Journal of Chromatography A, 1217 (2010) 6511–6521

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Development of a multi-residue analytical method, based on liquid chromatography–tandem mass spectrometry, for the simultaneous determination of 46 micro-contaminants in aqueous samples

Karsten Nödler[a,*], Tobias Licha[a], Kai Bester[b], Martin Sauter[a]

[a] Geoscience Centre of the University of Göttingen, Department Applied Geology, Goldschmidtstr. 3, 37077 Göttingen, Germany
[b] National Environmental Research Institute, Aarhus University, Frederiksborgsvej 399, 4000 Roskilde, Denmark

ARTICLE INFO

Article history:
Received 31 March 2010
Received in revised form 12 July 2010
Accepted 19 August 2010
Available online 26 August 2010

Keywords:
Liquid chromatography–tandem mass spectrometry
Pharmaceuticals
Extraction
Sorbents
Drying gas temperature
Switching electrospray ionization

ABSTRACT

A multi-residue analytical method based on high-performance liquid chromatographic separation, electrospray ionization with tandem mass spectrometric detection (HPLC/MS–MS) was developed for the simultaneous analysis of 46 basic, neutral and acidic compounds covering a wide range of polarity ($\log K_{OW}$ < 0–5.9). The compound list included selected iodinated contrast media, analgesics, anti-inflammatories, stimulants, beta-blockers, antibiotics, lipid regulators, anti-histamines, psychiatric drugs, herbicides, corrosion inhibitors and the gastric acid regulator pantoprazole. The main feature of the presented method was a simultaneous solid phase extraction (SPE) of all analytes followed by simultaneous separation and detection by HPLC/MS–MS with electrospray ionization in both positive and negative polarization within the same chromatogram. Optimization of electrospray drying gas temperature resulted in using a temperature gradient on the ion source. Six different polymeric sorbents for SPE were compared with respect to recoveries, taking into account the specific surface of each sorbent. Method quantitation limits (MQL) in surface and seawater ranged from 1.2 to 28 ng/L, in wastewater from 5.0 to 160 ng/L, respectively. In order to demonstrate the applicability of the method, river water, treated wastewater and seawater were analyzed.

1. Introduction

During the last decades, the field of instrumental analytical sciences has progressed rapidly. This advancement has laid the foundation for the analysis of numerous organic compounds in trace concentrations (ng/L range), acknowledging the widespread occurrence of organics with anthropogenic origin in all parts of the water cycle [1–3]. Several multi-residue analytical methods using high-performance liquid chromatography (HPLC) with mass spectrometric detection and different ionization techniques have been discussed in the literature (e.g. [4–10]) With respect to electrospray ionization, some compounds are preferably ionized in negative (ESI−), while others give better signals in positive mode (ESI+). To support better ionization often different eluents are used for ESI+ and ESI− respectively. Thus different chromatographic runs have to be performed for chemically different groups of compounds [5]. Additionally, fast switching between negative and positive mode has not been possible with older instruments in a stable way and up to now there are only a few studies reported in the literature using this feature [11–13]. A critical issue of connecting liquid chromatography with electrospray ionization is that acidic compounds can be best chromatographed in the undissociated form (eluent pH < pK_a), while they give best ESI− signals in their dissociated form (eluent pH > pK_a) [14]. This illustrates the importance and difficulty of carefully identifying a suitable HPLC column and a generic eluent composition for multi-residue analyses.

For this study, a multi-residue analytical method was chosen over a single group approach in order to reduce cost and time while simultaneously obtaining information on the occurrence and fate of a broad spectrum of xenobiotic compounds. With respect to simultaneous pre-concentration, combinations of various pharmaceuticals such as analgesics, anti-inflammatories, beta-blockers, lipid regulators, antibiotics, psychiatric drugs, anti-histamines and caffeine are common [4,5,15–17] as well as the combination of herbicides and pharmaceuticals [6]. Methods for single group analysis of iodinated contrast media (ICM) are well established [18], but their incorporation into multi-residue methods is rare and with respect to ICM currently applicable for iopromide only [15,17,19]. However, there is a huge difference in concentration e.g., iopromide occurs with thousands of ng/L on the one hand and the sedative diazepam with only a few ng/L in the same samples [19]. This

* Corresponding author. Tel.: +49 5513919332; fax: +49 551399379.
E-mail addresses: karsten.noedler@geo.uni-goettingen.de, karsten.noedler@gmx.de (K. Nödler).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.08.048

range of concentrations is highly demanding considering the linear range of the method and cross talk in the mass spectrometer. To the authors knowledge no multi-residue analytical method, which incorporates all of the compounds discussed here, has been developed to this date.

The objective of the presented work was to develop a multi-residue analytical method including simultaneous sample pre-treatment, simultaneous separation and detection of 46 basic, neutral and acidic analytes such as selected ICM, analgesics, anti-inflammatories, stimulants, beta-blockers, antibiotics, lipid regulators, anti-histamines, psychiatric drugs, herbicides, corrosion inhibitors and the gastric acid regulator pantoprazole. Pantoprazole has recently become an over-the-counter drug in the European Union and thus must be regarded as an emerging contaminant in the near future.

2. Experimental

2.1. Chemicals and materials

Methanol (LC/MS grade) was purchased from Fisher Scientific (Schwerte, Germany), ethyl acetate, acetonitrile, ammonium acetate and formic acid (all analytical grade) were purchased from VWR (Darmstadt, Germany). Ultrapure water was obtained from a combined water purification system consisting of Elix 5 (Progard 1 silver cartridge) and Milli-Q Gradient A10 (Quantum Ex Ultrapure Organex + Q-Gard 1 cartridge), both from Millipore (Schwalbach, Germany).

Reference standards of erythromycin A, diclofenac, paraxanthine, theobromine, theophylline, 1-methylxanthine, 3-methylxanthine, metoprolol, roxithromycin, sulfamethoxazole, bezafibrate, clofibric acid, carbamazepine, citalopram, fluoxetine, sertraline, tolyltriazole, pantoprazole, iohexol, primidone and 1H-benzotriazole were purchased from Sigma–Aldrich (Steinheim, Germany). Ibuprofen and caffeine were from Fisher Scientific (Schwerte, Germany), naproxen, paracetamol (acetaminophen), phenazone, atenolol, trimethoprim and diazepam from Fagron (Barsbüttel, Germany). Atrazine, desethylatrazine, desisopropylatrazine, diuron, isoproturon, mecoprop and metazachlor were purchased from Dr. Ehrenstorfer (Augsburg, Germany), gemfibrozil from Salutas (Barleben, Germany), tetrazepam from Welding Pharma (Hamburg, Germany) and benzoylecgonine from LGC Promochem (Wesel, Germany). Cetirizine and loratadine were from KSK-Pharma (Berghausen, Germany), iopamidol and iopromide from Bayer (Leverkusen, Germany) and iomeprol from Bracco Imaging (Konstanz, Germany). Sotalol and clarithromycin were obtained from pharmaceutical preparations for intravenous injection, respectively, from Carinopharm, Gronau (Leine), Germany (Carino Sotalol i.v. 40 mg) and Abbott, Wiesbaden, Germany (Klacid® i.v. 500 mg). A summary of all analytes is presented in Table 1.

To avoid internal reactions, individual standard solutions were prepared. Unless otherwise stated, individual standard solutions with concentrations up to 10 mg/mL were prepared in methanol. Cetirizine was dissolved in acetonitrile in order to prevent esterification with methanol. This reaction occurred rapidly when the compound was dissolved in methanol (<1 week). Caffeine, paraxanthine, theobromine, theophylline and the mono-methylxanthines were dissolved jointly in warm water (60 °C, 100 μg/mL each) and diluted to 10 μg/mL in methanol. Individual standard solutions of iopamidol, iopromide, iohexol and iomeprol were prepared in methanol containing 20% water (v/v). Multi-standard solutions for calibration were obtained by mixing individual standard solutions and gradual dilution with acetonitrile (to prevent cetirizine esterification). All individual reference standard solutions and multi-standard solutions were stored at −18 °C.

Paraxanthine-D_6, atenolol-D_7, erythromycin-N-methyl-^{13}C-D_3, ibuprofen-D_3 and lansoprazole were purchased from Sigma–Aldrich (Steinheim, Germany). Desmethoxyiopromide, sulfamethoxazole-$^{13}C_6$, carbamazepine-D_{10}, diazepam-D_5 and fluoxetine-D_6 were from LGC Promochem (Wesel, Germany). Isoproturon-D_6, atrazine-D_5 and mecoprop-D_3 were from Dr. Ehrenstorfer (Augsburg, Germany) while loratadine-D_4 was purchased from Biomol (Hamburg, Germany). The internal standard (E)-9-[O-(2-methyloxime)]-erythromycin (in the following called *ery-methyloxime*) was synthesized according to Schlüsener et al. [20]. However, instead of purification on silica gel the crude product containing significant amounts of erythromycin and anhydro-erythromycin has been washed ten times with acetonitrile. The identity and purity of the final product was verified by HPLC/MS–MS and neither erythromycin nor its anhydro-derivative was detected after washing. The purified macrolide internal standard showed two resolved peaks in HPLC–MS/MS with a peak area ratio of 1:9 probably caused by isomeric effects

Table 1
Analytes and their application/origin.

