharmaceutical analysis; Solid-phase extraction; Caffeine; Ibuprofen; Hydroxy-ibuprofen; Carboxy-ibuprofen; Diclofenac; Clofibric acid

1. Introduction

Solid-phase extraction (SPE) is widely used in the determination of contaminants (e.g. pesticides and pharmaceuticals) from environmental water samples [1–3]. Depending on the choice of sorbent, a wide range in polarity and chemical class may be covered. For the extraction of analytes of high polarity, polymeric sorbents often proved to be superior to alkylated silica (e.g. C_{18}-) sorbents [4–6]. A variety of hyper-crosslinked polystyrene-divinylbenzene (PS-DVB) based sorbents is commercially available, differing in the degree of linkage, porosity and surface area. Higher surface areas have been found to yield higher retention of analytes [7,8]. The exploration of the possibilities of functionalised polystyrenes for analytical SPE was intensified in the beginning of the 1990s with the introduction of acetyl- and hydroxymethyl-groups into PS-DVB resins [4]. Since then, a variety of polymers carrying different functionalities, e.g. carboxybenzoyl moieties [9], was developed and was reviewed recently [10,11]. In consequence, functionalised polymers became commercially available during the second half of the 1990s. They are either co-polymerisates of styrene and a hydrophilic component (e.g. methacrylate or *N*-vinylpyrrolidone) or the functional groups are introduced after polymerisation (e.g. by sulfonation). This results in mainly two effects: improved wetting characteristics for better mass transfer and additional possibilities for interactions with functional groups of the analytes and thus a higher retention. Due to these improvements, this generation of SPE-sorbents is increasingly used in the analysis of polar pesticides and pharmaceuticals in environmental water samples [12–14].

A tempting feature of these high surface PS-DVB, functionalised PS-DVB, and hydrophilic/lipophilic co-polymers is their capability to extract acidic analytes from water without acidification of the sample, together with neutral analytes of a wide polarity range. Pichon et al. [8] found recoveries >80% for acidic and neutral pesticides extracted jointly from water at pH 7 with the PS-DVB sorbent SDB-1. Furthermore, they showed that the co-extraction

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of humic and fulvic acids was significantly reduced at pH 7 as compared to extraction at pH 3. Own investigations [15] yielded recoveries of 40% or above for the extraction of acidic pharmaceuticals from alkaline (pH 8.3) seawater using the same sorbent. Up to date, few other studies reported on this potential of PS-DVB sorbents, e.g. [16]. More commonly, simultaneous extractions of acidic and base/neutral analytes, especially pesticides, were carried out with graphitised carbon black (GCB) sorbents [17–19]. However, significant drawbacks (desorption problems, presence of active oxygen complexes [20]) prevented a more widespread application of these sorbents. Modified PS-DVB sorbents combine the advantages of high retention of polar analytes and reproducible desorption and have recently been used for simultaneous extractions without pH adjustment [12,21].

The main advantages of the extraction with polymeric sorbents at neutral pH are: (i) simplified sample handling: no acidification step, no clean-up for the removal of humic and fulvic acids, (ii) possibility of on-line extraction, especially of large sample volumes, (iii) no enhanced risk of acidic hydrolysis of susceptible analytes during sample preparation, as observed for fenofibrate [22], (iv) no protonation of basic analytes. The resulting ability to extract a broad range of analytes simultaneously under the same conditions from one sample is essential when sampling and sample extraction is the limiting factor of the analytical procedure.

According to the differences in the chemical nature of the analytes, their determination often requires a separation into related groups. Especially in GC, various analytes are only accessible after derivatisation. Optimum sensitivity for different chemical groups, e.g. carboxylic acids, amines or steroids is achieved by specific derivatisation reactions. But also in LC–MS, separation and ionisation conditions can be specifically optimised when similar analytes are separated into groups. A separation can be achieved by the sequential elution of loaded SPE sorbent with solvents of different polarity as has been shown for GCB sorbents [17–19].

The intention of this work was to evaluate various different polymeric sorbents for their ability to extract acidic, neutral and basic analytes from water for a subsequent use in either LC–MS or GC–MS determination. In addition to three non-functionalised PS-DVB sorbents with surface areas of $\geq$1000 m^2/g (Bakerbond SDB-1, LiChrolut EN, Chromabond HR-P), two functionalised PS-DVB sorbents (Isolut Env+, Chromabond EASY) of high surface area (1000–1200 m^2/g) and two co-polymers composed of both lipophilic and hydrophilic monomers (Oasis HLB, abselut Nexus) of lower surface area (500–700 m^2/g) were included in the study. Based on the results of these recovery studies a method for the extraction of 1 l water samples, group separation of acidic and base/neutral analytes by sequential elution, derivatisation of the acidic analytes and determination by GC–MS was developed.

2. Experimental

2.1. Chemicals

Chemicals for standards, recovery experiments, derivatisation and buffering were purchased from the indicated companies: acetaminophen (paracetamol) from Aldrich (Taufkirchen, Germany), acetic acid (HPLC-grade) from Baker (Griesheim, Germany), mecoprop 2,2,4-trimethylpentylester and D_3-mecoprop from Dr. Ehrenstorfer (Augsburg, Germany), ammonium acetate (HPLC-grade) from Fluka (Neu Ulm, Germany), clofibric acid and propranolol hydrochloride from ICN Biomedicals (Eschwege, Germany), caffeine, *N*,*N*-diethyl-3-toluamid (DEET), methyl chloromethanoate, sodium sulfate p.a. granulated and triethylamine p.a. from Merck (Darmstadt, Germany), $^{15}N_2$-caffeine and triclosan from Promochem (Wesel, Germany), bezafibrate, 17ß-estradiol, estrone, fluoxetine hydrochloride, metoprolol tartrate and oxazepam from Sigma–Aldrich (Steinheim, Germany), carbamazepine, ibuprofen, sodium diclofenac from Synopharm (Barsbüttel, Germany). Solvents of organic trace analysis grade (acetone, ethyl acetate, *n*-hexane, methanol, toluene) and of gradient grade (methanol, water) were obtained from Merck.

