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Journal of Chromatography A, 1021 (2003) 133–144
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of acidic pharmaceuticals, antibiotics and ivermectin in river sediment using liquid chromatography–tandem mass spectrometry

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Received 6 March 2003; received in revised form 13 August 2003; accepted 29 August 2003

Abstract

Analytical methods have been developed for the determination of eight acidic pharmaceuticals and two metabolites, seven antibiotics and the parasiticide ivermectin in a selected river sediment. The sediments were solvent extracted with ultrasonic assistance. A solid phase extraction (SPE) clean-up step was performed thereafter. The acidic compounds clofibric acid, diclofenac, fenoprofen, gemfibrozil, ibuprofen, 2-hydroxy-ibuprofen, indomethacin, ketoprofen, naproxen and the parasiticide ivermectin were measured in the negative mode by LC–APCI–tandem MS, whereas the antibiotics clarithromycin, erythromycin, roxithromycin, sulfadiazine, sulfamethazine, sulfamethoxazole and trimethoprim were detected in the positive mode by LC–ESI–tandem MS. Bezafibrate could not be determined in the sediment using the method developed. The limit of quantification (LOQ) ranged from 0.4 to 8 ng g^{-1} for the acidic pharmaceuticals, sulfadiazine and ivermectin and was 20 ng g^{-1} for the other antibiotics.

Keywords: Sediments; Extraction methods; Environmental analysis; Antibiotics; Ivermectin; Antiphlogistics; Lipid regulators

1. Introduction

Pharmaceuticals are widely used for modern medicinal practices. Approximately 3,000 compounds are approved as constituents in medicinal products. Their annual consumption in Germany ranges between a few kilograms and several hundred tons per individual compound [1]. Pharmaceuticals enter the wastewater either by excretion with feces and urine or by direct disposal via toilets. In sewage treatment plants (STPs), the pharmaceutical residues are not totally eliminated and are thus discharged into the receiving waters [2–5]. In recent years, several analytical methods have been reported for the determination of about 100 pharmaceuticals in aqueous environmental matrices [3,6–14]. However, the knowledge of their occurrence in rivers and groundwater is still limited to a few countries. Data on the behavior and the fate of pharmaceuticals in rivers and lakes, including the sediment compartment, are extremely rare [15].

For sediment, only few analytical methods exist, mostly focusing on antibiotics such as tetracyclines [16–20], estrogens [21,22], and a few others [23,24]. To date, methods for the analysis of most human and veterinary pharmaceuticals in sediments are not available. Pharmaceuticals are commonly analyzed in environmental matrices using hyphenated mass spectrometry techniques. LC–tandem MS is the detection method of choice for the polar pharmaceuticals, since no time consuming derivatization is needed.

Macrolide and sulfonamide antibiotics have been reported to occur in the aqueous environment [5,24–26] and are relevant in the formation of antibiotic resistances in pathogenic bacteria [27–29]. Other pharmaceuticals such as analgesics, antiphlogistics and lipid regulators are consumed in large quantities and were frequently found in high concentrations in the aqueous environment [2,5,10,30]. The parasiticide ivermectin, used in human and veterinary medicine, was already found in sediments close to fish farms [24,31–34] due to its elevated lipophilicity ($\log P_{OW}$=3.22).

The aim of the current work was to develop reliable methods for the determination of selected acidic pharmaceuticals, antibiotics and ivermectin with the ultimate goal of having a method to investigate the selected compounds in water/sediment test systems according to OECD guideline 308 [35]. Since the sediment to be used following OECD had to be relatively carbon poor, the sediment selected for the

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doi:10.1016/j.chroma.2003.08.089

method development was a sandy loam with 2.4% organic matter. Comparably, Duft et al. [36] reported a median of organic matter of 2% for 200 different German sediments investigated in chironomide tests, showing that the selected sediment type is quite common.

2. Experimental

2.1. Reagents and standards

The lipid regulators bezafibrate, clofibric acid and gemfibrozil, the antiphlogistics diclofenac, fenoprofen, ibuprofen, indomethacin, ketoprofen and naproxen, the antibiotics erythromycin, sulfadiazine, sulfamethazine, sulfamethoxazole, sulfapyridine, trimethoprim, the surrogate standard oleandomycin and the parasiticide ivermectin were all purchased from Sigma (Deisenhofen, Germany). The surrogate standards abamectin and fenoprop were purchased from Riedel-de-Haen (Seelze Germany), clarithromycin from Abott (Wiesbaden, Germany) and 2-hydroxy-ibuprofen from μ-Mol (Luckenwalde, Germany). Roxithromycin was a courtesy of Roussel Uclaf (Romainville, France). The radiochemical ^{14}C-ibuprofen (labeled in the carboxylic group, specific radioactivity: 8.93 MBq mg^{-1}) was purchased from ARC (St. Louis, MO, US), ^{14}C-paracetamol (labeled in the phenyl-ring, specific radioactivity: 1.54 MBq mg^{-1}) was obtained from Sigma–Aldrich (Deisenhofen, Germany), and ^{14}C-trimethoprim (labeled in the pyrimidine ring, specific radioactivity: 1.48 MBq mg^{-1}) was a courtesy of Hoffmann-La. Roche (Basel, Switzerland). Chemical structures, CAS registry numbers and application fields of the compounds are shown in Table 1.

Stock solutions of the reference compounds were prepared in methanol and stored at −20 °C. Acetic acid, ammonium acetate, sodium dihydrogenphosphate, disodium hydrogenphosphate, acetone, acetonitrile, methanol, ethyl acetate were purchased from Merck (Darmstadt, Germany) and humic acid sodium salt (45–60% humic acid) from Roth (Karlsruhe, Germany). All reagents were of analytical grade and the applied solvents were of SupraSolv quality. High purity water was prepared by a Milli-Q water purification system (Millipore, Milford, MA, USA).

