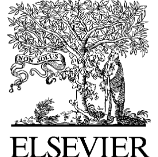

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Determination of pharmaceuticals, iodinated contrast media and musk fragrances in sludge by LC/tandem MS and GC/MS

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Abstract

Analytical methods have been developed that allow for the determination of antiphlogistics, lipid regulators, the antiepileptic carbamazepine, cytostatic agents, the psychiatric drug diazepam and iodinated contrast media (ICM) as well as two major polycyclic musk fragrances HHCB (galaxolide) and AHTN (tonalide) in activated and digested sludge. The procedures consist of ultrasonic solvent extraction (USE) using methanol/acetone or pressurized liquid extraction (PLE) using 100% methanol. Clean-up was performed with C_{18ec} material and silica gel followed by LC tandem MS (electrospray or atmospheric pressure chemical ionization) detection for pharmaceuticals and iodinated contrast media as well as GC/MS in the SIM mode for musk fragrances. Absolute recoveries from spiked activated sludge in general ranged from 88 ± 4 to $119 \pm 20\%$ for ICM and were 78 ± 15 and $87 \pm 10\%$ for the AHTN and HHCB, respectively. For the pharmaceuticals, absolute recoveries in activated sludge ranged between 43 and 78%. Subsequently, compensation of losses was carried out by using surrogate standards (acidic pharmaceuticals: fenoprop, neutral pharmaceuticals: dihydro-carbamazepine, musk fragrances: AHTN-D_3). With one exception the recoveries were also adequate in digested sludge ranging from 43% to 120%.
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Keywords: Antiepileptics; Cytostatic agents; Psychiatric drugs; Antiphlogistics; Lipid regulators; Contrast media; Musk fragrances; Sewage; Sewage sludge; GC/MS; SPE; PLE; LC/MS/MS

1. Introduction

In human medicine of many countries, pharmaceuticals and iodinated contrast media (ICM) are consumed in the ton-range per year. It can be expected that worldwide consumption of pharmaceuticals will increase due to a developing health care system and a higher life expectancy in industrial countries.

Musk fragrances are used in high quantities in cosmetic products. In 1996, about 5600 t of polycyclic musk fragrances (PMF) were used worldwide [1]; the production of HHCB alone was estimated to be 1000 t. In recent years, nitro musk fragrances have successively been replaced by PMF in some countries (e.g. Germany).

Human pharmaceuticals, iodinated contrast media (ICM) and ingredients of cosmetic products such as PMF are introduced into sewage to a high extent by households. Recently, their occurrence in municipal sewage treatment plants (STPs) and the receiving water has been reported in Europe, Brazil and North America by several authors [2–15]. Since pharmaceuticals, ICM and PMF are not totally removed during sewage treatment [9,10,14,16–20] they are discharged in appreciable quantities into receiving waters through STP effluents.

Concentrations of pharmaceuticals present in sewage sludge are needed to perform flow studies and total mass balances in STPs. Although many pharmaceuticals are relatively polar, a specific sorption might occur as found for

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fluoroquinolone antibiotics [14]. To the best of our knowledge, no analytical methods have been reported in literature for the analysis of the selected pharmaceuticals (see Table 1) in sewage sludge.

Most PMF are more hydrophobic than pharmaceuticals. Due to their elevated lipophilicity ($\log K_{OW}$ (AHTN) = 5.90–6.35) [1,18,21,22] PMF are, therefore, sorbed onto sludge and suspended matter. In literature, analytical methods are reported for analyzing PMF in sewage sludge using Soxhlet or pressurized liquid extraction (PLE) with dichloromethane, silica gel, alumina columns and gel permeation chromatography (GPC) clean-up and GC/MS [16,23,24]. Concentration levels for AHTN and HHCB in municipal sludge have been reported to be in the mg/kg range [23–25].

The objective of this paper is to present reliable analytical methods developed for the detection of antiphlogistics, lipid regulators, the antiepileptic carbamazepine, cytostatic agents, the tranquilizer diazepam, ICM, and two major polycyclic musk fragrances HHCB and AHTN in activated and digested sludge (see Table 1). The individual compounds were selected according to current data available on their occurrence in municipal sewage [2–15] (Table 1a and b; list of selected pharmaceuticals, iodinated contrast media and musk fragrances).

2. Experimental section

A scheme of the analytical methods is illustrated in Fig. 1a and b.

2.1. Sampling of Swiss sewage treatment plants

Anaerobically digested sludges ($n = 2$) and two activate sludges ($n = 2$) were collected from the mechanical–biological STPs in Kloten–Opfikon and Altenrhein near Zurich, Switzerland. The samples were collected in glass bottles, filtered through a glass fibre filter (GF 8, diameter 90 mm Schleicher & Schuell, Dassel, Germany) to obtain the solid fraction and were frozen at −20 °C. Afterwards, the sewage sludge samples were freeze-dried, ground in a mortar, mixed thoroughly and stored in amber bottles until analysis. The STP of Kloten–Opfikon serves a residential population of 55,000 population equivalents. The total solid sludge retention time is 10–12 days. The plant consists of primary clarification, denitrification, nitrification and sand filtration. The primary sludge from mechanical treatment and the excess sludge from biological treatment and filtration are mixed and fed into a mesophilic anaerobic digester. The conventional STP of Altenrhein treats the mixed sewage of 120,000 population equivalents. The plant is equipped with mechanical treatment, secondary treatment consisting of a denitrification and nitrification and tertiary sand filtration. For additional details see Joss et al. [26].