Hydroxy-ibuprofen (ibu-OH) and carboxy-ibuprofen (ibu-CX) were synthesised according to [23,24], respectively and characterised by GC–MS and proton nuclear magnetic resonance spectroscopy (^{1}H NMR). Chemicals and solvents of synthesis grade were purchased from Merck except for 2-*p*-tolylmalonic acid diethylester, methylmalonic acid diethylester (Aldrich, Taufkirchen, Germany), 3-chloroperbenzoic acid (85%), potassium *tert*-butoxide, lithium bromide, palladium/carbon (10%) (Lancaster, Mühlheim, Germany).

The standard stock solutions for the HPLC experiments of ca. 100 μg/ml were prepared by dissolving approximately 10 mg (range from 10 to 15 mg) of the pure compounds in 100 ml methanol. In case of GC experiments solutions of ca. 200 μg/ml were prepared by dissolving approximately 20 mg (range from 21 to 36 mg) of the pure compounds in 100 ml methanol (acidic compounds) or acetone (neutral compounds). The working standard solutions were obtained by further dilution with methanol or acetone (for spiking) or toluene (for GC–MS calibration of neutral compounds). All solutions were stored at 277 K (4 °C) in the dark.

2.2. Sampling

Surface water samples were taken along the river Elbe in the region of Hamburg/Germany. This part of the river is under tidal influence, but not brackish. All samples were taken at falling tide, i.e. at downstream flow direction. One sample was taken from the lake Alster in the centre of the city, draining into the Elbe. For sampling, a submersible stainless steel sampler was used, operated

with cleaned 2.5 l glass solvent bottles [25]. Sampling dates and positions are listed with the results (see Table 4, Fig. 5). The samples were stored in the sampling bottles (no longer than 6 h) at 277 K (4 °C) in the dark. Further sample treatment of 1 l aliquots was carried out as described below.

2.3. Sample preparation

2.3.1. Sorbent comparison

One liter tap water samples (pH = 7.8) were spiked with 200 μl of a solution of the target analytes in methanol ($c \approx 10$ μg/ml). The 6 ml polypropylene test cartridges (for details, see Table 1) were obtained from the following manufacturers: SDB-1 (Baker, Griesheim, Germany), Chromabond HR-P and EASY (Macherey-Nagel, Düren, Germany), abselut Nexus (Varian, Darmstadt, Germany), Isolute Env+ (IST/Separtis, Grenzach-Whylen, Germany), LiChrolut EN (Merck, Darmstadt, Germany), Oasis HLB (Waters, Eschborn, Germany) and used with the vacuum-operated column processing system spe-12G (Baker). Prior to extraction, the cartridges were washed/conditioned with 5 ml *n*-hexane, 5 ml ethyl acetate, 10 ml methanol and 10 ml tap water. Afterwards, they were connected via large volume adaptors (IST) to the sample bottles. The approximate flow rate was 15 ml/min. After the extraction the cartridge was rinsed with 5 ml of de-ionised water and dried by nitrogen flow. The elution was performed with 30 ml of methanol. After addition of 50 μl of the volumetric internal standard triclosan (31 μg/ml in methanol), the eluates were condensed to 0.5 ml in a Turbovap 500 Closed Cell Concentrator (Zymark, Hopkinton, USA). The condensed extracts were transferred to vials and 400 μl of HPLC-grade water were added.

2.3.2. Surface water

Six milliliters glass cartridges with 20 μm PTFE-frits (both IST) were packed with 500 mg of 60 μm Oasis HLB bulk sorbent (Waters) and washed/conditioned as described above. Surface water samples were filtered with GF/C glass fibre filters, 47 mm diameter, 1.2 μm exclusion size (Whatman, Maidstone, UK), using a modified filtration apparatus (Sartorius, Göttingen, Germany). Afterwards, pH was adjusted to 7 with sulfuric acid (25%) and 100 μl of the surrogate standard mix (0.12 μg/ml D_3-mecoprop + 0.65 μg/ml $^{15}N_2$ caffeine) were added. For recovery experiments, 1 l tap water samples were adjusted to pH 7 and spiked with 100 μl of the respective standard solutions and 100 μl of the surrogate standard. The extraction was carried out on a Baker manifold via large volume adaptors (IST) at a flow rate of ∼15 ml/min. After drying of the cartridges with nitrogen, the elution was carried out sequentially with 5 ml of *n*-hexane, 5 ml of ethyl acetate and finally with 14 ml of methanol. The ethyl acetate eluates were reduced in volume under a gentle stream of nitrogen, the solvent was changed to toluene and the volumetric internal standard (25 ng mecoprop 2,2,4-trimethylpentylester in toluene) was added. The final volume was set to 50 μl. The methanol eluates were condensed to 0.5 ml in a Turbovap unit, transferred to 2 ml vials and the solvent removed under a stream of nitrogen. The acidic analytes were transformed to their methyl esters (triclosan to its alkoxycarbonyl derivative) by derivatisation with methyl chloromethanoate as described in [15]. After addition of the volumetric internal standard (25 ng mecoprop 2,2,4-trimethylpentylester in toluene) the extract was concentrated to 50 μl.

2.4. HPLC analysis

Quantification of the extracts from the sorbent comparison studies was carried out on a Gynkotek HPLC-system (Gynkotek, Germering, Germany), which consisted of the autosampler GINA 50, the pump M480 and the UV/DAD detector UVD 340 S. The column used was a LiChroCART 125-4 filled with LiChrosper 100 RP-18 (5 μm) from Merck (Darmstadt, Germany). Separation of the analytes was achieved by a methanol/water (10 mM ammonium acetate, 0.1% triethylamine, acetic acid to pH 5) gradient programme (Fig. 1), flow 0.5 ml/min, detection at 230 nm except for paracetamol (254 nm), injected volume 50 μl. Linear regression coefficients from a six-point linear calibration curve (concentration range 0.5–10 μg/ml) were between 0.9982 and 0.9999 for all compounds. Precision was in the range of 2–6% (at 2.5 μg/ml, $n = 7$).