2.2. Sediment extraction

The sediment (50 g) was spiked with 800 ng of the surrogate standards. Fenoprop was used as surrogate standard for the acidic pharmaceuticals, oleandomycin and sulfapyridine for the antibiotics and abamectin for ivermectin. The sediments were then extracted successively in an ultrasonic bath with 4 × 45 ml of different organic solvents in PPCO flasks (Nalge Nunc International, Rochester, NY, USA). For the acidic pharmaceuticals and the parasiticide ivermectin, acetone/acetic acid (20/1 (v/v)) was used first and then three times ethyl acetate. Antibiotics were extracted using two times methanol and then acetone and ethyl acetate. The slurries of the solvent–sediment mixtures were thoroughly hand shaken and then ultrasonicated for 15 min. Afterwards the slurries were centrifuged for 7 min at 34,000 rad min^{-1} and the supernatant solvent phases were filtrated through paper filters, combined and evaporated by a rotary evaporator at 40 °C and 150–200 mbar until only one aqueous phase remained.

2.3. SPE/clean-up

The obtained sediment extracts were diluted with 500 ml of deep groundwater, which is known to be free of anthropogenic organic contamination. Additionally, the flasks used for rotary evaporation were rinsed using 3 ml of methanol which was then combined with the groundwater solution. Afterwards, a solid phase extraction (SPE) was performed using specific solid phases (see below) for the three different analyte groups. The SPE cartridges were conditioned prior to sample extraction with 6 ml of *n*-hexane, 2 ml of acetone, 10 ml of methanol and 10 ml of groundwater. Solid sample material which precipitated on the frits in the course of the SPE was removed by exchanging the uppermost frits in the SPE cartridges.

2.3.1. Acidic pharmaceuticals

Aqueous sediment extracts (500 ml), adjusted to pH 2 with 3.5 mol l^{-1} sulfuric acid, were enriched on pre-packed Oasis MCX cartridges (60 mg, 30 μm, Waters, Eschborn, Germany). The samples were passed through the SPE cartridges at a flow rate of 20 ml min^{-1}. Afterwards, the cartridges were dried with nitrogen for 20 min and were eluted with 4 × 1 ml of acetone. Then the eluates were reduced in a gentle nitrogen stream to approximately 100 μl. A volume of 200 μl methanol was added and the extracts were evaporated to 200 μl. Finally, the extracts were filled up to 500 μl with Milli Q water adjusted to pH 2.9 with acetic acid.

2.3.2. Antibiotics

The SPE of environmental waters is already described in detail in Hirsch et al. [6]. The original protocol was slightly modified by the addition of 12.5 mg l^{-1} humic acids to the groundwater prior to SPE. Aqueous sediment extracts were passed through SPE cartridges, which were then dried and eluted using 4 × 1 ml methanol. After removing the solvent, the residues were dissolved in 50 μl methanol and 450 μl phosphate buffer.

2.3.3. Ivermectin

Aqueous sediment extracts (500 ml) were mixed with 25 ml ammonium acetate buffer (1 mol l^{-1}, pH 4.0). The SPE was conducted on manually packed glass cartridges filled with 250 mg LiChrolute EN (40–120 μm) Merck (Darmstadt, Germany). Then, the cartridges were eluted with 4 × 1 ml ethyl acetate. The eluates were reduced to dryness in a gentle nitrogen stream and the residues were fi-

Table 1
CAS registry numbers, chemical structure and use/origin of acidic pharmaceuticals, antibiotics and parasiticides

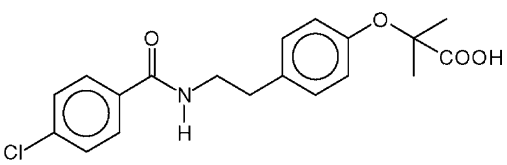
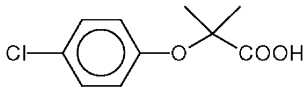
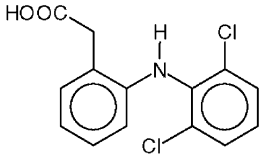
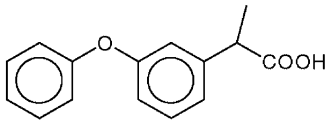
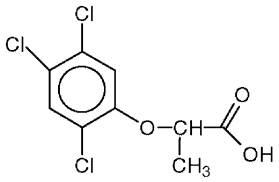
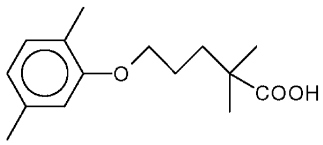
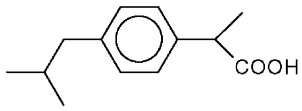
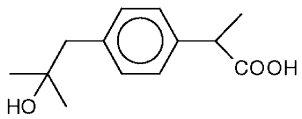
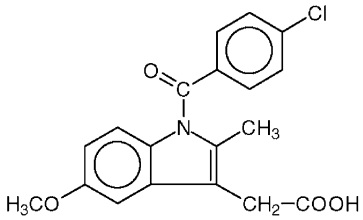
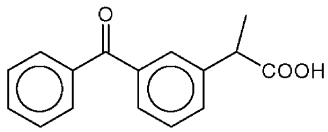
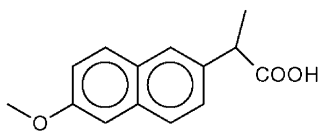
Compound	CAS RN	Structure	Use/origin
Acidic pharmaceuticals			
Bezafibrate	41859-67-0		Lipid regulator
Clofibric acid	882-09-7		Metabolite of several lipid regulators
Diclofenac	15307-86-5		Antiphlogistic
Fenoprofen	53746-45-5		Antiphlogistic
Fenoprop	93-72-1		Surrogate standard
Gemfibrozil	25812-30-0		Lipid regulator
Ibuprofen	15687-27-1		Antiphlogistic
2-Hydroxy-ibuprofen	51146-55-5		Metabolite of ibuprofen
Indomethacin	53-86-1		Antiphlogistic
Ketoprofen	22071-15-4		Antiphlogistic
Naproxen	22204-53-1		Antiphlogistic