2.2. Sampling of a German sewage treatment plant

The solid fraction of activated sludge was obtained by filtration through glass fibre filters (GF8 Schleicher & Schuell, Dassel, Germany) of the slurry of activated sludge samples taken from the nitrification tank of the Wiesbaden STP. The samples were collected in glass bottles, homogenized, filtered and used immediately. Digested sludge was collected from the respective digester. The Wiesbaden STP (population equivalent: 350,000) consists of a preliminary clarification, a denitrification-nitrification cascade with an internal recirculation of sludge, and a phosphate removal by addition of $Fe(II)Cl_2$ into the final clarification. The total solid sludge retention time is 11–13 days. Primary and secondary sludge is fed into a mesophilic anaerobic digester. Further details can be found in Andersen et al. [27].

3. Extraction and clean-up

3.1. PLE of musk fragrances

For extraction, an automated ASE 200 from DIONEX (Sunnyvale, CA) was used. An aliquot of freeze-dried sludge (0.2 g) was placed into 11 mL stainless steel extraction cells from Dionex and was thoroughly mixed with ∼10 g of quartz sand. The extraction solvent was methanol. The selected operating conditions included: extraction temperature, 100 °C; extraction pressure, 100 bar; pre-heating period, 5 min; static extraction period 5 min; number of extraction cycles, 2; solvent flush, 100% of cell volume; nitrogen purge 30 s. The final extraction volume (20 mL) was quantitatively transferred (rinsed with ∼80 mL of de-ionized water in 2–3 portions) to a 1000 mL volumetric flask and filled with 900 mL de-ionized water. The samples were shaken and spiked with 500 ng of the surrogate standard Tonalid-D_3 (AHTN-D_3 from Dr. Ehrenstorfer, Augsburg, Germany) (50 μL from a 10 ng/μL solution). Because PLE extractions were done at elevated temperature, potential thermal degradation of the PMF was checked by spiking and recovery experiments on purified sand. The spiked sand was extracted twice for 5 min at 100 °C. Procedural blanks (quartz sand) were extracted for each extraction series to control the laboratory contamination. Multiple sequential extractions of the same sludge sample were conducted to assure for quantitative extraction.

3.2. Ultrasonic solvent extraction (USE)

An aliquot (PMF: 0.2 g, others: 0.5 g) of freeze-dried sludge was extracted successively with 4 and 2 mL methanol and then two times (musk fragrance: three times) with 2 mL acetone. In a subsequent extraction step with 2 ml acetone none of the analytes could be detected anymore. In each extraction step, the sample slurry was ultrasonicated for 5 min. The sludge was centrifuged at 19,000 rad/min for 5 min and the supernatants combined. Surrogate standards (musks:

Table 1
Compounds selected for the present study

Substance	CAS number	Chemical structure	Application
7-Acetyl-1,1,3,4,4,6-hexamethyltetralin (AHTN), tonalide; CAS: 1506-02-1		1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-(*g*)-2-benzopyran (HHCB), galaxolide; CAS: 1222-05-5	
Carbamazepine; CAS: 298-46-4		Pentoxiphylline (oxpentiphylline); CAS: 6493-05-6	
Ifosfamide; CAS: 3778-73-2		Cyclophosphamide; CAS: 6055-19-2	
Propyphenazone; CAS: 479-92-5		Phenazone (antipyrin); CAS: 60-80-0	
Dimethylaminophenazone (aminopyrin); CAS: 58-15-1		Ketoprofen; CAS: 22071-15-4	
Diazepam; CAS: 439-14-5		Glibenclamide; CAS: 10238-21-8	
Diclofenac; CAS: 15307-86-5		Clofibric acid; CAS: 882-09-7	
2 OH-Ibuprofen; CAS: 51146-55-5		Ibuprofen; CAS: 15687-27-1	
Indomethacin; CAS: 53-86-1		Iomeprol; CAS: 78649-41-9	
Iohexol; CAS: 66108-95-0		Diatrizoate; CAS: 131-49-7	

Table 1 (*Continued*)

Substance	CAS number	Chemical structure	Application
Iopamidol; CAS: 60166-93-0		Iopromide; CAS: 73334-07-3	
Iothalamic acid; CAS: 2276-90-6		Ioxithalamic acid; CAS: 28179-44-4	

AHTN-D_3; neutral pharmaceuticals: dihydro-carbamazepine (Aldrich Chem. Co., Milwaukee, USA), acidic pharmaceuticals: fenoprop (Riedel-de-Häen, Seelze, Germany), ICM: desmethoxy-iopromide (DMI) (courtesy of Schering, Berlin, Germany)) were spiked into the slurry of the first extraction. The solvent was evaporated to a volume of ca. 200 μl, and the extracts were diluted with 150 mL groundwater for solid phase extraction (SPE). Specific solid phases were used for the SPE of the four different analytical groups (acidic and neutral pharmaceuticals, ICM and PMF). The SPE cartridges were conditioned prior to sample extraction with 6 mL *n*-hexane, 2 mL acetone, 10 mL methanol and 10 mL groundwater.