2.5. Gas chromatography–mass spectrometry

Separation of the analytes was carried out on a Varian 3400 GC (Varian Associates, Sunnyvale, USA) equipped with the split/splitless injector 1075 (60 s splitless, 523 K (250 °C)) and a HP-5MS column (Agilent Technologies, Palo Alto, USA; length 30 m, i.d. 0.25 mm, film thickness 0.25 μm). Carrier gas was helium 5.0 (75 kPa), the transfer-line was held at 523 K (250 °C). The GC was operated with an A 200 SE autosampler (CTC Analytics AG, Zwingen, Switzerland) (injected volume 2 μl). Standard temperature programme was 353 K (80 °C) [2 min] → (7 K/min) → 533 K (260 °C) [10 min], while for recovery studies it was 353 K (80 °C) [2 min] → (10 K/min) → 523 K (250 °C) [5 min]. The gas chromatograph was coupled to a Magnum ITD ion trap mass spectrometer (Finnigan MAT, Bremen, Germany) which was operated under the following conditions: electron impact (EI)-ionisation at 70 eV, manifold temperature 473 K (200 °C), emission current 10 μA, scan range 100–399 amu. Instrument calibration was carried out at four points (concentration range 1–1000 ng/ml), yielding a linear calibration curve with regression coefficients of 0.9988 or above. Precision was between 2 and 4% (at 20 ng/ml, $n = 6$). Quantification was performed using the surrogate internal standards (D_3-mecoprop for the acidic and $^{15}N_2$-caffeine for the neutral compounds) and relative recovery rates.

Table 1
Properties of the tested SPE-cartridges, recovery rates (RR) and relative standard deviations (R.S.D.) of three replicate extractions ($n = 3$)

Sorbent		Bakerbond SDB-1		Lichrolut EN		Isolute Env+		Chromabond HR-P		Chromabond EASY		Abselut Nexus		Oasis HLB	
Polymer-type		PS-DVB		PS-DVB-EVB		PS-DVB-OH		PS-DVB		PS-DVB-AX		PS-MA		PS-DVB-NVP	
Surface area (m^2/g)		1060		1200		1000		1200		650–700		500–650		810	
Particle size (μm)		40–120		40–120		90		50–100		40/80		65–80		30	
Amount (mg)		200		200		200		500		500		200		200	
Recoveries (%)	log Kow	RR[a]	R.S.D.[b]	RR	R.S.D.	RR	R.S.D.	RR	R.S.D.	RR	R.S.D.	RR	R.S.D.	RR	R.S.D.
Paracetamol	0.3	60	4	37	4	39	22	72	4	50	25	0	0	14	2
Caffeine	−0.1	99	4	91	2	99	9	94	3	99	3	25	2	97	3
DEET	2.0	96	3	100	3	94	6	91	2	100	3	91	3	100	3
Carbamazepine	2.7	100	3	97	2	104	3	95	5	99	3	95	1	101	2
Oxazepam	2.3	65	3	74	2	81	4	27	5	80	4	91	4	98	1
Fluoxetine	–	69	4	80	5	86	7	53	5	86	4	94	4	88	2
Metoprolol	0.6	81	6	79	13	50	14	52	4	79	3	97	2	96	7
Propranolol	1.9	68	4	65	8	36	22	50	6	70	1	90	2	98	4
Estrone	3.7	92	2	75	0	80	3	54	5	71	3	92	1	96	3
17ß-Estradiol	4.1	96	2	89	3	101	5	85	5	95	0	95	1	98	2
Clofibric acid	−1.3	54	3	29	1	48	10	25	4	27	3	23	3	83	6
Bezafibrate	−0.4	55	9	55	5	43	9	23	5	18	110	87	2	95	2
Ibuprofen	0.3	46	2	61	4	55	9	6	10	10	25	68	1	98	1
Diclofenac	−0.4	42	6	62	3	38	7	19	4	1	92	90	3	102	2

Conditions: 1 l tap water samples (pH 7.8) spiked at a concentration of 2–5 μg/l. log Kow: calculated values for pH 8 [29]. PS: polystyrene, DVB: divinylbenzene, EVB: ethylvinylbenzene, OH: hydroxy, AX: weak anion exchanger, MA: methacrylate, NVP: *N*-vinylpyrrolidone.

[a] $n = 1$.

[b] R.S.D. determined from an earlier series (elution volume 70 ml, $n = 3$).

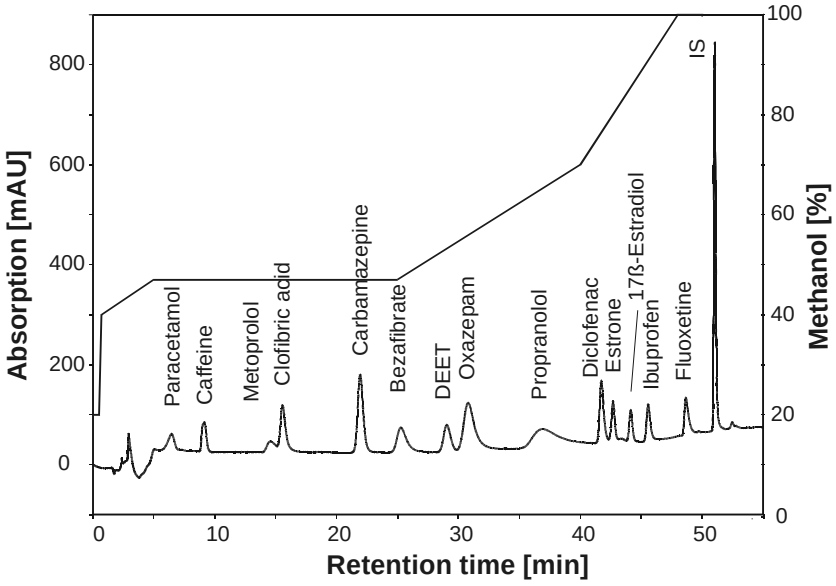

Fig. 1. HPLC–UV chromatogram of a standard solution ($c = 10\,\mu g/ml$).