Table 1 (*Continued*)

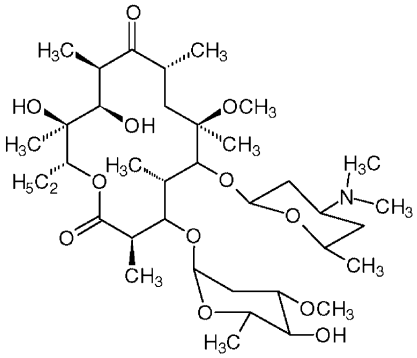
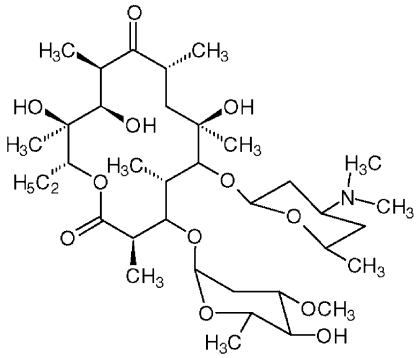
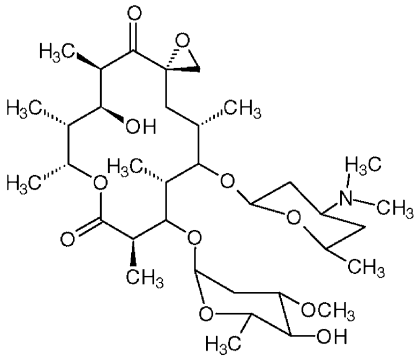
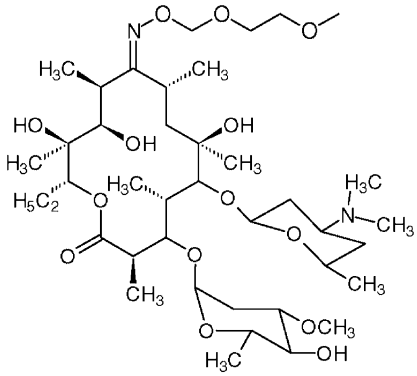
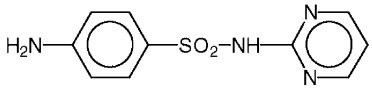
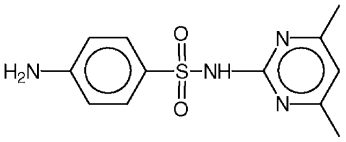
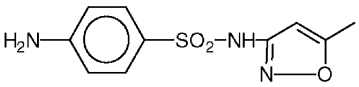
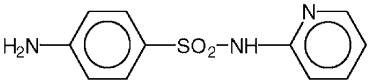
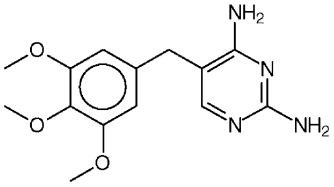
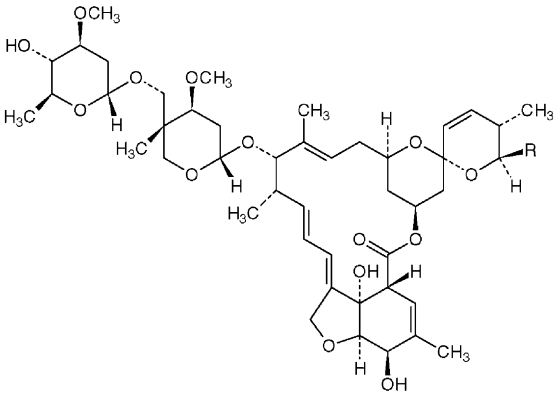
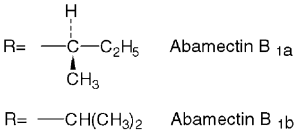
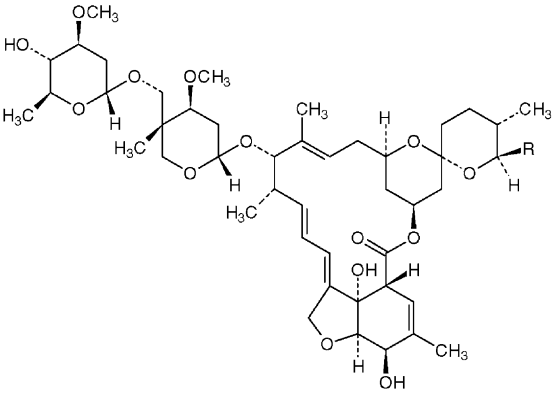
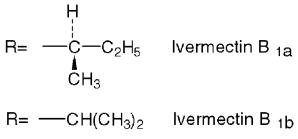
Compound	CAS RN	Structure	Use/origin
Antibiotics			
Clarithromycin	81103-11-9		Macrolide antibiotic
Erythromycin	114-07-8		Macrolide antibiotic
Oleandomycin	3922-90-5		Surrogate standard
Roxithromycin	80214-83-1		Macrolide antibiotic
Sulfadiazine	68-35-9		Sulfonamide antibiotic

Table 1 (*Continued*)

Compound	CAS RN	Structure	Use/origin
Sulfamethazine	57-68-1		Sulfonamide antibiotic
Sulfamethoxazole	723-46-6		Sulfonamide antibiotic
Sulfapyridine	144-83-2		Sulfonamide antibiotic
Trimethoprim	738-70-5		Chemotherapeutic agent
Parasiticides			
Abamectin	71751-41-2, B1a 65195-55-3	 	Surrogate standard
Ivermectin	B1a 71827-03-7, 70161-11-4, B1b 70209-81-3	 	Parasiticide

nally dissolved in 500 μl of a mixture of ammonium acetate buffer (15 mmol l^{-1}, pH 4.0) and acetonitrile (90:10 (v/v)).