Application or origin	Compound	Application or origin	Compound
Analgesics/anti-inflammatories	Diclofenac	Lipid regulators	Bezafibrate
	Ibuprofen		Clofibric acid
	Naproxen		Gemfibrozil
	Paracetamol	Anti-histamines	Cetirizine
	Phenazone		Loratadine
Stimulants/caffeine metabolites	Caffeine	Anticonvulsants/sedatives	Carbamazepine
	Paraxanthine		Diazepam
	Theobromine		Primidone
	Theophylline		Tetrazepam
	1-Methylxanthine	Selective serotonin reuptake inhibitors	Citalopram
	3-Methylxanthine		Fluoxetine
Antihypertensive agents	Atenolol		Sertraline
	Metoprolol	Herbicides/herbicide metabolites	Atrazine
	Sotalol		Desethylatrazine
Iodinated contrast media	Iohexol		Desisopropylatrazine
	Iomeprol		Diuron
	Iopamidol		Isoproturon
	Iopromide		Mecoprop
Antibiotics	Clarithromycin		Metazachlor
	Erythromycin	Corrosion inhibitors	1H-benzotriazole
	Roxithromycin		Tolyltriazole
	Sulfamethoxazole	Cocaine metabolite	Benzoylecgonine
	Trimethoprim	Gastric acid regulator	Pantoprazole

Table 2
Sample preparation scheme of the surface-normalized SPE experiments.

Sorbent	Specific surface (m^2/g)	Surface per cartridge (m^2)	Extracted standard solution (mL)	Rinse water (μL)	Re-dissolution (μL)
OASIS HLB	810	405	405	1620	1000
Bond Elut Plexa	450	90	90	360	500
AbsElut Nexus	575	115	115	460	500
Isolute ENV+	1000	500	500	2000	1000
Strata X	800	400	400	1600	1000
Bond Elut PPL	600	300	300	1200	1000

as erythromycin (purity ≥95%) from Fagron (Barsbüttel, Germany) was used as reactant in the synthesis. The more intensive peak was used as the internal standard and the resolution of both was used as a qualitative indicator for matrix-induced deterioration of separation efficiency for basic compounds.

An internal standard (IS) mix with concentrations of 40 ng/μL desmethoxyiopromide, 30 ng/μL fluoxetine-D_6, 20 ng/μL of lansoprazole, paraxanthine-D_6, erythromycin-N-methyl-^{13}C-D_3, ibuprofen-D_3 and 10 ng/μL of the other respective internal standard compounds was prepared in acetonitrile.

During the method development the following disposable cartridges (6 mL) for solid phase extraction (SPE) were used: OASIS HLB 500 mg from Waters, Eschborn, Germany; Bond Elut Plexa 200 mg, AbsElut Nexus 200 mg and Bond Elut PPL 500 mg all from Varian (Darmstadt, Germany); Strata X 500 mg from Phenomenex (Aschaffenburg, Germany) and Isolute ENV+ 500 mg from Separtis (Grenzach-Wyhlen, Germany). The intention was to identify the most suitable sorbent for extracting the selected analytes. Environmental samples can contain humic and fulvic acids. In comparison to acidified samples, co-extraction of these matrix compounds may be significantly lower at neutral pH [21]. Regarding that and the final method, which should be applicable to environmental analysis, all experiments on solid phase extraction were performed at pH values buffered by dihydrogen phosphate to 7 ± 0.2. Additionally, some analytes such as erythromycin A are susceptible to chemical degradation due to acidification and/or alkalinization [22,23].

2.2. Surface-normalized comparison of selected SPE-sorbents

A phosphate buffer containing 13.4 g potassium dihydrogen phosphate and 6.22 g disodium hydrogen phosphate dihydrate (both from VWR Darmstadt, Germany) per liter buffer solution was used in a ratio of 10 mL buffer solution per 1000 mL sample (pH 7 ± 0.2).

Six commercially available cartridges were tested. An approach following typical adsorption studies, i.e. technical adsorption on carbon black by using a surface-normalized approach in a matrix-free system, was applied [24]. In this way, a more realistic comparison for the individual sorbent capacities and mechanisms can be obtained. For this purpose, each cartridge was tested with buffered ultrapure water containing 1 μg/L of iohexol, iomeprol, iopamidol and iopromide, and 0.5 μg/L of any other analyte, respectively. A constant ratio of 1 mL solution per m^2 sorbent was applied resulting in a range of 500 mL for 500 mg Isolute ENV+ to 90 mL for 200 mg Bond Elut Plexa. Therefore, a constant analyte to surface ratio of 1 ng/m^2 ICM and 0.5 ng/m^2 for all compounds other than ICM was applied to all types of sorbent. For a better illustration, a sample preparation scheme of the experiment is shown in Table 2. In order to exclude effects of varying extraction kinetics of the sorbents, a low extraction speed of 3 mL/min was used. Prior to extraction, the SPE cartridges were conditioned with 4 mL of methanol and rinsed twice with 4 mL of ultrapure water successively. After extraction each cartridge was rinsed with twice the amount of ultrapure water given in Table 2, as the amount of rinsing water was also adjusted to the specific surface of the respective sorbent. The rinsed cartridges were dried by drawing air through the cartridges for 30–45 min and sequentially eluted with 2 × 2 mL of methanol and 2 × 2 mL ethyl acetate. The combined extract was spiked with 5 μL of internal standard by using a 10-μL syringe. Internal standards were applied to compensate any adverse ionization effects. Thus, for calculating the absolute recovery in this experiment, the IS-compensated signal of the analyte was used. The extract was evaporated to dryness at 40 °C with a gentle stream of nitrogen and re-dissolved in aqueous 5 mM ammonium acetate solution containing 4% methanol (see Table 2 for exact volume). The extract was then transferred into an autosampler vial and centrifuged (Christ RVC 2-18, purchased from Fisher Scientific Schwerte, Germany) for 30 min before analysis. For this experiment the HPLC–MS/MS was calibrated with diluted multi-standard solutions in aqueous 5 mM ammonium acetate solution containing 4% methanol.

2.3. Sampling and sample preparation

Water samples were collected from the river Leine (Göttingen, Germany), the Baltic Sea (Ahlbeck, Usedom) and a municipal wastewater treatment plant. The river sample was taken directly at the riverbank and the seawater sample at a pier, in 200 m distance from the seashore. Salinity of the seawater sample was 6.2‰. The wastewater sample was collected from the effluent of a municipal wastewater treatment plant (WWTP), which was located in a city with a population of around 120,000 inhabitants. The WWTP consisted of a mechanical treatment for the separation of solid material (i.e. a grit, fat separator and a primary settler) followed by activated sludge treatment, including nitrification and denitrification. Additionally, chemical P-removal was performed. Under dry weather discharge conditions, the mean hydraulic residence time was 20–24 h. WWTP effluent and river water were sampled in October 2009, the Baltic Sea in May 2009. 1-L (clear glass and screw cap) bottles were used for sampling (Fisher Scientific, Schwerte, Germany). Samples were allowed to settle at 4 °C in the dark for 12–24 h. Careful handling prevented resuspension of settled particles and the supernatant was used for analysis instead of a filtered aliquot.

OASIS HLB was selected for sample extraction. Samples were extracted in duplicate. Prior to extraction the sorbent was conditioned with 4 mL of methanol and rinsed twice with 4 mL of ultrapure water. The sample (500 mL for river and seawater and 100 mL for treated wastewater) was spiked with 10 μL of IS by using a 10-μL syringe and with buffer concentrate (10 mL/L sample, see Section 2.2). It was extracted with a flow rate (applied by vacuum suction) of 15 mL/min. All sample matrices had pH 7.0 ± 0.2 after buffering.

After extraction, the sorbent was rinsed with 2 × 1.5 mL of ultrapure water in order to remove the inorganic salt matrix. Afterwards, the sorbent was dried by drawing air through the cartridges under vacuum for 30 min. The analytes were eluted with 2 × 2 mL of methanol, followed by 2 × 2 mL of ethyl acetate. The extract was evaporated to dryness at 40 °C with a gentle stream of nitrogen and

Table 3
Log K_{OW} and pK_a of analytes, ESI-MS–MS conditions for the analysis of the selected compounds.