3. Results and discussion

3.1. Comparison of sorbents

The test compounds (Fig. 2) were chosen to cover a wide range not only in view of their chemical properties. Representatives of several environmentally relevant pharmaceutical classes were included: analgesics, lipid lowering and psychopharmaceutical agents, ß-blockers, as well as the stimulant caffeine and two estrogens. The results of the extraction experiments are summarised in Table 1. Carbamazepine and DEET were almost quantitatively (90–100%) recovered on all investigated sorbents. The same holds for caffeine, with one exception (nexus: 14%). In this case, and in the case of paracetamol, which showed low to acceptable recoveries on all sorbents (0–72%), it can be assumed that their ready water solubility limits their retention. The highest recovery for paracetamol (72%) was obtained on Chromabond HR-P. It should be noted though that the two Chromabond cartridge types contained 500 mg of sorbent versus only 200 mg in the other test cartridges. Recoveries of the benzodiazepine oxazepam were ranging from 60 to 100% (except for HR-P: 27%), being highest on the two hydrophilic/lipophilic co-polymers. The three basic analytes, carrying all a secondary amino-function, were recovered at 70% or higher (exception: Isolute Env+ and Chromabond HR-P), in the case of the hydrophilic/lipophilic co-polymers at 90–100%. For the two estrogens included in the present study good recoveries were obtained. Except for Chromabond HR-P, they were higher than 75%. On the PS-DVB sorbents recoveries for estrone were generally lower than those for 17ß-estradiol, whereas on the two hydrophilic/lipophilic co-polymers, both were almost quantitatively recovered. The largest differences in behaviour were observed for the acidic analytes. Best results were obtained with Oasis HLB: quantitative recoveries for bezafibrate, ibuprofen and diclofenac and still 83% for clofibric acid, the compound with the lowest log Kow (−1.3) under the given conditions. Oasis HLB was followed in performance by the second hydrophilic/lipophilic co-polymer, abselut Nexus, with recoveries of 70–90% for most acids but a clearly lower value for clofibric acid (23%). This is in accordance with the low extraction efficiency of this sorbent for the hydrophilic compounds paracetamol and caffeine. Among the PS-DVB sorbents, Bakerbond SDB-1, Lichrolut EN, and Isolute ENV+ showed a comparable behaviour, with recoveries in the range of 40–60% and no significantly lower value for clofibric acid, apart from Lichrolut EN (29%). Exceptionally low values (1–27%) were observed for the two Chromabond sorbents HR-P and EASY for the acidic compounds. EASY is a PS-DVB sorbent carrying a "weak *anion* exchanger" [26]. This would require a more specific elution protocol for the acidic compounds instead of pure methanol. The relative standard deviations (R.S.D.s) were below 10% in most cases (Table 1) except for EASY (up to 110%). Average values obtained were 2% (Nexus), 3% (HLB), 4% (SDB-1, EN), 5% (HR-P). Clearly higher values were determined for Env+ (9%) and EASY (20%), the latter mainly due to the problems with the acidic analytes mentioned above.

Most manufacturers typically recommend elution volumes of around 5 ml methanol for 200 mg cartridges. In order to assure a complete elution of all analytes, in the present work all cartridges were eluted with 30 ml of methanol. Additionally, for each sorbent type, one cartridge was eluted with further 40 ml of methanol to check whether a residual amount of analytes remained on the cartridge. In several cases this yielded additional recoveries. In Table 2, these additional recoveries are compared to the mean