3.3. Polycyclic musk fragrances (AHTN, HHCB)

The diluted sample extracts (150 mL), from PLE and USE, were enriched at neutral pH (7–7.5) on solid phase material, 500 mg RP-C_{18} Bulk Sorbent (Separtis GmbH, Grenzahl-Wyhlen, Germany) filled into glass cartridges. Samples were passed through the SPE cartridges at a flow rate of 20 mL/min. Subsequently, the cartridges were dried by a nitrogen stream for 1 h and the analytes were eluted four times with 1 mL of methanol. The methanol extracts were evaporated close to dryness with nitrogen and the residue was dissolved in 200 μL *n*-hexane. The same SPE enrichment procedure also was performed for the determination of PMF in water samples (1 L). For this, the surrogate standard AHTN-D_3 was spiked directly into the water sample. For further clean-up silica gel (silica gel 60, 70–230 mesh, from Merck Corp., Darmstadt, Germany) was deactivated with 1.5% water. A slurry of 1 g silica gel in hexane/acetone (85:15, v/v) was manually filled into a 3 mL glass cartridge. The SPE extracts were quantitatively transferred onto the silica gel column. The PMF were eluted using 5 mL of hexane/acetone (85:15, v/v), which was then evaporated to a final volume of ca. 300 μL. The instrumental standard PCB 30 (2,4,6-trichlorophenyl) (50 ng dissolved in 50 μL hexane, Promochem, Germany) was added before measurement.

3.4. Acidic pharmaceuticals

The diluted sample extracts (150 mL) from USE were adjusted to an *acidic* pH (2.0) with 3.5 mol/L H_2SO_4, and enriched on pre-packed Oasis MCX cartridges (60 mg, 30 μm, Waters, Eschborn, Germany). Samples were passed through the SPE cartridges at a flow rate of 20 mL/min. Afterwards, the cartridges were dried with a nitrogen stream for 20 min. The acidic drugs were eluted four times with 1 mL of acetone. The eluates were reduced to approximately 200 μL under a gentle stream of nitrogen, 200 μL methanol was added, and the extracts were again reduced to 200 μL using nitrogen. The extracts were finally diluted to 500 μL using Milli-Q water adjusted to pH 2.9 with acetic acid [28].

3.5. Neutral pharmaceuticals

The diluted sample extracts (150 mL) from USE were enriched at *neutral* pH (7–7.5) on RP-C_{18ec} material (Separtis GmbH, Grenzahl-Wyhlen, Germany) filled into glass cartridges. Samples were passed through the SPE cartridges at a flow rate of 20 mL/min. Subsequently, the SPE material was dried by a nitrogen stream for 1 h and the cartridges were eluted four times with 1 mL of methanol. The methanol extracts were evaporated close to dryness by a gentle nitrogen stream. Then 20 μL of methanol and 500 μL of a phosphate buffer (pH 7, 20 mM KH_2PO_4/Na_2HPO_4) were added.

3.6. Iodinated contrast media

For the clean-up of ICM, an SPE was used as for the other groups. First, 200 mg ENV+ solute® material and then, after adding a PTFE frit, 200 mg RP-C_{18ec} material (both from Separtis, Germany) were filled into the glass cartridges. The pH 2.8 was adjusted in the diluted sample extracts (150 mL) with H_2SO_4 ($c = 3.5$ mol/L). Then, the sample was passed through the glass cartridges at a reduced flow rate of 10 mL/min in a way that it first was in contact with the RP-C_{18ec} material to enable sorption of a high proportion of

0.5 g freeze-dried sludge

Ultrasonication 4 x 5 min
Methanol: 1 x 4 mL, 1 x 2 mL
Acetone: 2 x 2 mL
Addition of surrogate standard

Centrifugation
Supernatants: combine and evaporate to about 200 µL N_2

Acidic pharmaceuticals
Reconstitution in water (pH 2)
SPE: OASIS MCX
Elute with acetone
Evaporate to about 200µL (N_2)
Reconstitute in water, pH 2.9

Neutral pharmaceuticals
Reconstitution in water (pH 7)
SPE: RP-C_{18}ec
Elute with methanol
Evaporate to close dryness
Reconstitute in buffer (pH 7)

Iodinated contrast media
Reconstitution in water (pH 2.8)
SPE (two layers): RP-C_{18}ec/ENV+
Removal of upper RP-C_{18}ec layer
Elution with methanol
Evaporate to dryness (N_2)
Reconstitute in buffer (pH 7)

(a) LC-tandem MS (+ESI, -APCI, MRM)

0.2 g freeze-dried sludge

Ultrasonication: 4 x 5 min
Methanol: 1 x 4 mL, 1 x 2 mL
Acetone: 3 x 2 mL
Add surrogate standard

PLE: 100 °C, 100 bar
2 x 5 min, methanol
Final volume 20 mL

Centrifugation
Supernatants: combine and evaporate to about 200 µL (N_2)
Reconstitution in 150 mL groundwater

Reconstitution up to 1L with water
Add surrogate standard

SPE: RP-C_{18} ec
elution with methanol
Evaporation close to dryness (N_2) and add 200 µL n-hexane
Clean up on silica gel (n-hexane/acetone, 85/15, v/v)
Evaporation to about 300 µL and add instrumental standard

(b) GC-MS (SIM)

Fig. 1. (a) Scheme of the analytical methods for acidic and neutral pharmaceuticals and iodinated contrast media. (b) Scheme of the analytical methods for musk fragrances.

organic compounds which were co-extracted from the sludge. The cartridges were dried for 1 h with nitrogen, and after removing the upper RP-C_{18ec} layer the remaining solute® ENV+ was eluted four times with 1 mL methanol. Then the extracts were reduced to dryness under a gentle stream of nitrogen. The residue was dissolved in 20 µL methanol and 500 µL phosphate buffer (pH 7, 20 mM KH_2PO_4/Na_2HPO_4) was added.