2.4. HPLC conditions

Prior to injection all sediment extracts were filtrated through syringe filters (0.45 μm, Spartan 13/20, Schleicher & Schuell, Dassel, Germany). The Perkin-Elmer HPLC system consisted of a Series 2000 in-line degasser and eluent pump connected to an AS-2000a auto sampler. For all pharmaceuticals the LC columns used were 125 mm × 3 mm LiChrospher RP-18 columns (5 μm; Merck, Darmstadt, Germany), which were kept at 25 °C for antibiotics and acidic compounds and at 50 °C for ivermectin. The three analyte groups were separated using three eluent systems (Table 2) at a flow rate of 300 μl min^{-1} for the acidic pharmaceuticals, 400 μl min^{-1} for the antibiotics, following the method reported by Hirsch et al. [6], and 500 μl min^{-1} for ivermectin. Sample injection volumes were always 50 μl.

2.5. Tandem MS parameters

A Perkin-Elmer Sciex API 365 triple stage quadrupole mass spectrometer was used for detection. The acidic pharmaceuticals and the parasiticide ivermectin were determined using atmospheric pressure chemical ionization (APCI) in the negative ion mode at 425 °C, without splitting the eluent and a corona needle charge of 1450 V for the 2-hydroxy-ibuprofen, 2050 V for the other acidic pharmaceuticals and 1850 V for the avermectins. For the antibiotics, turbo electrospray ionization (ESI) was applied in the positive ion mode at 400 °C, a split rate of 1:10 and an ion source voltage of 4600 V. MS–MS parameters were optimized in the continuous flow mode. The conditions for the measurement of precursor ions were optimized in the single MS scan mode. Exclusively protonated or deprotonated molecular ions were used as precursors for subsequent MS–MS experiments. Product ion spectra were recorded by scanning Q3 over the relevant mass range. After the determination of the product ions, the conditions for the nitrogen-collision-induced dissociation were optimized. Detailed data on the measurement conditions of the individual compounds, their precursor and product ions are shown in Table 3. The fragmentation patterns are in accordance with mass spectra already reported for the acidic pharmaceuticals [7,37], antibiotics [6,38–42] and avermectins [43,44].

Table 2
Mobile phase compositions for the three separation methods

Time (min)	Composition of the mobile phase	
Acidic pharmaceuticals	Eluent A[a] (%)	Eluent B[a] (%)
0	40	60
3	100	0
13	100	0
18	40	60
28	40	60
Antibiotics	Eluent C[a] (%)	Eluent D[a] (%)
0	74	26
2	62	38
7	62	38
10	0	100
26	74	26
36	74	26
Ivermectin	Eluent E[a] (%)	
0	100	
5.5	100	

[a] Eluent A, acetonitrile; Eluent B, Milli-Q water acidified to pH 2.9 with acetic acid; Eluent C, aqueous solution of 900 ml 20 mmol l^{-1} NH_3 adjusted with acetic acid to pH 5.7 and mixed with 100 ml of acetonitrile; Eluent D = 200 ml of Eluent C + 800 ml acetonitrile; Eluent E = acetic acid was added to water containing 15 mmol l^{-1} ammonium acetate until pH 4.0 was obtained – a volume of 900 ml acetonitrile was then mixed with 100 ml of that solution.

2.6. Determination of recoveries

2.6.1. Sediment extraction

Aerobic sediment was taken from the Wickerbach creek, close to Frankfurt (Southwest Germany). It was sieved (2 mm mesh) and was characterized as loamy sand containing 77.5% sand, 12.6% silt, 9.9% clay and 2.4% organic matter. The sediment was autoclaved in portions of 50 g at 131 °C for 2 h to inactivate microorganisms and any enzymatic activity.

All recoveries were determined relative to non-enriched standard samples. For recovery experiments, autoclaved sediment samples (50 g) were spiked with the analytes at various concentrations and were shaken thoroughly to obtain even distribution of the analytes. The samples were then kept overnight (∼14 h) to distribute the analytes to the sediment. Surrogate standards were then added and the samples were analyzed as described above. The extraction efficiencies were checked by spiking ^{14}C-ibuprofen and ^{14}C-trimethoprim, respectively, to 50 g of autoclaved sediment. Sediment samples were extracted the next day (∼14 h later) and the total radioactivity in the solvent extract was measured by a liquid scintillation counter Tricarb 2500 TR (Packard, Dreieich, Germany).

2.6.2. SPE/clean-up

Individual recoveries for the SPE step were determined by spiking groundwater samples (500 ml) with analytes and the respective surrogate standards. All recoveries were calculated in comparison to an external, non-enriched standard sample. For calculation of the relative recoveries, the results were corrected with the recovery of the spiked surrogate standard.

2.7. Calibration and limit of quantification

Calibration included the entire sample preparation procedure after spiking groundwater samples. The calibration curves were prepared of 10 calibration points by spiking

Table 3
LC–APCI–MS–MS conditions for analysis of acidic pharmaceuticals, antibiotics and parasiticides