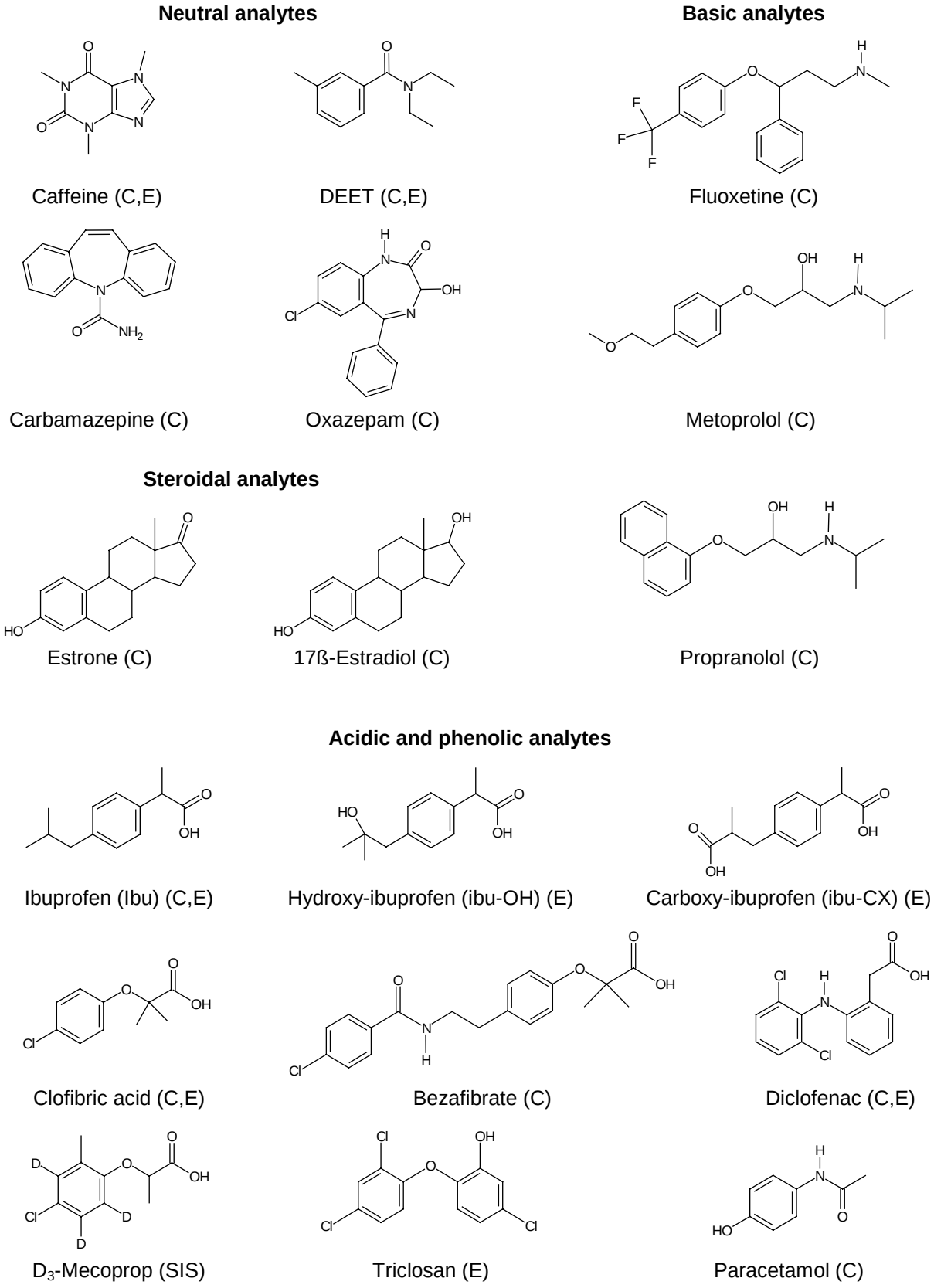

Fig. 2. Structures of the compounds investigated in the sorbent comparison (C) and in environmental samples (E), SIS: surrogate internal standard.

Table 2
Comparison of mean recovery rates (RR) (%) obtained by elution with 30 ml ($n = 3$) and additional recoveries (AR) by elution with further 40 ml ($n = 1$) of methanol

Sorbent	Bakerbond SDB-1			Lichrolut EN			Isolute Env+			Chromabond HR-P			Chromabond EASY			Abselut Nexus		Oasis HLB	
	RR[a]	AR	Σ	RR	AR	Σ	RR	AR	Σ	RR	AR	Σ	RR	AR	Σ	RR	AR	RR	AR
Paracetamol	60		60	37		37	39		39	72		72	50		50	0	No additional recovery	14	No additional recovery
Caffeine	99		99	91		91	99		99	94		94	99		99	25		97	
DEET	96		96	100		100	94		94	91		91	100		100	91		100	
Carbamazepine	100		100	97		97	104		104	95		95	99		99	95		101	
Oxazepam	65	4	68	74	7	81	81		81	27	16	43	80	1	81	91		98	
Fluoxetine	69	7	76	80	6	86	86	9	95	53		53	86	15	101	94		88	
Metoprolol	81	8	89	79	6	85	50	17	67	52	16	68	79	11	90	97		96	
Propranolol	68	14	82	65	17	82	36	22	58	50	10	60	70	16	86	90		98	
Estrone	92	2	94	75	11	86	80	9	89	54	11	65	71	10	81	92		96	
17ß-Estradiol	96	2	98	89	9	95	101		101	85	4	89	95	4	99	95		98	
Clofibric acid	54	6	61	29	9	38	48	10	58	25	11	36	27	24	51	23		83	
Bezafibrate	55	25	81	55	22	77	43	14	57	23	14	37	18	10	28	87		95	
Ibuprofen	46	26	72	61	11	72	55	17	72	6	9	15	10	21	31	68		98	
Diclofenac	42	31	72	62	11	73	38	14	52	19	14	33	1		1	90		102	

A fictitious overall recovery is given as the sum (Σ).
[a] $n = 1$.

recoveries obtained with 30 ml. No additional recoveries were observed for the two hydrophilic/lipophilic co-polymers Nexus and HLB. For the remaining PS-DVB type sorbents, only the polar neutral compounds were completely desorbed with the first 30 ml of solvent. Additional recovery of the comparatively lipophilic estrogens might be related to incomplete removal due to the high polarity of methanol. Possible explanations for the hindered desorption of the basic and acidic analytes are: (i) the existence of non-specified modifications of the PS-DVB matrix, e.g. a light sulfonation [27] which would explain desorption difficulties of the amino-compounds in case of ion-exchange interactions; (ii) incomplete removal of partly dissociated acids with neutral methanol.

Chromabond EASY, abselut Nexus and Oasis HLB are described as not requiring a solvent conditioning step. This was checked for the first two sorbents by running an extraction with a non-conditioned cartridge in parallel. For EASY, most recoveries are the same as with conditioning, except for the acidic compounds for which recoveries went down to 0%. Co-elution of sorbent matrix prevented the quantification of clofibric acid and metoprolol and led to erroneously high recoveries for propranolol and fluoxetine (163%). In the case of Nexus, all recoveries except that of estrone were reduced, some even drastically (e.g. caffeine, metoprolol, bezafibrate, diclofenac, ibuprofen). Quantification of clofibric acid and also fluoxetin was severely affected by co-elutions. Co-elutions clearly have to be attributed to the lack of a cartridge cleaning which is an important secondary effect of solvent conditioning. Furthermore, a higher flow resistance was caused by the hydrophobic polyethylene frits when they were not conditioned prior to extraction.

3.2. SPE/GC–MS method

After identification of Oasis HLB as the most suitable sorbent for the analytes of interest, a method for the extraction, separation and quantification of these compounds from water samples was developed, aiming at quantification limits below 1 ng/l. Initial experiments for the optimisation in terms of sensitivity and chromatographic performance led to the following choice of methods: acidic/phenolic compounds by GC–MS after derivatisation (methyl chloromethanoate), estrogens by GC–MS after a *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) based derivatisation, base/neutral nitrogen compounds by HPLC–MS–MS (ESI+). In this work, we focussed on a GC–MS method for the acidic compounds, including a few neutral analytes such as caffeine as a tracer for municipal sewage (Fig. 2). The resulting method is outlined in Fig. 3.