4. Chromatography and mass spectrometry

4.1. Detection of PMF by GC/MS

Separation and detection of the analytes was achieved using a GC/MS system: HEWLETT PACKARD 5890 Serie II coupled with HEWLETT PACKARD 5971 Mass Selective Detector. The gas chromatograph was equipped

Table 2
SIM masses of polycyclic musk fragrances, surrogate and instrumental standard

Substances	Retention time (min)	Ion I for SIM (*m/z*)	Ion II for SIM (*m/z*)
AHTN	15.30	243	187
HHCB	15.05	243	213
AHTN-D_3[a]	15.25	246	190
PCB 30[b]	13.28	258	256

[a] Surrogate standard.
[b] Instrumental standard.

with a capillary column (Restek, Bad Soden, Germany; XTI-5/30 m × 0.25 mm × 0.25 μm). The head pressure was 85 kPa He. Retention times and the selected ions are listed in Table 2.

GC injection parameters included: 3 μL splitless; 250 °C; GC temperatures: 50 °C isothermal 20 °C/min—160 °C, 4 °C/min—280 °C, 20 °C/min—300 °C, 300 °C isothermal 10 min. Temperature of transfer line (direct interface): 260 °C.

4.2. Detection of ICM, acidic and neutral pharmaceuticals by liquid chromatography tandem mass spectrometry (LC tandem MS)

Prior to injection, all sample extracts were filtered (0.45 μm, Spartan 13/0.45, Schleicher & Schuell, Dassel, Germany). The HPLC system consisted of a Perkin-Elmer L-6200 pump connected to an AS–2000a auto sampler. Eluents were degassed by an in-line degasser. The injection volume was always 50 μL. For ICM and neutral pharmaceuticals a 125 mm × 3 mm LiChrospher® RP-18ec column (5 μm) (Merck Corp., Darmstadt, Germany) was used with a flow rate of 0.4 mL/min. The acidic pharmaceuticals were chromatographed on a 125 mm × 3 mm LiChrospher® RP-18 column (5 μm) (Merck Corp., Darmstadt, Germany) with a flow rate of 0.4 mL/min. In any case, the columns were kept at 25 °C. The LC runs of the three analytical groups were operated with three eluent systems: eluent A (5 mmol/L aqueous ammonium acetate (pH 5.7) and acetonitrile (90:10, v/v)); eluent B (400 mL eluent A + 600 mL acetonitrile); eluent C (Milli-Q water pH 2.9 (acetic acid)); eluent D (acetonitrile).

ICM: 100% eluent A for 16 min.

Neutral pharmaceuticals: 100% eluent A for 1 min, change to 89% A/11% B within 2 min, change to 10% A/90% B within 14 min, change to 100% B within 5 min, change to 100% A within 3 min, 100% A for 11 min.

Acidic pharmaceuticals: 60% C/40% D, change to 100% D within 3 min, 100% D for 10 min, change to 60% C/40% within 5 min, 60% C/40% for 10 min.

A Perkin-Elmer Sciex API 365 triple stage quadrupole mass spectrometer was used for detection. The ICM and neutral pharmaceuticals were detected using turbo electrospray ionization (ESI) in the positive mode at 400 °C at a split rate of 1:10. For the acidic pharmaceuticals, the atmospheric pressure chemical ionization (APCI) was applied in the negative mode at 425 °C without splitting the eluent. In all LC runs the first 3 min were send to the waste, to minimize the impurities entering the MS. Orifice voltages generally varied between −15 and 40 V, depending on best signal of the ionization products in the used ionization mode. MS–MS parameters were optimized as follows. After determination of the best conditions for the isolation of the precursor ion (either $[MH]^+$ or $[M-H]^-$), the quadrupole and lens conditions for the collisional activation were optimized (precursor scan: 1-Da steps, 4-ms dwell time; product-ion scan 0.1 Da steps, 4 ms dwell time; multiple reaction monitoring (MRM): dwell time > 200 ms depending on the number of recorded mass traces). Precursor and product ion of the individual compounds are given in Table 3.

5. Method validation

5.1. Determination of recoveries

Freeze-dried sludge (activated or digested) was spiked with the analytes that were dissolved in a stock solution (each at 1 μg/μL methanol). The spiking levels were 100 ng/g for neutral pharmaceuticals, 200 ng/g for acidic pharmaceuticals and ICM and 1000 ng/g for AHTN and 2500 ng/g for HHCB. After spiking, the samples were stirred intensively to homogenize the analyte concentrations and to enable a sufficient contact of the analytes and standards with the matrix. Absolute recoveries were determined in relation to a non-enriched standard solution. Recoveries of the analytes in the individual clean-up steps were determined without matrices. Solvent extraction, PLE, clean-up with silica gel and SPE, and detection by GC/MS and LC tandem MS were performed as described above.