Charge at neutral water pH	Related IS[a]	Compound	Log K_{OW}[b]	pK_a[b]		Quantifier	Cap U (V)[d]	CE (V)[e]	Qualifier	Cap U (V)[d]	CE (V)[e]
				Acidic	Basic[c]						
Neutral	1	1H-benzotriazole	1.34 ± 0.25	8.4 ± 0.1	1.2 ± 0.3	120 > 65	+65	−11.5	120 > 92	+65	−9.5
	1	3-Methylxanthine	−0.79 ± 0.39	10.5 ± 0.2	1.3 ± 0.7	165 > 122	−55	+19.0	165 > 150	−55	+18.0
	6	Atrazine	2.63 ± 0.21		2.4 ± 0.5	216 > 174	+55	−9.0	216 > 104	+55	−18.0
	7	Benzoylecgonine[f]	2.72 ± 0.38	3.4 ± 0.4	10.8 ± 0.4	290 > 168	+60	−12.0	290 > 105	+60	−21.0
	1	Caffeine	−0.13 ± 0.37		0.7 ± 0.7	195 > 138	+55	−9.5	195 > 110	+55	−9.0
	7	Carbamazepine	2.67 ± 0.38	13.9 ± 0.2		237 > 194	+45	−11.0	237 > 179	+45	−27.0
	7	Cetirizine[f]	2.17 ± 0.84	2.9[g]	(1.5 and 8.3)[g]	389 > 201	+40	−14.5	389 > 166	+40	−34.5
	6	Desethylatrazine	1.50 ± 0.25		2.1 ± 0.5	188 > 146	+50	−8.5	188 > 104	+50	−16.0
	6	Desisopropylatrazine	1.16 ± 0.25		2.6 ± 0.5	174 > 104	+50	−13.5	174 > 132	+50	−8.5
	10	Diazepam	2.96 ± 0.55		3.4 ± 0.1	285 > 193	+65	−21.0	285 > 154	+65	−16.5
	12	Diuron	2.78 ± 0.33	13.6 ± 0.7		231 > 186	−40	+15.0	231 > 150	−40	+22.0
	3	Iohexol	−4.16 ± 0.85	11.4 ± 0.5		820 > 127	−60	+11.5	–		
	3	Iomeprol	−3.08 ± 0.86	11.4 ± 0.5		776 > 127	−50	+14.0	778 > 558	+60	−21.5
	3	Iopamidol	−2.09 ± 1	10.9 ± 0.5		776 > 127	−50	+14.0	778 > 558	+60	−21.5
	3	Iopromide	−2.95 ± 0.91	10.6 ± 0.7		790 > 127	−45	+8.0	792 > 573	+65	−14.5
	5	Isoproturon	2.32 ± 0.29		0.9 ± 0.5	207 > 72	+45	−10.0	207 > 165	+45	−7.0
	11	Loratadine	5.94 ± 0.77		4.8 ± 0.2	383 > 337	+55	−15.5	383 > 267	+55	−26.5
	7	Metazachlor	2.11 ± 0.48		1.3 ± 0.5	278 > 134	+30	−17.5	278 > 210	+30	−6.0
	1	Paracetamol	0.34 ± 0.21	9.9 ± 0.1	1.7 ± 0.5	152 > 110	+40	−20.0	–		
	1	Paraxanthine	−0.63 ± 0.59	8.5 ± 0.5	0.2 ± 0.7	181 > 124	+60	−8.0	181 > 96	+60	−10.5
	7	Phenazone	0.27 ± 0.33		0.7 ± 0.7	189 > 146	+60	−10.0	189 > 131	+60	−9.5
	4	Primidone	0.40 ± 0.52	12.3 ± 0.4		219 > 162	+35	−9.0	219 > 91	+35	−21.0
	10	Tetrazepam	3.13 ± 0.65		2.0 ± 0.2	289 > 225	+60	−24.0	289 > 253	+60	−9.5
	1	Theobromine	−0.72 ± 0.55	9.9 ± 0.5	0.6 ± 0.7	181 > 138	+55	−9.5	181 > 110	+55	−13.0
	1	Theophylline	−0.18 ± 0.31	8.6 ± 0.5	1.5 ± 0.7	181 > 124	+60	−8.0	181 > 96	+60	−10.5
	1	Tolyltriazole	1.89[h]	Not available		134 > 77	+55	−15.0	134 > 106	+55	−5
Anionic	12	Bezafibrate	3.46 ± 0.45	3.3 ± 0.1		360 > 274	−40	+13.0	360 > 154	−40	+21.0
	12	Clofibric acid	2.72 ± 0.27	3.2 ± 0.1		213 > 127	−25	+12.0	213 > 85	−25	+8.5
	12	Diclofenac	4.06 ± 0.41	4.2 ± 0.1		294 > 250	−30	+8.5	296 > 214	+30	−24.0
	12	Gemfibrozil	4.39 ± 0.49	4.8 ± 0.5		249 > 121	−30	+11.0	–		
	12	Ibuprofen	3.72 ± 0.23	4.4 ± 0.1		205 > 161	−25	+5.5	–		
	13	Mecoprop	2.83 ± 0.27	3.2 ± 0.2		213 > 141	−30	+12.5	–		
	12	Naproxen	3.00 ± 0.24	4.8 ± 0.3		231 > 185	+40	−10.0	231 > 170	+40	−21.5
	4	Sulfamethoxazole	0.89 ± 0.42	5.8 ± 0.5	1.4 ± 0.1	254 > 156	+35	−13.5	254 > 108	+35	−17.5
Cationic	2	Atenolol	0.10 ± 0.28		9.2 ± 0.4	267 > 145	+55	−20.0	267 > 190	+55	−11.0
	9	Clarithromycin	3.16 ± 0.78	13.1 ± 0.7	8.2 ± 0.7	748 > 158	+60	−13.5	748 > 590	+60	−6.0
	10	Citalopram	2.51 ± 0.74		9.6 ± 0.3	325 > 109	+60	−17.5	325 > 262	+60	−11.0
	8	Erythromycin	2.83 ± 0.78	13.1 ± 0.7	8.2 ± 0.7	734 > 158	+60	−16.0	734 > 576	+60	−6.0
	14	Fluoxetine	4.09 ± 0.45		10.1 ± 0.1	310 > 148	+25	−6.5	–		
	2	Metoprolol	1.79 ± 0.4		9.2 ± 0.4	268 > 116	+55	−11.0	268 > 191	+55	−10.0
	9	Roxithromycin	2.75[i]		8.8[i]	837 > 158	+70	−17.0	837 > 679	+70	−6.0
	14	Sertraline	4.81 ± 0.41		9.5 ± 0.4	306 > 159	+25	−19.0	306 > 275	+25	−7.5
	2	Sotalol	0.32 ± 0.37	9.6 ± 0.5	9.2 ± 0.4	273 > 255	+40	−7.5	273 > 213	+40	−17.0
Neutral/cationic	7	Trimethoprim	0.79 ± 0.38		7.2 ± 0.1	291 > 230	+65	−15.0	291 > 123	+65	−15.5
Neutral/anionic	1	1-Methylxanthine	−0.58 ± 0.59	7.7 ± 0.7	1.2 ± 0.7	165 > 108	−55	+19.0	165 > 80	−55	+25.0
	15	Pantoprazole	1.69 ± 1.12	7.7 ± 0.7	3.6 ± 0.3	384 > 200	+25	−8.5	384 > 138	+25	−21.0

[a] Corresponding to Table 4.
[b] SciFinder predicted values unless otherwise noted.
[c] 'Basic' pK_a implies pK_a of the corresponding acid.
[d] Capillary voltage.
[e] Collision energy.
[f] Dipolar ion.
[g] Hanocq et al. [26].
[h] Giger et al. [27].
[i] Beausse [28].

re-dissolved in 1 mL of aqueous 5 mM ammonium acetate solution, containing 4% methanol. Prior to analysis, the extract was transferred into an autosampler vial and centrifuged for 30 min.

2.4. Liquid chromatography–tandem mass spectrometry (HPLC–MS/MS)

2.4.1. Liquid chromatography

The HPLC system consisted of a Varian ProStar 410 autosampler and a high-pressure gradient system of two Varian ProStar 210 pumps. For chromatographic separation a Polaris C18-Ether column 150 mm × 2 mm i.d., 3 μm particle size (Varian, Darmstadt, Germany) was used. The flow was 200 μL/min. The separation was operated at 30 °C and the injection volume was set to 100 μL. In comparison to studies with similar dimensions concerning HPLC column and flow rate, the applied injection volume of 100 μL was relatively high (e.g. in comparison to 10 μL [6], 25 μL [7]). Therefore, the extracts were re-dissolved in a solution of elution strength slightly lower than the starting eluent in order to enable sufficient retention and excellent peak shape of early eluting analytes (e.g. sotalol). A direct dissolution in acidic start gradient was not applied in order to preserve acid instable compounds.

Eluent A was 0.015% formic acid + 5% methanol in ultrapure water, eluent B was methanol. The elution started isocratically for 50 s with 100% A, followed by a gradient of 10 s to 95% A. This rapid step was due to the minimum flow rate with reliable accuracy of the used pump given by the manufacturer (10 μL/min), which corresponds to 5% of eluent B at a total flow rate of 200 μL/min. Accordingly, this sharp gradient must be considered as a switch-on of pump B in order to overcome its range of unreliable accuracy as fast as possible, rather than as part of the eluting gradient. This step was followed by a 39-min linear gradient to 95% B. This was held for 5 min followed by a 1-min linear gradient to 100% A, which was maintained for 11 min to equilibrate the system.