3.2.1. pH-dependence

In contrast to the sorbent comparison experiments, the pH was adjusted to 7. This additional step became necessary by the inclusion of the ibuprofen metabolites into the set of target analytes. The recovery of carboxy-ibuprofen (ibu-CX) was strongly affected by pH, at values above 7 recoveries were minimal (0.04% at pH 8). At pH 7, they were around 30–40%, increasing further with decreasing pH to 74% at pH 2. While this effect is expected for acidic compounds in general, it is interesting to note that in this case it was only observed for ibu-CX, the most hydrophilic compound (log Kow at pH 7: −2.8 [29]). Obviously, the concept of a simultaneous extraction of acidic and base/neutral analytes at neutral pH reaches its limitations here. For the other acids there was hardly any pH-effect on recoveries within the investigated range. Expectedly, the neutral compounds were not influenced by variations in pH.

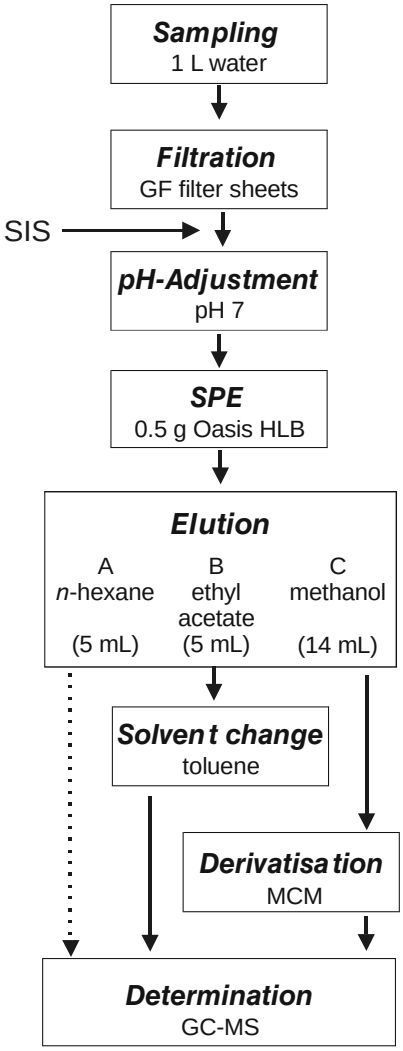

Fig. 3. Analytical procedure for the determination of neutral and acidic analytes.

3.2.2. Sequential elution

The elution of the loaded and dried cartridges was carried out sequentially with different solvents to divide the target analytes in separate groups. The initial elution with 5 ml of *n*-hexane removed lipophilic matrix components but none

Table 3
Recovery rates (RR) for extractions of 1 l of tap water, pH 6.8, spiking level 20–30 ng/l, relative standard deviations (R.S.D.; $n = 3$), linear regression coefficients (r^2; concentration range 0.2–200 ng/l) and reproducibility as coefficients of variation (CV; $n = 6$) for the extraction method, instrumental limits of quantification (LOQ, s/n = 9), ions used for quantification (underlined) and as qualifiers for GC–MS analysis, for the acidic compounds as the methyl (-Me), dimethyl (-di-Me) esters or the methoxycarbonyl derivative (-COOMe)

Compound	RR (%)	R.S.D. (%)	r^2	CV (%)	LOQ (ng/l)	Ions (*m/z*, amu)
Ibuprofen	74	5	0.9999	10	0.05	161, 220 (-Me)
Ibu-OH	92	2	0.9999	10	0.38	119, 178 (-Me)
Ibu-CX	30	12	0.9992	34	0.21	145, 205 (di-Me)
Clofibric acid	108	21	1	19	0.26	128, 228 (-Me)
Diclofenac	87	1	1	4	0.08	214, 242 (-Me)
Triclosan	66	6	0.9992	14	0.16	252, 346 (-COOMe)
D_3-Mecoprop (SIS)	94	2	1	–	–	172, 231 (-Me)
DEET	82	4	0.9996	13	0.16	119, 190
Caffeine	95	4	1	9	0.25	109, 194
$^{15}N_2$-Caffeine (SIS)	99	4	1	–	–	110, 196

of the target analytes from the sorbent and thus served as a clean-up step. The following elution with 5 ml of ethyl acetate removed the neutral/basic analytes such as caffeine and DEET while the final elution with 14 ml of methanol yielded the acidic analytes, e.g. ibuprofen and clofibric acid. Most acidic analytes were eluted within the first 5 ml of methanol, only diclofenac required an additional 9 ml for maximum recovery.

3.2.3. Recoveries, linearity, quantification limits

Recovery rates for the present method were determined at an environmentally relevant concentration of ~20 ng/l and were in the range of 70–100% (Table 3). For triclosan, it is slightly lower which is owed to the fact that a small proportion of this analyte is already eluted in the ethyl acetate fraction. Ibu-CX has an exceptionally low recovery due to the reasons discussed above. R.S.D.s were generally low (1–6%) with two exceptions. For ibu-CX the tight control of the pH is crucial for the extraction accuracy. Already small variations alter recovery in a way that affects the R.S.D. In the case of clofibric acid the high variations originate rather from the derivatisation than from the extraction. In comparison to the results from the sorbent testing experiments, recoveries were lower for some compounds, e.g. ibuprofen (74% versus 98%). The prepacked cartridges used in the sorbent comparison contained 200 mg HLB of a particle size of 30 μm while the bulk material used for laboratory packing of glass cartridges is exclusively available in a 60 μm quality. Using the same amount of sorbent (200 mg), recoveries were lower for the 60 μm material. So either the two sorbents do not only differ in particle size (and thus number of theoretical plates) but also in their extraction properties or the observed effect is caused by charge-to-charge variations. In this context it is interesting to note that for the Oasis HLB sorbent the recoveries obtained under similar conditions for some of the investigated analytes vary considerably in different publications. Farré et al. [28] for example used 300 mg of Oasis HLB (particle size not specified) for 1 l water samples at pH = 7 and flow rates of 10 ml/min and recovered ibuprofen at only 38%.