5.2. PLE

The sequential extraction of 2 × 5 min enabled a quantitative extraction from different sludges. Overall method accuracy was determined by recovery studies of spiked sewage sludge at different concentrations. Duplicates of spiked sludge at 5, 7.5, 10 and 12.5 μg/g were extracted and analyzed. Relative recoveries for activated sludge samples were 78 ± 6% for AHTN and 109 ± 12% for HHCB. The precision of the entire procedure for sewage sludge was determined extracting 6 replicates containing native PMF. The overall precision of the method, indicated by the relative standard deviation (R.S.D.), was 10% for AHTN and 11% for HHCB. No thermal degradation was observed at the working temperature.

5.3. Calibration, limits of quantification and blank samples

Calibration was performed without matrix over the whole procedure after spiking the solvents used for extraction with

Table 3
Precursor and product ions of pharmaceuticals used for LC tandem MS detection

Substance	Retention time (min)	Precursor ion (*m*/*z*)[a,b]	Product ions (*m*/*z*)
Iodinated contrast media			
Iopamidol	5.0	777.8	387.1
Iopromide	8.1	791.8	572.8
Diatrizoate	5.1	614.6	233.1
Iothalamic acid	5.1	614.6	486.7
Ioxithalamic acid	4.9	644.6	302.2
Iomeprol	5.5	777.8	405.0
Iohexol	5.2	821.8	804.0
DMI[c]	6.0	761.8	543.1
Neutral pharmaceuticals			
Pentoxiphylline	15.2	279.2	99.1, 182.2
Phenazone	14.5	189.2	56.1, 77.1
Propyphenazone	20.5	231.1	201.0, 189.2
Dimethylaminophenazonee	17.4	232.2	113.1, 111.1
Diazepam	23.9	287.0	269.0, 241.0
Carbamazepine	19.4	237.1	194.2, 179.2
Ifosfamide	16.5	260.0	92.1, 154.1
Cylophosphamide	17.0	260.0	106.0, 140.1
Glibenclamide	22.8	494.0	369.1, 169.0
Caffeine	10.9	195.1	138.0, 110.0
Dihydro-carbmazepine[c]	19.6	239.1	194.1, 181.1
Acidic pharmaceuticals			
Clofibric acid	9.7	213.1	127.0, 85.1
Ibuprofen	16.0	205.1	159.1, 175.0
Ibuprofen-OH	4.2	221.2	177.1, 133.1
Diclofenac	15.6	294.1	249.8, 214.4
Indomethacin	15.6	356.2	312.1, 296.6
Bezafibrate	10.6	360.0	273.5, 153.9
Ketoprofen	9.9	253.0	209.1, 197.1
Fenoprop[c]	14.4	267.1	194.9, 159.0

[a] $[M+H^+]$ for iodinated contrast media and neutral pharmaceuticals.
[b] $[M-H^-]$ for acidic pharmaceuticals.
[c] Surrogate standard.

the reference standards. A nine-point calibration was used, ranging from 10 ng/g to 20 μg/g. Limits of quantification (LOQ) allowing for the quantification of analytes in native samples were set as the second or third lowest calibration point of the linear correlation as long as the calculated signal/noise ratio of the analytes in the native samples extracts was at least 10 for that concentration. Blank samples, quartz sand and groundwater were spiked with the surrogate standards and were included in each series of the analysis. The absolute recoveries were calculated with analyte areas in relation a non-enriched standard sample containing the analytes and the surrogate standards without any sample preparation. The analyte concentrations in native samples were calculated in relation to the surrogate standards spiked at the beginning, while the instrumental standard PCB 30 for musk fragrances was used only to confirm the reliability of the GC/MS measurement.

In the following text, the term *absolute recovery* is assigned to values calculated without using the areas of the surrogate standards or PCB 30, and the term *relative recovery* is used for values corrected with the areas of the surrogate standards. The latter compensated for losses during sample preparation, while the former exhibited the total recovery.

Table 4
Absolute mean recoveries by ultrasonic solvent extraction for activated sludge ($n=3$), digested sludge ($n=3$) and groundwater ($n=3$) after spiking with 1000 ng/g (AHTN) and 2500 ng/g (HHCB) to the sludge and 1 μg/L to the water

	LOQ		Activated sludge	Digested sludge	Water
	Sludge (ng/g)	Water (ng/L)	Absolute recovery (mean ± R.S.D. 1σ (%))	Absolute recovery (mean ± R.S.D. 1σ (%))	Absolute recovery (mean ± R.S.D. 1σ (%))
AHTN	250	20	78 ± 15	74 ± 20	82 ± 9
HHCB	250	20	87 ± 10	64 ± 12	78 ± 8
AHTN-D_3*		20	109 ± 7	105 ± 4	88 ± 7

LOQ: limit of quantification.