2.4.2. Mass spectrometry (MS/MS)

A Varian 1200 L triple quadrupole with electrospray interface (ESI) was used for detection and quantification. A spray voltage of 5.5 kV in positive mode, −4.5 kV in negative mode and shield voltages of 0.5 and −0.5 kV were used, respectively. Detection was performed in multiple reaction monitoring (MRM) and with the exception of paracetamol, fluoxetine, ibuprofen, iohexol, gemfibrozil, mecoprop and the internal standards two transitions per analyte were monitored [25]. Compounds were quantified using their most intensive MRM. Minimum dwell times of the individual MRM were 40 and 20 ms for quantifier and qualifier, respectively. Fragmentation experiments, optimization of capillary voltage and collision energy were achieved by direct injection of stock solutions with a concentration of 5 μg/mL. Individual MS/MS parameters of analytes and IS are shown in Tables 3 and 4. Argon 5.0 was used as the collision gas with a pressure of 0.27 Pa. Following the manufacturer's guidelines the pressure of the drying and nebulizing gases were 180×10^3 and 386×10^3 Pa, respectively.

2.5. Performance of the multi-residue method

Method quantitation limits (MQL) were calculated throughout the complete method by linear regression of a 10-point calibration incorporating SPE and HPLC/MS–MS analysis (confidence interval 0.95). The highest and lowest concentration levels were extracted in triplicate, all intermediate concentrations in duplicate. Single injection of each extract was applied. For calculating the MQL of surface and seawater 500 mL buffered aqueous standard solutions were prepared and extracted according to the method described in Section 2.3. The extracts were analyzed according to Section 2.4. With respect to treated wastewater 100 mL were used. For both sample volumes 10 equidistant absolute levels corresponding to a low concentration range were prepared: 5–50 ng for the iodinated contrast media, fluoxetine, sertraline, 1-methylxanthine and 3-methylxanthine and 1.25–12.5 ng for all other compounds.

The linear range of the method was evaluated using artificial samples of 500 mL Milli-Q water spiked with the analytes. In contrast to instrumental linearity studies, SPE was included to account for breakthrough of analytes during extraction. Linearity ranges for 100 mL sample volume were calculated from the 500 mL results as empirical worst-case values, hereby possibly underestimating the real range of linearity for the smaller volume.

Table 4
ESI-MS–MS conditions of the internal standards.

Internal standard	MRM	Cap U (V)[a]	CE (V)[b]
1 Paraxanthine-D_6	187 > 127	+60	−9.0
2 Atenolol-D_7	274 > 145	+55	−17.5
3 Desmethoxyiopromide	760 > 127	−40	+8.5
4 Sulfamethoxazole-$^{13}C_6$	260 > 162	+35	−13.5
5 Isoproturon-D_6	213 > 171	+50	−8.0
6 Atrazine-D_5	221 > 179	+55	−10.5
7 Carbamazepine-D_{10}	247 > 204	+45	−13.0
8 Erythromycin-N-methyl-^{13}C-D_3	738 > 162	+65	−16.5
9 Ery-methyloxime	763 > 158	+60	−20.0
10 Diazepam-D_5	290 > 198	+65	−24.5
11 Loratadine-D_4	387 > 269	+55	−26.5
12 Ibuprofen-D_3	208 > 164	−25	+6.0
13 Mecoprop-D_3	218 > 146	−35	+12.0
14 Fluoxetine-D_6	316 > 154	+25	−6.5
15 Lansoprazole	370 > 252	+25	−8.0

[a] Capillary voltage.
[b] Collision energy.

3. Results and discussion

3.1. Surface-normalized comparison of selected SPE-sorbents

In contrast to other comparative recovery studies, the sorbents were tested considering their specific surface areas. The results are presented in Fig. 1. With respect to the used sorbents, there is no universal correlation of recovery and log K_{OW} of neutral compounds (Fig. 1a and b). However, recoveries clearly decreased with increasing basic character of the compounds (Fig. 1c and d). Standard deviation (SD) of the triple-extractions was ≤10%, except for erythromycin A on AbsElut Nexus (14%) and diuron on Isolute ENV+ (11%).

The recoveries of cationic compounds and ICM turned out to be the critical ones, since recoveries of neutral and anionic compounds were mostly in the same range for all sorbents. Out of all analytes, fluoxetine and sertraline were exceptional cases, since both compounds exhibited very poor recoveries for all of the tested sorbents (3–38 and 2–23%, respectively). Apart from these compounds, AbsElut Nexus (*a*cidic *b*asic *s*creen *ELUT*), a polystyrene-divinylbenzene (PS-DVB)/methyacrylate copolymer, and Bond Elut Plexa exhibited the highest recoveries of cationic compounds with a median of 69%. With Bond Elut Plexa and AbsElut Nexus recoveries of the ICM iopamidol, iomeprol and iohexol were unacceptably low (0–21%). Surprisingly, Bond Elut PPL showed the best recoveries for all of the ICM tested (≥82%). This sorbent was never mentioned in the literature for extraction of ICM [18]. Verification of this sorbent for a specialized analytical method for both non-ionic and ionic ICM may be promising since recoveries of all anionic compounds were also high (≥84%). Unfortunately, this sorbent exhibited very low recoveries for beta-blockers, macrolide antibiotics and SSRI (<10%). With the exception of fluoxetine and sertraline, OASIS HLB demonstrated high recoveries of cationic compounds (65% median recovery) and all ICM except iopamidol

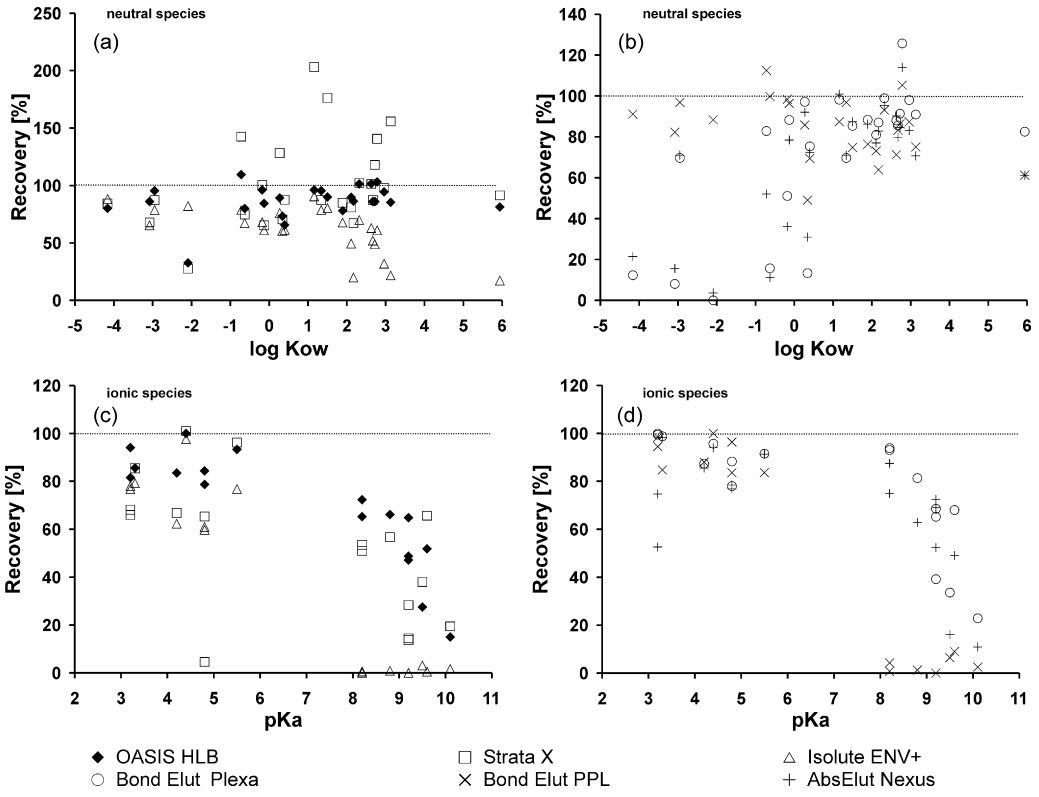

Fig. 1. Recovery rates (RR) of compounds on different SPE-sorbents; correlation of RR, log K_{OW} and pK_a. In case of multiple pK_a per compound, the one that was relevant at experimental conditions was chosen. Charts a + c illustrate RR of OASIS HLB, Strata X and Isolute ENV+, charts b + d illustrate RR of Bond Elut Plexa, Bond Elut PPL and AbsElut Nexus. High RR (>120%) of Strata X in chart 'a' are most likely attributed to over-compensation by internal standardization. Standard deviation (SD) of the triple-extractions was ≤10% except for erythromycin A on AbsElut Nexus (14%) and diuron on Isolute ENV+ (11%).

(33%) were recovered with yields of >80% by this sorbent. For a multi-residue analytical method of the required analytes, OASIS HLB represented the best compromise, since it was the only sorbent with sufficient recoveries of ICM *and* basic compounds.