Compound	Retention time (min)	MS-parameters			Precursor and product ions (*m*/*z*)	
		Orifice (V)	Ring (V)	CID energy[b] (eV)		
(a) Acidic pharmaceuticals						
2-Hydroxy-ibuprofen	4.2	−6	−110	18	221.2	$[M–H]^-$
					177.1	$[M–H–CO_2]^-$
					133.1[a]	
Clofibric acid	9.7	−3	−84	30	213.1	$[M–H]^-$
					127.0[a]	$[C_6H_4ClO]^-$
					85.1	$[C_5H_5O_2]^-$
Ketoprofen	9.9	−1	−80	19	253.0	$[M–H]^-$
					209.1	$[M–H–CO_2]^-$
					197.1[a]	
Naproxen	10.5	−1	−80	55	229.0	$[M–H]^-$
					185.0	$[M–H–C_2H_3O]^-$
					169.0[a]	$[M–H–C_2H_3O_2]^-$
Bezafibrate	10.6	−1	−92	31	360.0	$[M–H]^-$
					273.5[a]	$[M–H–C_4H_6O_2]^-$
					153.9	$[M–H–C_{12}H_{14}O_3]^-$
Fenoprop	14.4	−1	−81	25	267.1	$[M–H]^-$
					194.9[a]	$[M–H–C_3H_4O_2]^-$
					159.0	
Fenoprofen	14.4	−3	−92	63	241.1	$[M–H]^-$
					197.2	$[M–H–CO_2]^-$
					182.3	
					211.0	
					93.0[a]	$[M–H–C_9H_8O_2]^-$
Diclofenac	15.6	−1	−95	23	294.3	$[M–H]^-$
					249.8[a]	$[M–H–CO_2]^-$
					214.4	
Indomethacin	15.6	−5	−90	21	356.2	$[M–H]^-$
					312.1[a]	$[M–H–CO_2]^-$
					296.6	$[M–H–C_2H_3O_2]^-$
Ibuprofen	16.0	−1	−80	17	205.1	$[M–H]^-$
					159.1[a]	$[M–H–CO_2]^-$
					175.0	
Gemfibrozil	17.5	−7	−110	36	249.3	$[M–H]^-$
					121.0[a]	$[M–H–C_7H_{12}O_2]^-$
(b) Antibiotics						
Sulfadiazine	2.7	8	130	30	251.0	$[M + H]^+$
					155.7[a]	[Sulfonamidyl cation]$^+$
					108.2	[Amidophenyl cation]$^+$
Sulfapyridine	3.1	14	140	34	250.2	$[M + H]^+$
					183.6[a]	$[M–H_2SO_2 + H]^+$
Sulfamethoxazole	3.4	30	185	31	254.2	$[M + H]^+$
					156.0[a]	[Sulfonamidyl cation]$^+$
					108.1	[Amidophenyl cation]$^+$
Sulfamethazine	3.6	27	180	34	279.2	$[M + H]^+$
					204.0[a]	$[M–\text{anilline} + H_2O + H]^+$
					124.0	[Aminodimethyl pyrimidine + H]$^+$
Trimethoprim	4.9	28	170	42	291.3	$[M + H]^+$
					260.8[a]	$[M–OCH_3 + H]^+$
					230.0	$[M–2OCH_3 + H]^+$
Oleandomycin	11.9	9	170	−30	688.7	$[M + H]^+$
					544.6[a]	$[M–\text{oleandrose} + H]^+$
					157.9	[Desosamine + H]$^+$
Erythromycin	13.8	16	190	37	716.4	$[M–H_2O + H]^+$
					558.4[a]	$[M–\text{desosamine}–H_2O + H]^+$
					158.2	[Desosamine + H]$^+$
Clarithromycin	18.0	14	180	37	748.6	$[M + H]^+$
					590.5[a]	$[M–\text{desosamine} + H]^+$
					158.2	[desosamine + H]$^+$

Table 3 (*Continued*)

Compound	Retention time (min)	MS-parameters			Precursor and product ions (*m*/z)	
		Orifice (V)	Ring (V)	CID energy[b] (eV)		
Roxithromycin	18.0	10	180	56	837.4	$[M + H]^+$
					679.4[a]	[*M*–desosamine + H]$^+$
					158.2	[desosamine + H]$^+$
(c) Parasiticides						
Abamectin	2.8	−9	−174	45	871.5	$[M–H]^-$
					835.5	$[M–2H_2O–H]^-$
					565.4[a]	[*M*–oleandrose anhydride–H]$^-$
Ivermectin	3.9	−15	−175	40	873.5	$[M–H]^-$
					837.6	$[M–2H_2O–H]^-$
					567.5[a]	[*M*–oleandrose anhydride–H]$^-$

[a] Product ion used for quantification.
[b] CID, collision induced dissociation.

groundwater samples of 500 ml. The SPE and the subsequent measurement were performed as described above. For calibration the analyte concentrations were plotted versus the corresponding analyte peak areas or the respective ratios of analyte and surrogate standard peak areas. The LOQ was defined as the second lowest calibration point in the linear regression, as long as the signal-to-noise (S/N) ratios of the sample extracts were >10 [45]. In each series an enriched blank sample and a non-enriched standard sample were included. For the acidic pharmaceuticals, matrix matched calibration samples were prepared by the addition of a blank sediment extract to the groundwater before enrichment to consider matrix effects.

3. Results and discussion

Spiking experiments are valuable tools for the determination of recovery rates and extraction yields allowing for a characterization and validation of analytical methods. Two major challenges have to be faced for the determination of the extraction yield of organic analytes in sediments. First, the analytes should attain sorption–desorption equilibrium by incubation during a defined contact time prior to extraction. Furthermore, the microbial activity of sediments might potentially transform the analytes. Additionally, microbial activity might influence the binding of the analytes to the sediment, e.g. by incorporation into the organic matter. The microbial activity and enzymatic activity in the sediments has to be eliminated, in order to minimize the influence of any bio-transformation process in the sediments on the extraction yields. Several techniques can be used for sterilization of the sediments, e.g. γ-radiation, autoclaving, amendment with poisons and chemo-oxidants [46,47]. Although autoclaving has a certain impact on the sediment, the organic matter, the cation-exchange capacity and finally the resulting adsorption isotherms remain essentially unaltered [47].

The impact of bio-transformation on the recovery rates was determined by spiking the ^{14}C-labeled pharmaceuticals ibuprofen, trimethoprim and paracetamol onto non-autoclaved and autoclaved sediment. The recovery rates, which were obtained after a contact time of 14 h, are shown in Table 4. Ibuprofen was rapidly bio-transformed and decarboxylated which led to low recovery rates in non-autoclaved sediment. The radioactivity initially present as paracetamol was bound almost entirely to the sediment matter. It can be assumed that this was due to the incorporation of unchanged or metabolized paracetamol in the sediment matter by microbial activity, as reported recently for paracetamol in soil [48]. For trimethoprim, the recovery rate in non-autoclaved soil was still slightly reduced. In contrast, all recovery rates determined in autoclaved soil were almost quantitative showing the necessity of the bio-inactivation step to avoid enormous analyte losses caused by bio-transformation.