6. Results and discussion

6.1. Method validation

6.1.1. Recoveries and detection limits

6.1.1.1. Polycyclic musk fragrances. For the determination of absolute recoveries, the extraction was performed by USE as described. The absolute recovery for activated sludge of AHTN was 78 ± 15% and for HHCB 87 ± 10% (Table 4). For digested sludge, recoveries were 74 ± 20% and 64 ± 11% for AHTN and HHCB, respectively. The slightly lower recoveries in the digested sludge could have been caused by the significant addition of primary sludge (ca. 70%) to the digester, since the method was not tested for primary sludge. In both cases (activated and digested sludge), compensation with AHTN-D_3 did not result in a higher relative recovery, because the surrogate standard was found quantitatively with recoveries of around 100%. It is important to note that the recoveries given were calculated by subtraction of the native contamination of the sludge from the concentrations found for the spiked samples. Since AHTN-D_3 was originally not present in the native sludge its recovery could be determined more accurately (absolute recoveries: 109 ± 7%, 105 ± 4%) than for the non-labeled AHTN and HHCB where error propagation has to be applied due to the subtraction. Furthermore, higher uncertainties should be expected for digested sludge, because of its higher native musk contamination (Table 7). The SPE clean-up itself only lead to small losses, as concluded from the absolute recoveries of HHCB and AHTN at 78% and 82% in groundwater, respectively (Table 4). A chromatogram is shown in Fig. 2.

6.1.1.1.1. Comparison of PLE and USE. Both extraction methods were compared by extracting the same sewage sludge. The extraction efficiency obtained by the two methods was in good agreement. The concentrations of AHTN obtained by PLE ($n = 2$) and ultrasonic ($n = 3$) were 3.82 (3.70, 3.94) and 3.76 ± 0.26 μg/g, respectively. The concentrations of HHCB obtained by PLE and USE were 7.16 (7.14, 7.18) and 7.31 ± 0.51 μg/g, respectively. Considering the statistical errors, the two extraction methods led to comparable results.

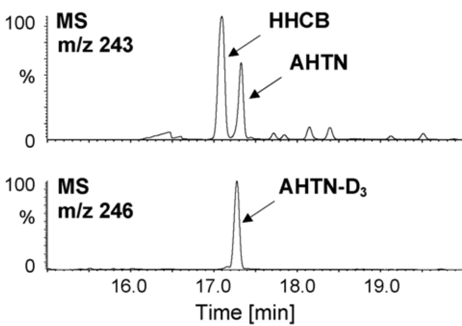

Fig. 2. Single ion monitoring (SIM) mode for HHCB and AHTN. AHTN-D_3 was used as surrogate standard.

6.1.1.2. Neutral pharmaceuticals. The absolute recoveries of neutral pharmaceuticals (except for diazepam, see below) varied over the total method from 52 ± 4% (carbamazepine) to 78 ± 7% (pentoxiphylline) for activated sludge and between 47 ± 9% and 67 ± 9% for digested sludge at a spiking level of 100 ng/g (Table 5). Using the surrogate standard dihydro-carbamazepine, these losses were efficiently compensated, as the relative recoveries ranged within 88 ± 10% and 125 ± 11% for activated and digested sludge, respectively. A relative standard deviation of less than 15% and the good relative recoveries indicated the appropriateness of the analytical method for native samples. The losses seem to be caused mainly by the extraction, since the SPE used as clean-up was quantitative with recoveries over 90% (data not shown). However, ion-suppression could not be ruled out totally. The recoveries of diazepam were two-fold lower than for the other compounds. Even the relative recoveries for diazepam must be classified as semi-quantitative with 58 ± 11% (activated sludge) and 48 ± 10% (digested sludge),

Table 5
Neutral pharmaceuticals: absolute (without correction) and relative mean recoveries (with correction using the recovery of the surrogate standard) for activated sludge ($n = 6$) and digested sludge ($n = 4$) after spiking with 100 ng/g

Substance	LOQ	Activated sludge		Digested sludge	
	Sludge (ng/g)	Absolute recovery (mean ± R.S.D. 1σ (%))	Relative recovery (mean ± R.S.D. 1σ (%))	Absolute recovery (mean ± R.S.D. 1σ (%))	Relative recovery (mean ± R.S.D. 1σ (%))
Pentoxiphylline	20	78 ± 7	125 ± 11	67 ± 7	133 ± 13
Phenazone	20	57 ± 4	90 ± 7	51 ± 6	102 ± 12
Propyphenazone	20	59 ± 7	98 ± 10	47 ± 9	95 ± 13
Dimethylamino-phenazone	20	70 ± 11	111 ± 17	51 ± 16	103 ± 20
Carbamazepine	20	52 ± 4	83 ± 6	43 ± 3	85 ± 11
Ifosfamide	20	59 ± 7	95 ± 11	53 ± 3	105 ± 5
Cylophosphamide	20	66 ± 7	106 ± 11	58 ± 5	116 ± 10
Glibenclamide	50	55 ± 6	88 ± 10	47 ± 6	95 ± 11
Caffeine	50	63 ± 9	100 ± 14	65 ± 13	129 ± 12
Diazepam	20	37 ± 6	59 ± 11	25 ± 3	48 ± 10
Dihydro-carbamazepine[a]		63 ± 8	–	52 ± 9	–

[a] Surrogate standard.