During the elution step of Strata X and Isolute ENV+, turbidity of the extracts indicated possible elution/dissolution of sorbent material. Since the turbidity endured in the re-dissolved extracts of both sorbents, analytes and internal standards may be sorbed to these particles and were removed by centrifugation. Additionally, adverse ionization effects in ESI may eventually be caused by soluble cartridge material. Fig. 1a shows remarkable recoveries of up to 200% for the Strata X. This may be attributed to over-compensation of the effects mentioned above with the internal standard, since the analytical system was calibrated with a diluted stock solution of the reference standards. Thus, the calibration did not represent any matrix effect caused by the sample or sample pre-treatment [29,30]. This may underestimate the extraction quality of Strata X, since a sorbent-specific matrix would be compensated during a calibration, which includes the extraction process. The partially disappointing recoveries with Isolute ENV+ may also be influenced by a similar effect.

3.2. Liquid chromatography–tandem mass spectrometry (HPLC–MS/MS)

3.2.1. Liquid chromatography

The applied gradient allowed sufficient retention and separation of analytes on a Polaris C18-Ether HPLC column. A chromatogram of a standard solution is presented in Fig. 2. During the optimization process, formic acid concentrations of eluent A were varied over the concentration range 0.01–0.1%. The influence of formic acid concentration (and thus pH) on response of ibuprofen-D_3 (MRM 208 → 164) is presented in Fig. 3. It clearly demonstrates a striking signal suppression of ibuprofen-D_3 in presence of more acidic pH values (higher formic acid concentrations). This is most likely attributed to a lower dissociation rate of ibuprofen at higher acid concentrations [14]. Determination of acidic pharmaceuticals in negative mode (ESI−) is usually performed with organic solvents and ultrapure water, or aqueous ammonium acetate, usually with alkaline pH values [5]. However, Busetti et al. [7] used a low formic acid concentration of 0.01% for the analysis of naproxen, ibuprofen, clofibric acid and gemfibrozil with negative ESI on a Micromass Quattro Ultima Quadrupole instrument. Yang et al. [12] reported suppressed ionization of phenolic acids in the presence of 0.1% formic acid in one eluent while using negative ionization. On the other hand, ionization of adenosine and coumarins were enhanced in positive polarity due to the acid. When using switching electrospray ionization it is obvious that the gradient composition must represent an appropriate compromise. Therefore, a formic acid concentration of 0.015% was found to be optimal in the multi-residue analytical method described here.

3.2.2. Mass spectrometry (MS/MS)

Selected results of the optimization experiments of ESI drying gas temperature are shown in Fig. 4. The optimization of the drying gas temperature was achieved by analyzing a multi-standard solution with 100 ng/mL for each analyte in 5 mM ammonium acetate and 4% methanol according to 2.4. Signal response (peak area) and relative standard deviation (3 injections per temperature) of each respective quantifier MRM was investigated at drying gas temperatures of 260–360 °C with 20 K increments. The drying gas temperature had significant effects for only a few of the compounds; however, effects were remarkable. Fig. 4a shows the results for those analytes for which signals increased with temperature (with the highest signal set to 100%). Since drying efficiency is a function of the organic nature of the mobile phase, eluents with

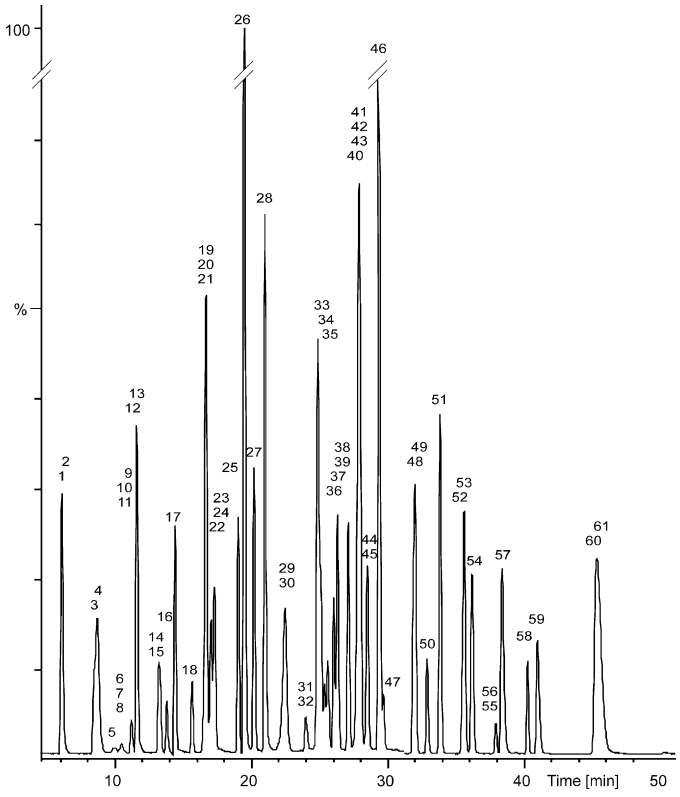

Fig. 2. Chromatogram (Total Ion Current, TIC) of a standard solution. Analytes and internal standards in order of elution: (1) sotalol, (2) iopamidol, (3) atenolol-D_7, (4) atenolol, (5) 3-methylxanthine, (6) iomeprol, (7) 1-methylxanthine, (8) iohexol, (9) desmethoxyiopromide, (10) paracetamol, (11) iopromide, (12) theobromine, (13) trimethoprim, (14) paraxanthine-D_6, (15) paraxanthine, (16) theophylline, (17) metoprolol, (18) caffeine, (19) desisopropylatrazine, (20) benzoylecgonine, (21) 1H-benzotriazole, (22) phenazone, (23) sulfamethoxazole-$^{13}C_6$, (24) sulfamethoxazole, (25) primidone, (26) citalopram, (27) desethylatrazine, (28) tolyltriazole, (29) erythromycin-N-methyl-^{13}C-D_3, (30) erythromycin, (31) fluoxetine-D_6, (32) fluoxetine, (33) pantoprazole, (34) sertraline, (35) clarithromycin, (36) ery-methyloxime, (37) roxithromycin, (38) carbamazepine-D_{10}, (39) carbamazepine, (40) lansoprazole, (41) atrazine-D_5, (42) metazachlor, (43) atrazine, (44) isoproturon-D_6, (45) isoproturon, (46) cetirizine, (47) diuron, (48) diazepam-D_5, (49) diazepam, (50) naproxen, (51) tetrazepam, (52) loratadine-D_4, (53) loratadine, (54) bezafibrate, (55) ibuprofen-D_3, (56) ibuprofen, (57) clofibric acid, (58) gemfibrozil, (59) diclofenac, (60) mecoprop-D_3 and (61) mecoprop.

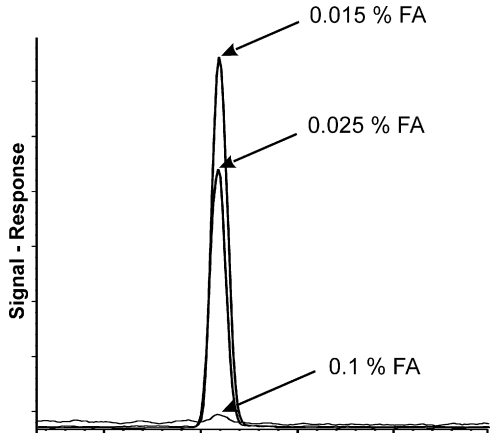

Fig. 3. Correlation of signal response and formic acid (FA) fraction of eluent A (%), 20 ng ibuprofen-D_3 using the reaction of the negatively charged $(M-H^+)^-$ ion for detection.

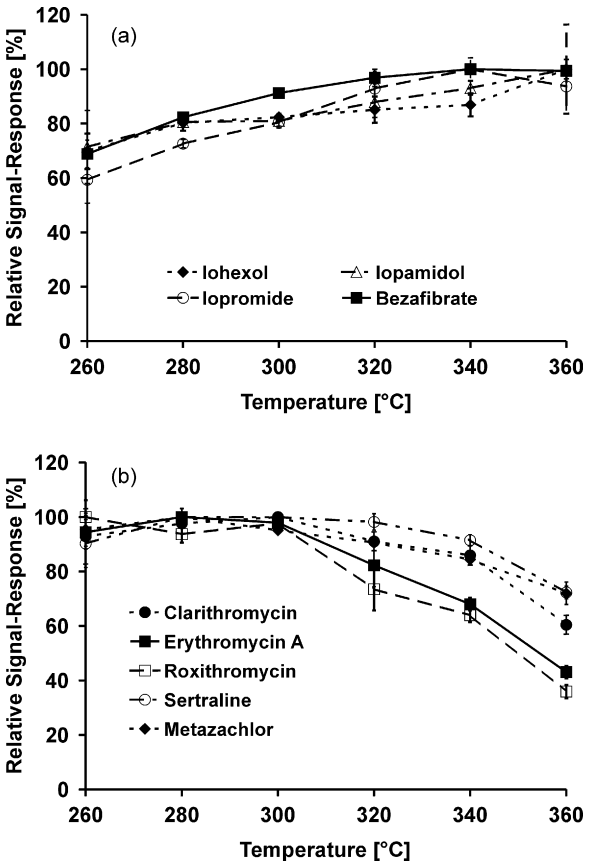

Fig. 4. Correlation of signal response and drying gas temperature of selected compounds.

high organic fractions may evaporate more easily than eluents with low organic fractions. Therefore, the most promising enhancement due to a high drying gas temperature was expected at gradient conditions, which are tendentially unfavorable for evaporation. Increasing signal responses of iohexol, iopromide and iopamidol, which were eluting with methanol fractions below 20%, confirmed the expectation. However, with respect to the analytes eluting with high methanol fractions (>50%), the excessive improvement of bezafibrate was the only remarkable example.