In general, solvent extracts of the sediments contained a high level of natural matrix components. Hence, it was essential to include further clean-up steps to minimize chromatographic interferences and ion suppression. A general scheme of the analytical method developed is shown in Fig. 1. Chromatograms of the three analyte groups in spiked sediment are shown in Fig. 2. Interfering matrix signals were only observed in the detection of diclofenac, however they occurred on a relatively low level. For the antibiotics and the parasiticides no significant matrix interferences could be detected in the chromatogram.

Table 4
Recoveries and confidence intervals ($P = 95\%$, $n = 4$) of ibuprofen, paracetamol and trimethoprim in non-autoclaved and autoclaved sediments

	^{14}C-ibuprofen (%)	^{14}C-paracetamol (%)	^{14}C-trimethoprim (%)
Non-autoclaved	25 ± 2	10 ± 1	68 ± 3
Autoclaved	94 ± 3	95 ± 2	86 ± 3

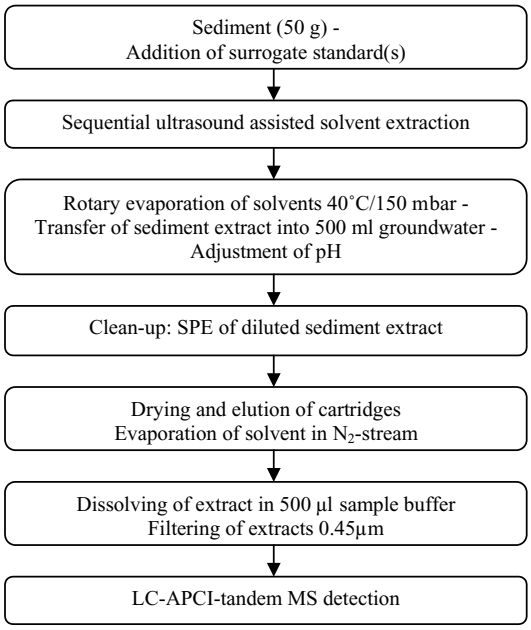


Fig. 1. Scheme of the analytical procedure for the analysis of pharmaceuticals in river sediment.

Table 5
Recoveries (%) and confidence intervals ($P = 95\%$, $n = 4$) for ibuprofen and trimethoprim after extraction of 50 g autoclaved sediment spiked with 3 ng g^{-1} ^{14}C-labeled ibuprofen and 20 ng g^{-1} ^{14}C-labeled trimethoprim

	Ibuprofen	Trimethoprim
First extraction	75 ± 1	62 ± 4
Second extraction	17 ± 3	18 ± 2
Third extraction	0	7 ± 3
Fourth extraction	0	0
Sum	92 ± 4	86 ± 4

3.1. Acidic pharmaceuticals

3.1.1. Sediment extraction

The efficiency of the solvent extraction was determined for ibuprofen using a ^{14}C-labeled standard. An excellent recovery of 92 ± 4% was obtained within the first two extraction steps at a spiking level of 3 ng g^{-1} (Table 5). Further extraction sequences did not increase the recovery. A similar behavior in the solvent extraction procedure can be assumed for the other acidic pharmaceuticals, but due to the lack of appropriate radiolabeled standards it could not be verified.

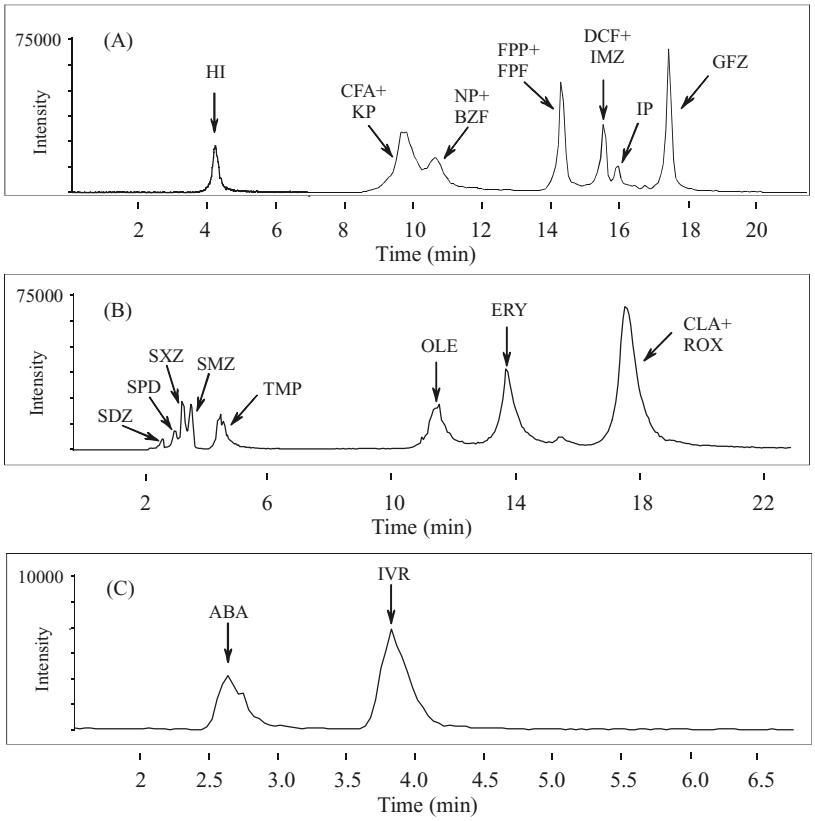


Fig. 2. Overlay of MRM traces recorded for sediment samples spiked at a level of 3 ng g^{-1}: (A) acidic pharmaceuticals (HI, 2-hydroxy-ibuprofen; CFA, clofibric acid; KP, ketoprofen; NP, naproxen; BZF, bezafibrate; FPP, fenoprop; FPF, fenoprofen; DCF, diclofenac; IMZ, indomethacin; IP, ibuprofen; GFZ, gemfibrozil); (B) antibiotics (SDZ, sulfadiazine; SPD, sulfapyridine; SXZ, sulfamethoxazole; SMZ, sulfamethazine; TMP, trimethoprim; OLE, oleandomycin; ERY, erythromycin; CLA, clarithromycin; ROX, roxithromycin); (C) parasiticides (ABA, abamectin; IVR, ivermectin).