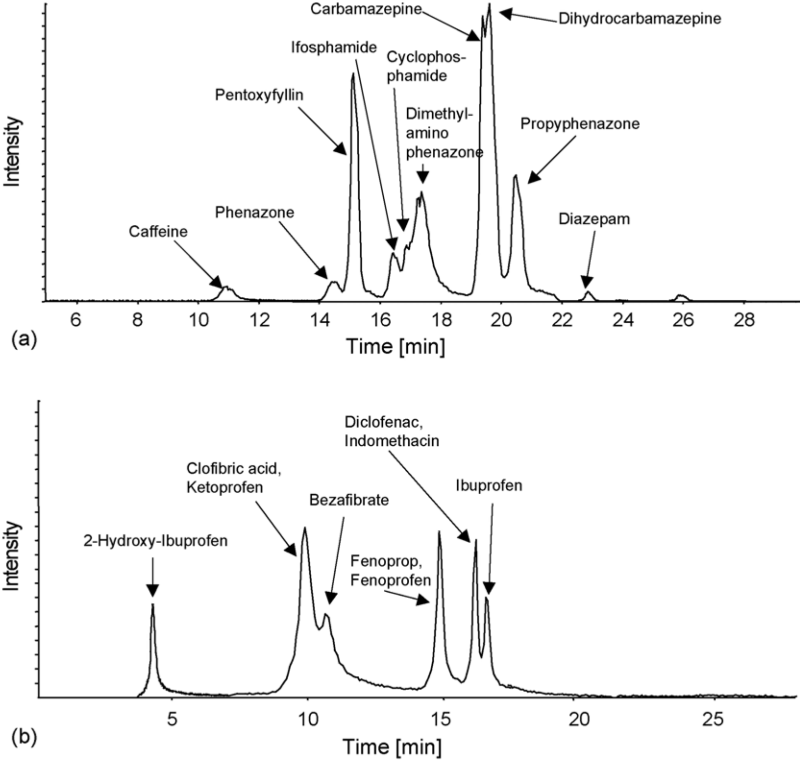

Fig. 3. Chromatogram of neutral (A) and acidic (B) pharmaceuticals in spiked activated sludge recorded in by LC tandem MS.

whereas the R.S.D. were surprisingly low. The LOQs were determined to be 20 or 50 ng/g. Chromatograms of spiked activated sludge are shown in Fig. 3.

6.1.1.3. Iodinated contrast media. The absolute recoveries in activated sludge were almost quantitative over the total method ranging from 88 ± 4% (iothalamic acid) to 119 ± 30% (iopamidol) at a spiking level of 200 ng/g (Table 6). A significantly reduced recovery of 48 ± 5% was observed only for ioxithalamic acid. For digested sludge, recoveries of most ICM were generally lower, ranging from 54 ± 7% (ioxithalamic acid) to 85 ± 11% (iomeprol). Only iopromide was found with a higher recovery of 120 ± 12%. It has to be noted that the analytical method was primarily evaluated for activated sludge and is not adequately appropriate for primary sludge. Obviously, the increased percentage of primary sludge fed to the digester and the increased TOC are responsible for the losses likely to occur during SPE or due to ion-suppression. It is already known from the water analysis that elevated TOC leads to a reduced efficiency of the SPE [29]. Compensation using the surrogate standard DMI led, for activated sludge and for digested sludge in many cases to an over-determination. Hence, except for ioxithalamic acid DMI was not suitable for compensation of losses during analysis of the sludge samples, although it has been confirmed as an excellent surrogate standard for water samples [8]. The LOQ was determined to be 50 ng/g for all target compounds.

6.1.2. Acidic pharmaceuticals

Absolute recoveries of the acidic pharmaceuticals ranged between 43 ± 5% and 72 ± 6% for activated sludge and 52 ± 5% to 76 ± 6% for digested sludge. Considering the statistical error, a significant difference was not found for activated and digested sludge. In both cases, the use of fenoprop as surrogate standard was effective in compensating for losses. Hence, except for bezafibrate, the relative recoveries exceeded 70%. It was not clear which treatment step was responsible for these losses. It can be assumed that either the solvent extraction, the SPE (affected by compounds co-extracted from the sludge) or ion-suppression was responsible for the non-quantitative recoveries. For groundwater, recoveries for the SPE were quantitative [28]. The LOQ was determined to 20 or 50 ng/g for the target compounds. A chromatogram of spiked activated sludge is shown in Fig. 3.

6.2. Application to environmental samples

Several environmental sludge samples were analyzed to assess the performance of the developed methods. In Switzer-

Table 6
Iodinated contrast media and acidic pharmaceuticals: absolute (without correction) and relative mean recoveries (with correction using the recovery of the surrogate standard) for activated sludge ($n = 3$) and digested sludge ($n = 3$) after spiking with 200 ng/g

