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Solid phase extraction coupled to liquid chromatography-tandem mass spectrometry analysis of sulfonamides, tetracyclines, analgesics and hormones in surface water and wastewater in Luxembourg

J.-Y. Pailler *, A. Krein, L. Pfister, L. Hoffmann, C. Guignard

Department of Environment and Agro-Biotechnologies, Centre de Recherche Public - Gabriel Lippmann, 41 rue du Brill, L-4422 Belvaux, Luxembourg

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ABSTRACT

In the early 1990s different studies highlighted the relationship between pharmaceuticals, human health and the environment. Among the emerging contaminants, antibiotics are obviously of high concern, because of their potential for inducing antibiotic resistance. In addition, natural and synthetic hormones are relevant because of their potential endocrine-disrupting effects on wildlife. This investigation focuses on the analysis of four classes of veterinary and human pharmaceuticals (sulfonamides, tetracyclines, analgesics and hormones) in surface water and wastewater in Luxembourg. The selected eleven pharmaceuticals include four sulfonamides (sulfathiazole, sulfamethoxazole, sulfadimethoxine and sulfamethazine), two tetracyclines (tetracycline and oxytetracycline), two analgesics (ibuprofen and diclofenac), and three hormones (2 naturals, estrone and β-estradiol, and a synthetic one, 17-α-ethinyl estradiol). The most innovative parts of this study are the simultaneous extraction of the above-mentioned pharmaceuticals as well as tracking their behaviour during flood events in a small river catchment. The method includes pre-concentration by solid phase extraction using Oasis® HLB (Hydrophilic Lipophilic Balance) which gave superior results compared to Chromabond® C-18EC, Chromabond® EASY and Bond Elut® PLEXA cartridges, also evaluated in this investigation. The analysis of the investigated pharmaceutical compounds is carried out by high performance liquid chromatography coupled to a triple quadrupole mass spectrometer. The limits of quantification were 1 ng L^{-1}, except for β-estradiol (2 ng L^{-1}) and 17-α-ethinyl estradiol (6 ng L^{-1}). Recovery rates range from 70 to 94%, with relative standard deviations between 4 and 19%. Application of this method to river concentration and flood events revealed high concentrations of ibuprofen (10–4000 ng L^{-1}), with highest levels during flood events, while concentrations of estrogens (1–240 ng L^{-1}) and sulfonamides (1–20 ng L^{-1}) were comparatively low.

1. Introduction

Accumulation of emerging micropollutants might cause adverse effects on human health and wildlife (Erickson, 2002). A vast number of pharmaceuticals, including sulfonamides, tetracyclines, hormones or anti-inflammatory drugs has been detected in different matrices as meat (Kaufmann et al., 2008), milk (Turnipseed et al., 2008) or water (Hernández et al., 2007; Snyder et al., 2003; Ternes, 2001). Sulfonamides (sulfathiazole, sulfamethoxazole, sulfadimethoxine or sulfamethazine) are commonly used as bacteriostatic reagents for treatment of humans (inhibition of folic acid synthesis in bacteria) and as growth promoters in animals (Yang et al., 2005). Tetracyclines are broad-spectrum antibiotics. Due to those bacteriostatic properties they are very popular for treating many different bacterial infections, such as urinary tract infections, acne, gonorrhea, clamydia and others. Hormones, natural or synthetics are monitored due to their endocrine disrupting effects (Mauduit et al., 2006; Rodriguez-Mozaz et al., 2004). Non-steroidal anti-inflammatory drugs (NAID) are commonly used as antipyretics and for reducing inflammations. Because of their high solubility and often-poor degradability, these polar pharmaceuticals deserve particular attention (Petrovic et al., 2003). Regarding water analysis, earlier studies have shown that these compounds are only partially eliminated during conventional (coagulation, sand filtration) wastewater treatment (Kim et al., 2007; Renew and Huang, 2004).