With respect to the analytes, drying gas temperature is basically limited by the thermal stability of individual compounds. The substances that were most susceptible to thermal decay were the macrolide antibiotics erythromycin A, clarithromycin and roxithromycin (Fig. 4b). Direct LC/MS injection experiments with the chosen macrolides at different drying gas temperatures demonstrated the temperature dependent decay of the respective $[M+H]^+$ into its $[M+H-C_8H_{15}O_3]^+$ derivative (elimination of cladinosyl). This equates to the macrolide qualifier reaction presented in this MS–MS method. Signal responses of sertraline and metazachlor were also negatively affected by increased drying gas temperature, but to a lesser extent.

Reproducibility, expressed as the relative standard deviation of each compound with three injections per temperature, also depended on a change in drying gas temperature. The temperature range of 280–340 °C showed RSD < 10% for all analytes whereas 260 and 360 °C revealed values of up to 15 and 16%, respectively. Thus, 340 °C was used during the first 19 min of the analytical run to optimize sensitivity of the ICM. 280 °C was used from 19 to 55 min to prevent thermolabile compounds from thermal degradation and 340 °C from 55 to 57 min to prepare the system for the following run.

Table 5
Performance of the developed multi-residue method.

Compound	500 mL sample volume				100 mL sample volume		
	MQL (ng/L)	Linear range[a] (ng/L)	R^2[b]	RSD[c] (%)	MQL (ng/L)	Linear range[d] (ng/L)	RSD[c] (%)
1H-benzotriazole	4.7	2.5–1400	0.9947	6.1	18	12.5–7000	5.2
1-Methylxanthine	21	10–1000	0.9934	4.1	130	50–5000	7.5
3-Methylxanthine	28	10–1000	0.9903	5.6	135	50–5000	7.8
Atenolol	3.5	2.5–2000	0.9983	4.9	12	12.5–10000	3.1
Atrazine	1.4	2.5–2000	0.9969	1.9	8.9	12.5–10000	2.4
Benzoylecgonine	2.3	2.5–1000	0.9942	3.2	8.8	12.5–5000	2.4
Bezafibrate	3.5	2.5–900	0.9868	4.9	7.3	12.5–4500	2.0
Caffeine	4.3	2.5–2000	0.9937	6.1	39	12.5–10000	12
Carbamazepine	2.2	2.5–1600	0.9900	3.1	8.8	12.5–8000	2.4
Cetirizine	2.2	2.5–750	0.9901	2.9	12	12.5–3750	3.2
Citalopram	3.2	2.5–2000	0.9978	4.5	35	12.5–10000	8.5
Clarithromycin	7.5	2.5–2000	0.9959	11	18	12.5–10000	5.0
Clofibric acid	3.4	2.5–1000	0.9964	4.7	15	12.5–5000	4.1
Desethylatrazine	1.7	2.5–2000	0.9987	2.3	8.7	12.5–10000	2.4
Desisopropylatrazine	5.6	2.5–1400	0.9949	8.1	15	12.5–7000	4.2
Diazepam	1.4	2.5–2000	0.9963	1.9	11	12.5–10000	3.0
Diclofenac	2.0	2.5–1000	0.9850	2.7	15	12.5–5000	4.1
Diuron	3.3	2.5–1000	0.9890	4.6	14	12.5–5000	3.8
Erythromycin	7.5	2.5–2000	0.9928	6.2	29	12.5–10000	8.4
Fluoxetine	16	10–2000	0.9984	5.5	82	50–10000	5.9
Gemfibrozil	2.0	2.5–1000	0.9861	2.7	17	12.5–5000	4.5
Ibuprofen	3.6	2.5–2000	0.9920	5.0	19	12.5–10000	5.4
Iohexol	21	12.5–4000	0.9913	6.0	160	60–20000	9.3
Iomeprol	19	12.5–4000	0.9970	5.1	145	60–20000	8.4
Iopamidol	19	12.5–4000	0.9945	5.2	135	60–20000	7.9
Iopromide	19	12.5–4000	0.9949	5.2	105	60–20000	6.0
Isoproturon	3.0	2.5–2000	0.9981	4.2	19	12.5–10000	5.1
Loratadine	2.7	2.5–1400	0.9905	3.8	11	12.5–7000	2.8
Mecoprop	1.2	2.5–2000	0.9972	1.5	5.0	12.5–10000	1.4
Metazachlor	1.8	2.5–1000	0.9938	2.5	12	12.5–5000	3.1
Metoprolol	4.1	2.5–2000	0.9959	5.9	23	12.5–10000	6.3
Naproxen	4.8	2.5–900	0.9950	6.9	24	12.5–4500	6.9
Pantoprazole	4.8	2.5–1600	0.9977	6.8	36	12.5–8000	11
Paracetamol	3.7	2.5–2000	0.9905	5.1	26	12.5–10000	7.5
Paraxanthine	3.2	2.5–2000	0.9954	4.4	13	12.5–10000	3.5
Phenazone	2.0	2.5–1000	0.9896	2.7	18	12.5–5000	4.9
Primidone	2.7	2.5–2000	0.9977	3.7	14	12.5–10000	3.7
Roxithromycin	9.5	2.5–1400	0.9954	2.0	15	12.5–7000	4.2
Sertraline	16	10–1200	0.9981	3.3	115	50–6000	17
Sotalol	4.8	2.5–1800	0.9950	6.9	32	12.5–9000	9.4
Sulfamethoxazole	2.6	2.5–2000	0.9988	3.5	14	12.5–10000	3.8
Tetrazepam	2.5	2.5–2000	0.9955	3.4	15	12.5–10000	4.2
Theobromine	5.1	2.5–750	0.9971	7.4	24	12.5–3750	6.9
Theophylline	3.4	2.5–2000	0.9912	4.7	18	12.5–10000	5.0
Tolyltriazole	4.9	2.5–1800	0.9931	7.0	35	12.5–9000	10
Trimethoprim	2.5	2.5–2000	0.9890	3.4	15	12.5–10000	4.2

[a] Experimental values; 15 point calibration: extraction of 6.25–2000 ng ICM; 5–1000 ng fluoxetine, sertraline and mono-methylxanthines; 1.25–1000 ng for all other compounds; concentration range with $R^2 \geq 0.9850$ was defined as linear range.
[b] Corresponds to linear range of 500 mL sample volume.
[c] Coefficient of the variation of the procedure, calculated from MQL determination (linear regression, $n = 22$).
[d] Calculated values from 500 mL sample volume.

If mentioned at all, the optimization process of electrospray drying gas temperature is infrequently described in the literature and studies with quantified temperature/response relations are an exception [31]. As demonstrated, signal response of compounds from different chemical classes to varying drying gas temperatures can be highly individual. If supported by the MS instrument, the implementation of a temperature gradient can significantly increase the sensitivity of a given method. This application is primarily relevant for multi-residue analytical methods, as described in this study.

3.3. Performance of the developed multi-residue analytical method

Method quantitation limits (MQL) and linear range of the method were determined according to Section 2.5. They are presented in Table 5. In comparison to studies on organic pollutants in seawater the sample pre-concentration factor applied in this study was rather low. Sample volumes of 1–100 L were used frequently for target and non-target analysis in seawater [32–36]. However, MQL of the developed method are adequate for environmental analysis in the low ng/L concentration range. In contrast to other studies on multi-residue analytical methods [6–8], a high number and variety of individual compounds were analyzed here simultaneously, with only one injection, by using a generic gradient and switching electrospray ionization. Furthermore, the linear range and correlation factors presented in Table 5 were obtained as a result of the total analytical procedure and do not represent instrument levels only [6,7]. This may allow a more realistic assessment of the method's linearity within the scope of an application. However, one drawback of the presented method, as well as for all multi-residue methods in general, is their compromise nature. In comparison with single group analysis, MQL of analytes can be significantly higher as it is exemplary for ICM [37]. Fortu-

nately, if present in environmental samples, the concentrations of these compounds are usually several times higher than the MQL of the developed method [18,37]. The identified linear ranges of all analytes are generally in accordance with concentrations of micro-contaminants frequently detected in environmental samples [18,27,37–44]. Coefficients of the variation of the procedure are, apart from a few exceptions, less than 10%, indicating a good precision of the total method.