Substance	LOQ	Activated sludge		Digested sludge	
	Sludge (ng/g)	Absolute recovery (mean ± R.S.D. 1σ (%))	Relative recovery (mean ± R.S.D. 1σ (%))	Absolute recovery (mean ± R.S.D. 1σ (%))	Relative recovery (mean ± R.S.D. 1σ (%))
ICM					
Iopamidol	50	119 ± 30	201 ± 58	64 ± 11	120 ± 20
Iopromide	50	107 ± 33	120 ± 55	120 ± 12	206 ± 17
Diatrizoate	50	91 ± 9	153 ± 14	69 ± 12	118 ± 20
Iothalamic acid	50	88 ± 4	148 ± 6	75 ± 13	130 ± 22
Ioxithalamic acid	50	48 ± 5	80 ± 8	54 ± 7	94 ± 11
Iomeprol	50	103 ± 2	173 ± 4	85 ± 11	146 ± 18
Iohexol	50	105 ± 12	177 ± 20	82 ± 10	141 ± 16
DMI[a]		59 ± 5	–	58 ± 5	–
Acidic pharmaceuticals					
Clofibric acid	20	59 ± 3	92 ± 5	55 ± 6	86 ± 12
Ibuprofen	20	54 ± 4	83 ± 8	49 ± 6	76 ± 12
Ibuprofen-OH	20	70 ± 11	108 ± 15	47 ± 6	73 ± 12
Diclofenac	20	52 ± 8	80 ± 12	49 ± 7	76 ± 13
Indomethacin	20	55 ± 3	84 ± 6	57 ± 9	89 ± 14
Bezafibrate	20	43 ± 6	67 ± 9	56 ± 5	87 ± 10
Ketoprofen	50	72 ± 5	111 ± 9	76 ± 5	119 ± 13
Fenoprop[a]		63 ± 10	–	52 ± 7	–

[a] Surrogate standard.

land, AHTN and HHCB occurred in activated sludge at concentrations ranging between 2.3 and 8.5 μg/g and in digested sludge between 6.6 and 15 μg/g. In most cases, the concentrations of HHCB are about two times higher than those of AHTN. In German activated sludge samples, the two musk fragrances HHCB and AHTN were found in concentration levels ranging from 1.4 μg/g (AHTN) to 6.5 μg/g (HHCB). Since during digestion, the solid mass is reduced up to 50–70%, digested sludge should be in general more contaminated with PMF than the activated sludge as long as no appreciable degradation occurs. Although the data basis is very low, it can be assumed that the PMF selected are not significantly degraded during anaerobic digestion. The concentrations measured are in the same range as determined by Kupper et al. [25]. In their study, mean values in digested sludge from 16 Swiss STPs were 7.3 μg/g for AHTN and 20.3 μg/g for HHCB (Table 7). In addition to PMF, only the antiphlogistic diclofenac were quantified in the current study above the LOQ with concentrations ranging between 0.20 and 0.45 μg/g in five activated sludge samples randomly taken from three German municipal STPs. In the digested sludge sample, diclofenac was present with 0.22 μg/g. All the other selected neutral and acidic pharmaceuticals and ICMs were not detected above the LOQ and hence, sorption onto sludge seems to be negligible. Even for diclofenac sorption can be assumed to be not a relevant removal process. Obviously, non-ionic interactions are unlikely due to their elevated polarity, and specific interactions as known for the fluoroquinolones [14] do not occur. To confirm these assumptions, a study was used to quickly determine the solid-water distribution coefficient (K_d) of the spiked PMF and pharmaceuticals for primary and secondary sludge of the German STP under anaerobic conditions [30]. In that study, the concentrations of the pharmaceuticals and the musk fragrances in the sludge were analyzed with the analytical methods described here, however using only a very small quantity (about 50 mg) of filtered primary sludge. In general, the K_d values were below 100 L/kg (corresponding to a sorbed fraction of below 5% in sewage) for the pharmaceuticals spiked into the primary and secondary sludge slurries. Only for diclofenac in the primary sludge, a higher K_d value of about 450 L/kg was found, which should be the result of low pH 6.6 leading to a higher proportion of protonated diclofenac. Hence, the K_d study [29] underlined that sorption is not relevant for the polar pharmaceuticals and ICM. However, prior to a profound evaluation, the data basis has to be enlarged for native and non-spiked sludge samples using the analytical methods developed. On the other hand, for the musk fragrances log K_d values up to 3.7 were measured, indicating

Table 7
Concentrations of PMF and diclofenac in activated and digested sludge in μg/g

Sampling site	HHCB (μg/g)	AHTN (μg/g)	Diclofenac (μg/g)
Activated sludge			
STP-K (CH)	5.4/8.5/7.8/5.9	3.8/4.3/3.7/2.3	–
STP-A (CH)	4.5/4.7	2.9/3.9	–
STP-KO (GER)	–	–	0.37
STP-WI (GER)	6.5/5.3/5.2	1.4/2.6/1.6	0.20/0.27/0.28
STP-EHR (GER)	–	–	0.45
Digested sludge			
STP-K (CH)	15	6.6	n.d.
STP-KO (GER)	–	–	0.22

(–): Not analysed.

that in some cases up to 50–60% is sorbed onto sludge particles.

7. Conclusion

The analytical methods developed for the analysis of pharmaceuticals and musk fragrances in activated and digested sludge allowed for a quantification as low as 20 or 50 ng/g. The methods were developed for activated and digested sludge, while for primary sludge the methods are not appropriate unless the sludge quantity used for extraction is reduced to at least 100 mg. It is strongly recommended to determine individual recoveries for unknown sludge samples to avoid under-determinations since the sludge composition of other STPs might be different and contain, for instance, a higher percentage of primary sludge or is influenced by unique industrial sewage. The data basis using the methods developed has to be enlarged to elucidate, whether the analytical methods are influenced by different sludge compositions.

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