Linearity of the method was given in the concentration range expected in environmental samples (0.2–200 ng/l). Linear regression coefficients (four point calibration) were 0.9992 or above. The repeatability expressed as coefficients of variation was in the range of 4–19%, except for ibu-CX (34%) for the mentioned reason. A ruggedness testing was carried out for reasonable variations in the following parameters: pH of the sample, extraction flow rates, extracted volume, cartridge drying times, elution volumes, derivatisation conditions and sample matrix (tap-, river-, lake-water). The method was robust against most variations, while a tight control of the conditions was crucial for the sample pH (only for ibu-CX), the elution volumes, and the derivatisation (only for clofibric acid). Instrumental limits of quantification (LOQs; signal to noise ratio of 9) as determined from standard runs were in the range of 0.05–0.38 ng/l (Table 3).

3.3. Surface water samples

The present method demonstrated its ruggedness and applicability to environmental samples in the processing of a first set of surface water samples from the river Elbe and the lake Alster at Hamburg/Germany (Table 4). The majority of the analytes was detected in all samples as shown examplarily in the chromatogram of a river water sample (Fig. 4). Only triclosan which is prone to adsorption to particles due to its more lipophilic character (log Kow = 5.8 [29]) was not detected in more than one sample above the LOQ since the method covers dissolved analytes only. On the right bank of the river (samples H-08, H-09, H-10; for sampling positions, see Fig. 5), opposite the discharge of the central STP of the city, the concentrations of most target analytes were rather similar to each other within the investigated distance. This indicates a homogeneous water body with no significant transformation processes occurring within this stretch. Only the concentrations of the ibuprofen group were deviating at

Table 4
Sampling positions, dates, and concentrations (ng/l) of the investigated analytes in surface water samples from Hamburg, Germany

Sample	H-02	H-08	H-09	H-10	H-14	H-15	H-07
Position	Right bank 626.7 km	Right bank 626.7 km	Right bank 630 km	Right bank 637.7 km	Left bank 628.6 km	Left bank 622.3 km	Lake Alster
Date	23 October 2002	7 November 2002	7 November 2002	7 November 2002	19 November 2002	19 November 2002	5 November 2002
Clofibric acid	4.0	6.3	4.7	4.7	3.2	7.6	2.4
Ibuprofen	5.6	6.0	5.1	11	8.7	32	4.9
Ibu-OH	31	41	23	50	32	101	18
Ibu-CX	<LOQ	15	12	21	11	32	9.5
Diclofenac	38	32	31	33	42	67	26
Triclosan	nd	nd	nd	<LOQ	<LOQ	4.1	nd
Mecoprop	7.6	6.6	6.8	6.7	7.0	6.3	22
Caffeine	98	104	103	104	150	148	176
DEET	38	26	25	24	16	20	7.0

nd: not detected, LOQ: limit of quantification.

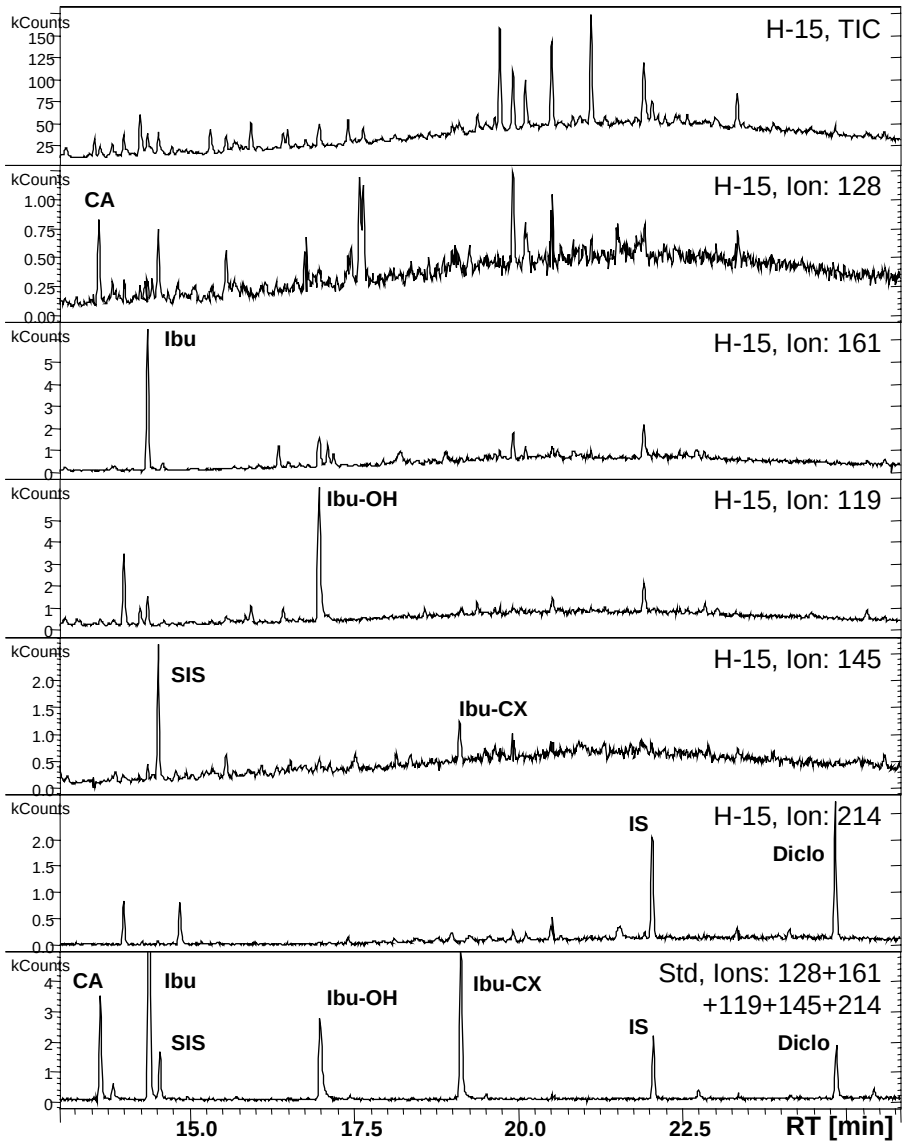

Fig. 4. GC–MS chromatogram of the methanolic fraction of a river water sample (H-15) in comparison to a standard solution (Std; $c \approx 200$ ng/ml) after derivatisation, displaying the total ion current (TIC) and extracted ion traces.