3.4. Matrix effects

Undesirable matrix effects such as signal suppression and enhancement are often the concern of LC/MS–MS studies. The goal of the presented study was at this stage to study the possibilities of a single gradient method. However, signal suppression was observed for almost all analytes in extracts from environmental samples.

Mechanisms of matrix effects, as well as strategies to overcome them, were discussed in the literature [45–48]. As stated, ESI is more susceptible than i.e. LC–MS interfaces, which are based on heated nebulization such as atmospheric pressure chemical ionization (APCI) and, very recently, electron impact (EI). However, limited distribution of EI and a higher sensitivity for many frequently detected compounds in environmental samples justify the frequent use of ESI in multi-residue analytical methods [4,10].

A simple strategy to reduce matrix effects in SPE-based methods requires rinsing the loaded SPE cartridge with water, or low concentrated organic acids containing little portions of organic solvents [42]. In the presented method, rinsing the sorbent twice with 1.5 mL of ultrapure water showed no significant influence on recovery of analytes, but was very effective in removal of inorganic salt matrix, as verified by ion chromatography. Particularly for the analysis of seawater matrix, this step is recommendable, since otherwise the formation of sodium adducts would result in reduced sensitivity of selected compounds [47]. Furthermore, compared to extraction at acidic pH values, extraction at neutral pH minimizes potential co-extraction of typical organic components of environmental samples like humic and fulvic acids [21].

The application of isotope-labeled internal standards was identified as a very powerful tool to compensate adverse matrix effects with ESI for various aqueous matrices [6,7,9,10]. However, for a multi-residue analysis with a wide range of chemically diverse compounds as presented here, the availability of suitable surrogates and their considerable costs make a suitable selection of internal standards with excellent performance for all matrices quite challenging. In the presented analytical method a high number of 15 IS were used as a strategy to overcome matrix effects, which represented a good compromise between precision and surrogate availability.

3.5. Application to environmental samples

To demonstrate the applicability of the method, real samples with different matrices were analyzed in duplicates. For that purpose, three different water types were used, from low organic concentration in seawater samples (commonly 0.1 mg/L total organic carbon, TOC) to a highly complex organic matrix in treated effluent with ca. 10 mg/L TOC. Additionally, the samples differed with respect to their ionic strength (from 0.01 to 0.5 mol/L). The results are presented in Table 6. Expanded uncertainties were calculated according to Konieczka and Namieśnik [49]. They are comparable between samples over wide concentration ranges, i.e. 1H-benzotriazole between 29 and 3846 ng/L. Furthermore, analytes in the sub ng/L as well as analytes in the μg/L concentration range were successfully quantified in the same sample.

36 of the calibrated 46 analytes were detected in at least one sample. As expected, the lowest concentrations of micro-contaminants were detected in seawater. The WWTP effluent exhibited the highest individual concentrations and the river presented the highest variety of compounds. 1H-benzotriazole, tolyltriazole, carbamazepine, cetirizine, clarithromycin, sulfamethoxazole, iomeprol and iopromide were detected in all three samples.

Table 6
Concentrations of analytes in three different sample matrices expressed as mean result of duplicate extraction ± expanded uncertainty[a] in ng/L.

Compound	River sample	Seawater sample	Treated effluent sample
1H-benzotriazole	670 ± 41	29 ± 3.3	3846 ± 277
1-Methylxanthine	61 ± 14	nd[c]	–
3-Methylxanthine	–[b]	nd	–
Atenolol	66 ± 3.6	–	245 ± 16
Atrazine	2 ± 0.8	–	–
Benzoylecgonine	–	–	–
Bezafibrate	29 ± 2.5	–	168 ± 11
Caffeine	101 ± 8.7	58 ± 5.4	–
Carbamazepine	265 ± 10	26 ± 1.6	1429 ± 46
Cetirizine	20 ± 2.0	4 ± 1.3	105 ± 8.2
Citalopram	54 ± 4.7	–	431 ± 41
Clarithromycin	77 ± 12	14 ± 9.4	520 ± 35
Clofibric acid	21 ± 2.4	–	103 ± 9.3
Desethylatrazine	6 ± 1.0	–	–
Desisopropylatrazine	7 ± 3.4	–	–
Diazepam	10 ± 0.9	–	27 ± 6.1
Diclofenac	156 ± 6.0	–	1492 ± 89
Diuron	–	–	–
Erythromycin	22 ± 2.7	–	173 ± 23
Fluoxetine	–	nd	–
Gemfibrozil	–	–	–
Ibuprofen	–	–	–
Iohexol	1214 ± 88	–	5574 ± 718
Iomeprol	1258 ± 106	40 ± 11	6272 ± 572
Iopamidol	785 ± 100	–	5569 ± 619
Iopromide	268 ± 30	25 ± 11	2670 ± 208
Isoproturon	43 ± 2.0	–	35 ± 11
Loratadine	–	4 ± 4.0	–
Mecoprop	26 ± 1.2	–	159 ± 5.2
Metazachlor	–	–	–
Metoprolol	337 ± 22	–	2513 ± 161
Naproxen	–	–	–
Pantoprazole	13 ± 2.8	nd	149 ± 23
Paracetamol	1992 ± 198	–	–
Paraxanthine	36 ± 3.3	22 ± 2.4	–
Phenazone	27 ± 1.7	–	56 ± 23
Primidone	60 ± 4.3	–	216 ± 20
Roxithromycin	16 ± 6.4	–	78 ± 11
Sertraline	–	nd	–
Sotalol	195 ± 9.0	–	1314 ± 130
Sulfamethoxazole	93 ± 4.4	7 ± 1.5	509 ± 35
Tetrazepam	–	–	–
Theobromine	–	23 ± 4.2	–
Theophylline	21 ± 3.0	–	–
Tolyltriazole	741 ± 45	37 ± 3.6	4803 ± 404
Trimethoprim	95 ± 4.2	–	681 ± 28

[a] expanded uncertainties calculated according to Konieczka and Namieśnik [49].
[b] Not detected.
[c] Not determined.

The river water was particularly interesting because the sampling location was about 2 km downstream of the sampled WWTP and both samples were taken at approximately the same time. It is remarkable that significant amounts of paracetamol, caffeine and its degradation products were detected in the river but not in the treated effluent. Micro-contaminants are suggested to be source specific and thus offer the potential to be employed as indicators for source delineation in monitoring ground and surface water quality, its decline or improvement. This especially applies to selected pharmaceuticals, pesticides and stimulants [50–52]. Since caffeine is generally readily biodegradable in WWTPs, it has been used as an indicator for the input of raw sewage into surface waters [52]. Elimination rates of paracetamol in WWTPs are also very high (up to 99%) [53]. Therefore, there is an indication of inflow of raw sewage

into the river. The same applies to the analyzed seawater sampling location since caffeine, paraxanthine and theobromine were detected.

Concentrations of iomeprol, iopamidol and iopromide are in the typical range of these compounds in German river water and WWTP effluents [37]. Considering concentrations of several μg/L ICM in WWTP effluents, detection of ICM in seawater was not surprising. Concentration ranges of antibiotics and other compounds such as atenolol, metoprolol, diclofenac and phenazone were also found within the typical range of these compounds in river water and WWTP effluents [38–41].

In Germany, the herbicide atrazine has been banned for almost two decades now. However, this compound as well as its metabolites, desethylatrazine and desisopropylatrazine, were detected in the river sample. The occurrence of desethylatrazine cannot be attributed to former usage of atrazine with absolute certainty, since this compound is also a metabolite of the formerly used herbicide propazine [54]. However, depending on local herbicide patterns, a desethylatrazine/atrazine-ratio (DAR) > 1 can indicate a breakdown of atrazine in soil and groundwater [50]. In the presented case, the DAR is ~3. Therefore, if attributed to atrazine, a recent application of the herbicide is unlikely and a non-point source of these compounds is likely. This outcome was expected; with the developed method it is possible to interpret this ratio in the very low concentration range.

Kosonen and Kronberg [42] revealed that high concentrations of anti-histamines in WWTP wastewaters correlate with an outbreak of allergic reactions, due to a high abundance of plant pollen in the air in April and May. Furthermore, they identified cetirizine as a relatively stable compound in the aquatic environment, which can be detected far from the discharge point. This can explain its detection in seawater, since sampling took place in late spring. Cetirizine concentrations in WWTP effluent and river water are comparable to those of the Finnish study.

The widely used corrosion inhibitors 1H-benzotriazole and tolyltriazole were detected in all matrices at expected concentrations. They have frequently been detected in surface waters and WWTP effluents [27,43,44]. Due to typical WWTP effluent concentrations, combined with the mobility and persistence of these compounds, a widespread distribution in marine environments has to be predicted.