Given the low analyte concentrations found in water samples, a pre-concentration by solid phase extraction (SPE) is required prior to analysis. Reverse phase sorbents are probably the best phases for estrogens but inappropriate for more polar or ionised molecules such as ibuprofen, diclofenac, sulfonamides or tetracyclines (Ahrer et al., 2001; Quintana and Reemtsma, 2004; Wissiack et al., 2000). In recent papers authors tested three different extraction/purification strategies, single polymeric cartridges (Oasis® HLB and Oasis® MCX) and tandem HLB–MCX (Díaz-Cruz et al., 2008; Gros et al., 2009).

* Corresponding author.
E-mail address: pailler@lippmann.lu (J.-Y. Pailler).

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Table 1
Physico-chemical properties and parameters of the quantitative determination of pharmaceuticals by SPE-LC/MS–MS.

Analyte	pK_a's	Log K_{ow}	r^2	Linearity (ng L^{-1})	LOD (LOQ) ng L^{-1}
IBU	4.9	4	0.9998	1–5000	0.3 (1.0)
DCL	4.1	4.5	0.9991	1–100	0.3 (1.0)
E_1	10.7	3.7	0.9995	1–100	0.3 (1.0)
E_2	10.7	4.1	0.9987	1–100	1.0 (3.0)
EE_2	10.2	4.5	0.9996	1–100	2.0 (6.0)
STZ	2.0; 7.1	0.7	0.9991	1–100	0.3 (1.0)
SMT	2.1; 7.5	0.9	0.9970	1–100	0.3 (1.0)
SMX	1.8; 5.6	0.9	0.9995	1–100	0.3 (1.0)
SDM	2.1; 6.1	1.6	0.9992	1–100	0.3 (1.0)
T	3.3; 7.8; 9.6	-1.3	0.9996	1–100	0.3 (1.0)
OTC	3.2; 7.5; 8.9	-3.6	0.9979	1–100	0.3 (1.0)

pKa's and Log K_{ow}'s were referred from Qian and Adams, 2004 (for pKa's of STZ, SMT, SMX, SDM, T and OTC), Choi et al., 2007 (for Log K_{ow}'s of STZ, SMT, SMX, SDM, T and OTC), Gros et al., 2006 (for pKa's and Log K_{ow}'s of IBU and DCL), Carpinteiro et al., 2004 (for Log K_{ow}'s of E_1, E_2 and EE_2) and Lewis and Archer, 1979 (for pKa's of E_1, E_2 and EE_2).

For the extraction part of this work the physical properties (Table 1) including the pK_a's and log K_{ow} are essential, since they govern the prevalence of ions or neutral forms of pharmaceuticals as a function of the pH. At this step the pH control is the key to the maximum interaction with polymeric sorbents, and molecules have to be in their neutral forms. Due to the difficulties in separating neutral and ionic compounds in the same extraction protocol, literature refers to separate methods dedicated to the same class of compounds, such as tetracyclines (Zhu et al., 2001), sulfonamides (Balakrishnan et al., 2006), or estrogens (Gabet et al., 2007; Wang et al., 2008). In this study, a different approach was considered and the four classes of molecules were extracted simultaneously. A similar strategy was developed recently for other matrices like meat (Granelli et al., 2008), swine wastewaters (Ben et al., 2008), or for other molecules (Chang et al., 2008; Gros et al., 2009; Kim et al., 2007).

This paper describes a simple method for extracting and quantifying common pharmaceuticals from surface waters by solid phase extraction and high performance liquid chromatography coupled with a tandem mass spectrometer (LC/MS–MS). The innovative part of this study is the simultaneous extraction of all compounds as well as its application for studying dissolved pharmaceuticals in different longitudinal profiles along the Alzette river in Luxembourg including a waste water treatment plant (WWTP) of 300,000 inhabitant equivalents. Another objective of this study is the investigation of rapid variations of dissolved pharmaceutical concentrations, and the fluxes of common pharmaceuticals during flood events in the small Mess catchment, a tributary of the Alzette. Limited information is