the three sampling locations. The variation in concentrations between two samples taken at the same location at an interval of 2 weeks (H-02 and H-08) was low for most compounds. On the left bank of the river, one sample was taken upstream (H-15) and a second one downstream (H-14) of the confluence of the southern arm of the river, bearing the discharge of the municipal STP, into the main course. Interestingly, concentrations were higher in the upstream sample, except for caffeine, DEET and mecoprop, which were present in similar concentrations in both samples. This result may be explained by the fact that this part of the river is strongly influenced by tides. This means that the flow direction is changing according to tides and the same water body is moving back and forth various times before it reaches the sea. In this way, higher concentrations are observed upstream of the discharge in case that the sampled water body (from the previous tidal cycle) has received higher inputs. Compared to the samples from the right bank of the river Elbe, concentrations of some compounds appeared to be up to 50% higher on the left bank (e.g. caffeine and diclofenac), while for the herbicide mecoprop they were in the same range. Since the samples on the left and on the right bank were taken on different days, this finding may only serve as an indication for the influence of the STP discharge on concentrations of pharmaceuticals and personal care products (PPCPs). The herbicide mecoprop as an indicator for non-STP-derived emissions was rather evenly distributed in all river samples.

In lake water (H-07), concentrations of the pharmaceuticals were lower than in the river but not as much as could be expected. No regular sewage emissions are reported for the lake and its tributaries. Only rarely, as a result of heavy rain

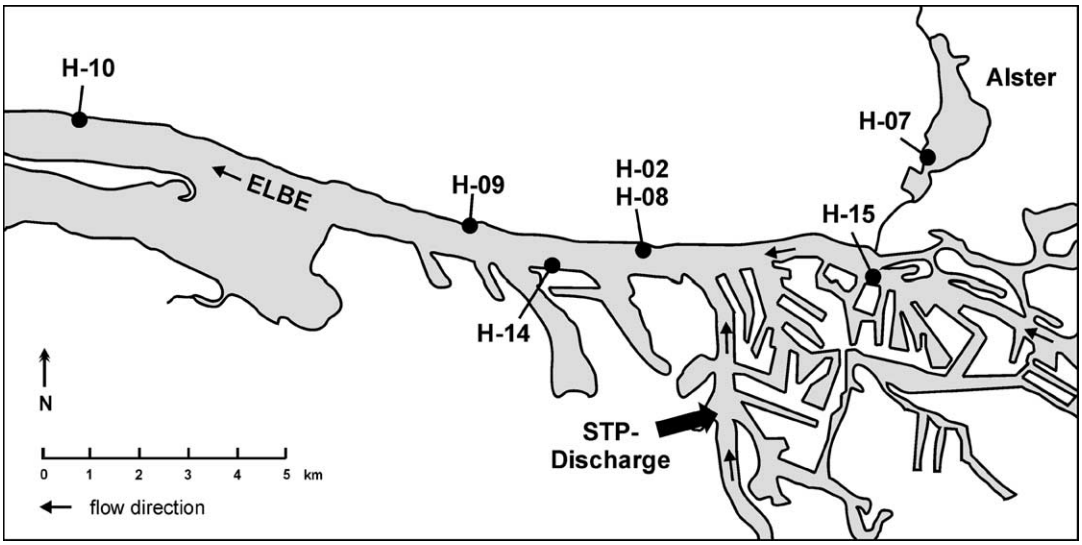

Fig. 5. Sampling positions at the river Elbe and the lake Alster at Hamburg/Germany.

events, the municipal sewage system is overloaded and raw sewage is discharged into channels connected to the lake. It is rather unlikely that these amounts account for the detected levels. The concentration of caffeine in lake Alster is even higher than in Elbe river water that is directly affected by STP-discharge. Even taking into account the low water exchange of the lake, these findings may be an indication for additional sources. The concentration of mecoprop was three-fold higher in the lake than in river Elbe water. In addition to agriculture in the upper reaches of the contributing river the application of mecoprop on lawns in the vast gardens and parks along the lake and its tributaries is a likely source.

4. Conclusions

The comparison of different types of polymeric SPE-sorbents demonstrated the potential of these materials to extract acidic, neutral and basic analytes simultaneously even at neutral pH. Under these conditions the best performance was achieved with two hydrophilic/lipophilic co-polymers. Oasis HLB yielded quantitative recoveries for all tested compounds except for paracetamol. These features, in conjunction with a sequential elution protocol, enable the reduction of sample numbers (which is especially useful in cases where sampling is a limiting factor, e.g. marine large volume samples) and facilitate sample handling. Thus, a broad array of PPCPs ranging widely in chemical structure can be covered by a single extraction. Based upon the results of the sorbent testing, a trace analytical method for the determination of a set of acidic and neutral PPCPs was developed, allowing recoveries above 70% at a pH of 7. Only the di-carboxy compound ibu-CX was not satisfactorily recovered at this pH, indicating the sorbent's limitations. Quantification limits were in the sub ng/l-range for 1 l aqueous samples. The application of the method to surface water samples demonstrated its robustness and delivered data on the distribution of these compounds in the aquatic environment.

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