Table 6
LOQ's ($ng\,g^{-1}$), recoveries (%) and confidence intervals ($P = 95\%$, $n = 3$) of various pharmaceuticals for the individual SPE and for the sediment at two spiking levels (20 and $3\,ng\,g^{-1}$)

	LOQ ($ng\,g^{-1}$)	SPE (spiking level, $1\,\mu g\,l^{-1}$)		Sediment (spiking level, $20\,ng\,g^{-1}$)		Sediment (spiking level, $3\,ng\,g^{-1}$)	
		Absolute recovery (%)	Relative recovery (%)	Absolute recovery (%)	Relative recovery (%)	Absolute recovery (%)	Relative recovery (%)
Acidic pharmaceuticals							
Bezafibrate	–	102 ± 1	102 ± 7	206 ± 14	146 ± 22	205 ± 45	152 ± 17
Clofibric acid	0.4	90 ± 4	90 ± 4	99 ± 4	70 ± 5	107 ± 6	79 ± 10
Diclofenac	8	116 ± 25	115 ± 25	81 ± 18	57 ± 12	125 ± 85	92 ± 48
Fenoprofen	1	93 ± 4	92 ± 5	110 ± 9	77 ± 11	137 ± 34	102 ± 8
Gemfibrozil	0.4	94 ± 4	94 ± 3	67 ± 15	47 ± 5	64 ± 17	47 ± 5
Ibuprofen	0.4	98 ± 2	98 ± 6	87 ± 10	61 ± 3	85 ± 17	63 ± 5
2-Hydroxy-ibuprofen	0.4	75 ± 13	75 ± 12	56 ± 10	40 ± 4	69 ± 16	51 ± 18
Indomethacin	0.4	94 ± 5	94 ± 5	80 ± 17	57 ± 5	123 ± 45	91 ± 18
Ketoprofen	0.4	109 ± 1	109 ± 7	151 ± 17	107 ± 22	170 ± 12	126 ± 23
Naproxen	0.4	102 ± 3	102 ± 6	134 ± 2	95 ± 12	131 ± 17	97 ± 6
Fenoprop[a]	–	100 ± 7	–	142 ± 17	–	135 ± 24	–
Antibiotics							
Clarithromycin	20	59 ± 14	98 ± 23	39 ± 10	69 ± 5	25 ± 7	42 ± 11
Erythromycin	20	62 ± 12	103 ± 19	43 ± 16	77 ± 15	22 ± 18	38 ± 30
Roxithromycin	20	55 ± 12	92 ± 20	41 ± 14	71 ± 9	36 ± 14	62 ± 24
Oleandomycin[b]	–	67 ± 7	–	55 ± 10	–	59 ± 2	–
Sulfadiazine	3	87 ± 8	100 ± 5	82 ± 7	121 ± 9	55 ± 18	79 ± 8
Sulfamethazine	20	90 ± 8	103 ± 6	49 ± 2	71 ± 4	40 ± 45	59 ± 74
Sulfamethoxazole	20	94 ± 7	108 ± 6	65 ± 8	99 ± 22	75 ± 41	113 ± 93
Trimethoprim	20	66 ± 16	75 ± 15	73 ± 3	99 ± 23	26 ± 2	38 ± 12
Sulfapyridine[c]	–	87 ± 9	–	71 ± 11	–	71 ± 30	–
Parasiticides							
Ivermectin	0.4	68 ± 17	90 ± 8	41 ± 16	102 ± 20	37 ± 6	119 ± 14
Abamectin[d]	–	75 ± 24	–	40 ± 8	–	31 ± 6	–

Surrogate standards (in superscript letters): (a) acidic pharmaceuticals, (b) macrolides, (c) sulfonamides and (d) ivermectin.

3.1.2. SPE/clean-up

The individual recoveries of the SPE were almost quantitative for all acidic compounds, with the exception of the ibuprofen metabolite 2-hydroxy-ibuprofen which attained a recovery of 75 ± 12% (Table 6). Therefore, the SPE exhibited high suitability for the acidic analytes. The method allowed for quantification with LOQ's ranging from 0.4 to $1\,ng\,g^{-1}$. For diclofenac, a S/N ratio >10 could only be ensured above the level of $8\,\mu g\,g^{-1}$, which was then defined as the LOQ for diclofenac. The correlation coefficients (r^2) were always higher than 0.99.

3.1.3. Entire sample preparation of the sediments

Over the entire sample preparation, clofibric acid, diclofenac, fenoprofen, ibuprofen and indomethacin showed good absolute recoveries ranging from 80 ± 17 to 110 ± 9% at a spiking level of $20\,ng\,g^{-1}$. Mainly similar recoveries were found at a spiking level of $3\,ng\,g^{-1}$. However, for some compounds, the 95% confidence interval increased significantly at the lower spiking level. The proposed surrogate standard fenoprop was not appropriate to compensate for the losses of these compounds.

Gemfibrozil and 2-hydroxy-ibuprofen had recoveries over the entire procedure not higher than 67 ± 15% and 56 ± 10%. However, due to the low statistical error, a semi-quantitative determination of these compounds should at least be possible. Once again, fenoprop was unable to compensate for these losses.