4. Conclusions

Sensitive detection of 46 compounds, belonging to different chemical classes, was successfully applied with only one injection. In the presented multi-residue analytical method, the use of switching electrospray ionization and SPE requires compromises in terms of a generic gradient and sorbent material, respectively. However, experimental MQL and linear ranges of analytes are in accordance with typical environmental concentrations and analytes in the sub ng/L together with analytes in the μg/L concentration range can be quantified in the same sample. If supported by the instrument, the application of a drying gas temperature gradient is certainly worth testing. Depending on the analyzed compound classes, implementation of such a gradient can significantly improve the sensitivity of the method. This is primarily interesting for multi-residue analytical methods as described in this study. The simplified purification step of the IS ery-methyloxime may increase its attraction, as alternative to expensive isotope-labeled compounds. In contrast to other comparative studies, six SPE-sorbents were tested considering their specific surface areas. As the specific surfaces of commercially available sorbents differ, the approach applied here allows a more realistic comparison of different sorbents. With respect to TOC and ionic strength, three distinct water types were analyzed in order to demonstrate the applicability of the method. Expanded uncertainties were comparable between samples over wide concentration ranges. Analyte concentrations were found within the typical range of the respective sample matrices.

Acknowledgements

This study was funded by the European Commission (contract number 518118-1, GABARDINE, “Groundwater Artificial Recharge Based on Alternative Sources of Water: Advanced Integrated Technologies and Management”), and by the German Federal Ministry of Education and Research (promotional reference No. 02WM0802, SMART, “Sustainable and Integral Management of Available Water Resources Using Innovative Technologies”).

KSK-Pharma (Berghausen, Germany) supported this research with cetirizine and loratadine, Bayer (Leverkusen, Germany) with iopamidol and iopromide and Bracco Imaging (Konstanz, Germany) with iomeprol. We thank Ulf Diederichsen and Olaf Senge for their support and ideas during the synthesis and purification of (E)-9-[O-(2-methyloxime)]-erythromycin.

References

[1] T. Ternes, Water Sci. Technol. 55 (2007) 327.
[2] R.P. Schwarzenbach, B.I. Escher, K. Fenner, T.B. Hofstetter, C.A. Johnson, U. von Gunten, B. Wehrli, Science 313 (2006) 1072.
[3] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, Environ. Sci. Technol. 36 (2002) 1202.
[4] M. Petrović, M.D. Hernando, M.S. Díaz-Cruz, D. Barceló, J. Chromatogr. A 1067 (2005) 1.
[5] M. Gros, M. Petrović, D. Barceló, Anal. Bioanal. Chem. 386 (2006) 941.
[6] R. Rodil, J.B. Quintana, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, J. Chromatogr. A 1216 (2009) 2958.
[7] F. Busetti, K.L. Linge, A. Heitz, J. Chromatogr. A 1216 (2009) 5807.
[8] M.J. Gómez, M. Petrović, A.R. Fernández-Alba, D. Barceló, J. Chromatogr. A 1114 (2006) 224.
[9] J.M. Marín, E. Gracia-Lor, J.V. Sancho, F.J. López, F. Hérnandez, J. Chromatogr. A 1216 (2009) 1410.
[10] A. Wick, G. Fink, T.A. Ternes, J. Chromatogr. A 1217 (2010) 2088.
[11] M.F. Tutunji, H.M. Ibrahim, M.H. Khabbas, L.F. Tutunji, J. Chromatogr. B 877 (2009) 1689.
[12] W. Yang, C. Feng, D. Kong, X. Shi, X. Zheng, Y. Cui, M. Liu, L. Zhang, Q. Wang, Food Chem. 120 (2010) 886.
[13] F. Gao, M. Zhang, X. Cui, Z. Wang, Y. Sun, J. Gu, J. Pharm. Biomed. 52 (2010) 149.
[14] B. Ardrey, Liquid Chromatography–Mass Spectrometry: An Introduction, John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England, 2003.
[15] B.J. Vanderford, R.A. Pearson, D.J. Rexing, S.A. Snyder, Anal. Chem. 75 (2003) 6265.
[16] M. Pedrouzo, S. Reverté, F. Borrull, E. Pocurull, R.M. Marcé, J. Sep. Sci. 30 (2007) 297.
[17] S.D. Kim, J. Cho, I.S. Kim, B.J. Vanderford, S.A. Snyder, Water Res. 41 (2007) 1013.
[18] S. Pérez, D. Barceló, Anal. Bioanal. Chem. 387 (2007) 1235.
[19] Y. Yoon, J. Ryu, J. Oh, B.-G. Choi, S.A. Snyder, Sci. Total Environ. 408 (2010) 636.
[20] M.P. Schlüsener, K. Bester, M. Spiteller, Anal. Bioanal. Chem. 375 (2003) 942.
[21] V. Pichon, C. Cau Dit Coumes, L. Chen, S. Guenu, M.-C. Hennion, J. Chromatogr. A 737 (1996) 25.
[22] A. Hassanzadeh, J. Barber, G.A. Morris, P.A. Gorry, J. Phys. Chem. A 111 (2007) 10098.
[23] J. Paesen, K. Khan, E. Roets, J. Hoogmartens, Int. J. Pharm. 113 (1995) 215.
[24] H. Sontheimer, B. Frick, J. Fettig, G. Hörner, C. Hubele, G. Zimmer, Adsorptionsverfahren zur Wasserreinigung, DVGW-Forschungsstelle am Engler-Bunte-Institut der Universität Karlsruhe, Karlsruhe, 1985.
[25] EU Commission Decision No. 657/2002, Off. J. Eur. Commun. L221 (2002) 8.
[26] M. Hanocq, P. Croisier, M. van Damme, C. Aelvoet, Anal. Lett. 22 (1989) 117.
[27] W. Giger, C. Schaffner, H.-P.E. Kohler, Environ. Sci. Technol. 40 (2006) 7186.
[28] J. Beausse, Selected drugs in solid matrices: a review of environmental occurrence, determination and properties of principal substances, Energy Research Centre of the Netherlands, 2004, 36 pp. Available from: http://www.ecn.nl/docs/society/horizontal/hor_desk_26_pharmaceuticals.pdf.
[29] K. Bester, G. Bordin, A. Rodriguez, H. Schimmel, J. Pauwels, G. VanVyncht, Fresenius J. Anal. Chem. 371 (2001) 550.
[30] C.R. Mallet, Z. Lu, J.R. Mazzeo, Rapid Commun. Mass Spectrom. 18 (2004) 49.
[31] M. Takino, S. Daishima, K. Yamaguchi, J. Chromatogr. A 904 (2000) 65.
[32] K. Bester, H. Hühnerfuss, Mar. Pollut. Bull. 26 (1993) 423.
[33] H.-R. Buser, M.D. Müller, N. Theobald, Environ. Sci. Technol. 32 (1998) 188.
[34] K. Bester, N. Theobald, Water Res. 34 (2000) 2277.
[35] S. Weigel, J. Kuhlmann, H. Hühnerfuss, Sci. Total Environ. 295 (2002) 131.

[36] S. Weigel, U. Berger, E. Jensen, R. Kallenborn, H. Thoresen, H. Hühnerfuss, Chemosphere 56 (2004) 583.
[37] T.A. Ternes, R. Hirsch, Environ. Sci. Technol. 34 (2000) 2741.
[38] R. Hirsch, T. Ternes, K. Haberer, K.-L. Kratz, Sci. Total Environ. 225 (1999) 109.
[39] M. Maurer, B.I. Escher, P. Richle, C. Schaffner, A.C. Alder, Water Res. 41 (2007) 1614.
[40] T.A. Ternes, Water Res. 32 (1998) 3245.
[41] E. Zuccato, D. Calamari, M. Natangelo, R. Fanelli, Lancet 355 (2000) 1789.
[42] J. Kosonen, L. Kronberg, Environ. Sci. Pollut. Res. 16 (2009) 555.
[43] A. Kiss, E. Fries, Environ. Sci. Pollut. Res. 16 (2009) 702.
[44] D. Voutsa, P. Hartmann, C. Schaffner, W. Giger, Environ. Sci. Pollut. Res. 13 (2006) 333.
[45] J.-P. Antignac, K. de Wasch, F. Monteau, H. De Brabander, F. Andre, B. Le Bizec, Anal. Chim. Acta 529 (2005) 129.
[46] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[47] K. Bester, Anal. Bioanal. Chem. 391 (2008) 15.
[48] A. Cappiello, G. Famiglini, P. Palma, E. Pierini, V. Termopoli, H. Trufelli, Anal. Chem. 80 (2008) 9343.
[49] P. Konieczka, J. Namieśnik, J. Chromatogr. A 1217 (2010) 882.
[50] M.A. Townsend, D.P. Young, Int. J. Environ. Anal. Chem. 78 (2000) 9.
[51] E. Godfrey, W.W. Woessner, M.J. Benotti, Ground Water 45 (2007) 263.
[52] I.J. Buerge, T. Poiger, M.D. Müller, H.-R. Buser, Environ. Sci. Technol. 40 (2006) 4096.
[53] M.J. Gómez, M.J. Martínez Bueno, S. Lacorte, A.R. Fernández-Alba, A. Agüera, Chemosphere 66 (2007) 993.
[54] E.M. Thurman, M.T. Meyer, M.S. Mills, L.R. Zimmerman, C.A. Perry, Environ. Sci. Technol. 28 (1994) 2267.