Ketoprofen and naproxen exhibited absolute recoveries of 151 ± 17% and 134 ± 2%, respectively. These overdeterminations could be effectively compensated by the surrogate standard fenoprop at both spiking levels, whereas this was not possible for bezafibrate. Therefore, bezafibrate cannot be measured quantitatively using the developed method. The main reason for the overdeterminations was presumably an enhanced ionization in the APCI-interface, due to the presence of matrix compounds. Contamination of the sediments used or increased recoveries in the SPE due to matrix effects were checked and could be excluded.

3.2. Antibiotics

3.2.1. Sediment extraction

Recoveries for the solvent extraction procedure were determined with ^{14}C-labeled trimethoprim, which was the only commercially available radio-labeled antibiotic (Table 5). After three extraction sequences, 86 ± 4% of trimethoprim were recovered at a spiking level of $20\,ng\,g^{-1}$ in autoclaved

sediment. The fourth extraction sequence did not enhance the recovery.

3.2.2. SPE/clean-up

In the SPE step, the recoveries of the macrolides in spiked groundwater are enhanced, compared to those in deionized water as already stated by Hirsch et al. [6]. It was assumed that the presence of the natural salt content was the main reason for this effect. Later on it was found that the presence of humic substances also improved the recoveries of the macrolides. Hence, humic substances were added to all aqueous samples, to attain recoveries comparable to those in aqueous sediment extracts which contained humic substances as natural matrix components. Individual recoveries of the macrolides ranged between 55 ± 12 and 67 ± 7% for the SPE (Table 6). These relative low recoveries were effectively compensated by the surrogate standard oleandomycin, leading to relative recoveries between 92 ± 20 and 103 ± 19%. All sulfonamides investigated were recovered almost quantitatively after SPE with a low statistical error, while the recovery of trimethoprim was 66 ± 16%. A compensation with the surrogate standard sulfapyridine led to relative recoveries between 100 ± 5 and 108 ± 6% for the sulfonamides and 75 ± 15% for trimethoprim.

3.2.3. Entire sample preparation of the sediments

For the macrolides the recoveries for the sediments ranged between 39 ± 10 and 55 ± 10% at a spiking level of 20 ng g^{-1} and were only in tendency, but not significantly, lower as the recoveries for the SPE alone. Hence, it can be assumed that the solvent extraction of the macrolides was almost quantitative, comparably to trimethoprim. The low recoveries were compensated to some extent by the surrogate standard oleandomycin, leading to relative recoveries between 69 ± 9 and 77 ± 15%. For the sulfonamides and trimethoprim the recoveries for the sediments ranged from 49 ± 2 to 82 ± 7% at a spiking level of 20 ng g^{-1}. A compensation with the surrogate standard sulfapyridine, provided excellent relative recoveries for sulfadiazine, sulfamethoxazole and trimethoprim, whereas for sulfamethazine the recoveries were lower at 71 ± 4%.

However, at the lower spiking level of 3 ng g^{-1}, for most macrolides and sulfonamides, the recoveries decreased and the statistical error increased enormously. Thus, reliable data could not be achieved, except for sulfadiazine with a relative recovery of 79 ± 8%. Hence, only for sulfadiazine a LOQ of 3 ng g^{-1} could be ensured. For all other antibiotics investigated the LOQ had to be set at 20 ng g^{-1} due to the limited reproducibility and low recoveries at the lower spiking level. Correlation coefficients (r^2) determined of the calibration were always higher than 0.99 for all analytes.

3.3. Ivermectin

A new LC tandem MS method was developed for the determination of the parasiticide ivermectin in sediment. As described by Reising, abamectin was applied as a surrogate standard, and allowed for an effective compensation of virtually all analyte losses, since both compounds show very similar properties [49]. Ivermectin forms adducts with cations, such as ammonium and sodium [43]. The cleavage of adducts in LC tandem MS could be effectively realized with the application of the APCI interface.

3.3.1. SPE/clean-up

Ivermectin and the surrogate standard abamectin were recovered after the SPE with 68 ± 17% and 75 ± 24%, respectively. A compensation with the surrogate standard led to good relative recoveries of 90 ± 8%.

3.3.2. Entire sample preparation of the sediments

For the entire sample preparation of the sediments, recoveries between 31 ± 6 and 41 ± 16% were attained at both spiking levels. Losses during the sample preparation were presumably caused by the sediment extraction and the SPE clean-up (Table 6). Both compounds have a high affinity for sediments due to their high lipophilicity and due to their ability to form adducts with dissolved cations, e.g. sodium, ammonium and possibly with undissolved cations in the inorganic sediment matter.

However, excellent relative recoveries were obtained for ivermectin after compensation with abamectin, exhibiting its good suitability as surrogate standard. The LOQ for ivermectin was set as 0.4 ng g^{-1} and the calibration curve provided correlation coefficients (r^2) always exceeding 0.99.

4. Conclusion

The described methods allow the determination of the selected pharmaceutical residues in sediments down to the lower ng g^{-1} range. Due to the effective SPE clean up, up to 50 g sediment samples could be used for extraction. The surrogate standards were crucial for reliable data, since they frequently compensated for analyte losses. Since sediments from different origins can vary widely in their composition, the methods developed have to be confirmed carefully for the analysis of sediments with a significantly higher percentage of organic matter. For those sediments higher LOQs might occur. The presented method allowed the determination of various pharmaceuticals in sediments of laboratory batch experiments. Determination of these analytes in real sediments should also be possible. However, since the concentrations of the analytes in natural sediments are still widely unknown it has to be clarified whether the LOQ's are sufficiently low to determine the natural contamination of river sediments with pharmaceutical residues.

Acknowledgements

This work was supported by grants of the German Environmental Agency (Project no. 299 67 401). The

experiments of this work were carried out mainly at the ESWE-Institute in Wiesbaden (Germany). We thank Derek McDowell for the fruitful discussions and the revision of the manuscript. Additionally, we acknowledge Nadine Herrmann and Matthias Bonerz for their support in the laboratory work and Michael Meller, Jörg Römbke and Hans Schallnaß for their support in the radiotracer experiments.

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