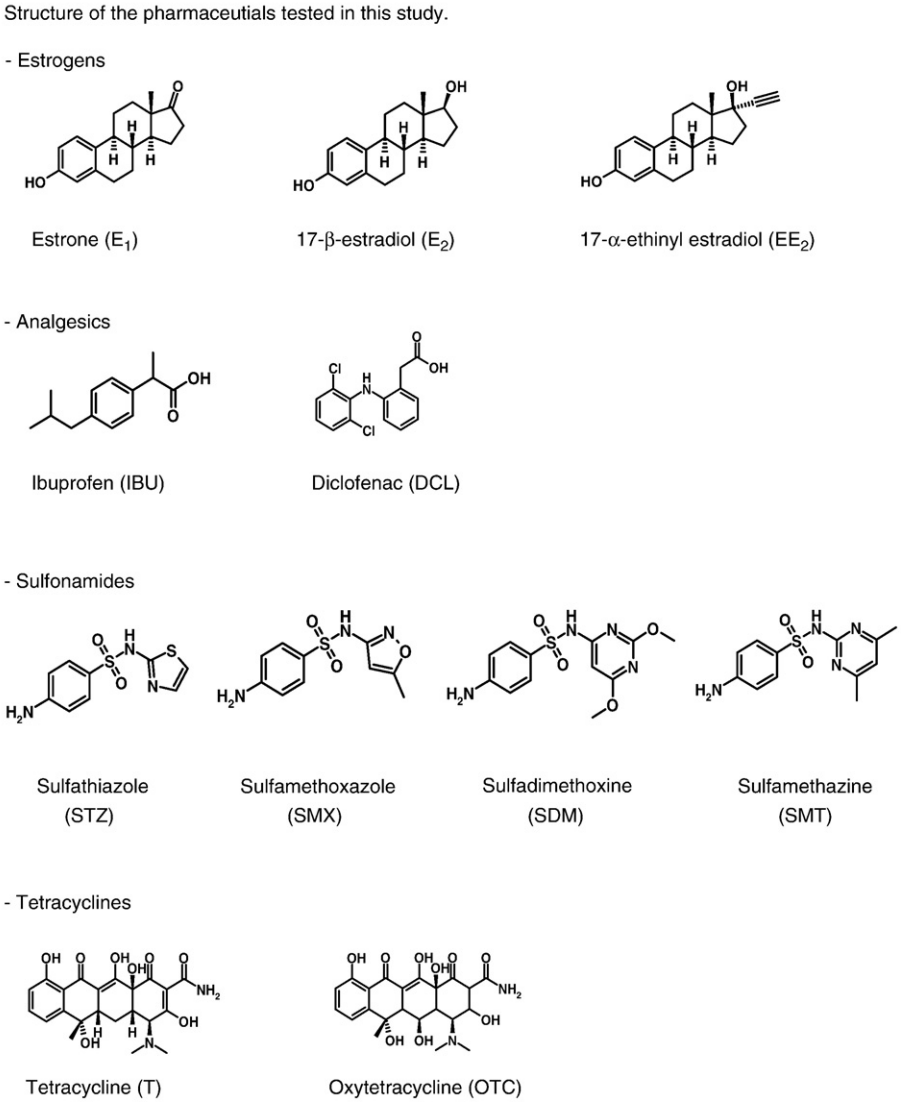

Fig. 1. Structure of the pharmaceuticals tested in this study.

known to the authors regarding the substance behaviour as a direct answer to runoff generation during flood events for small catchments. Many headwaters are heterogeneous mainly as a result of variable subsurface configurations (soil conditions, bedrock). Therefore, this study investigates the relationship between runoff generation and corresponding substance behaviour. Moreover, knowledge of the concentrations and the behaviour of pharmaceuticals are fundamental for calculating pollutant loads exported from the catchments under investigation.

2. Materials and methods

2.1. Chemicals

Calibration standards, all of analytical grade, were purchased from the following companies: sulfathiazole (STZ), sulfadimethoxine (SDM), sulfamethazine (SMT), tetracycline (T) and oxytetracycline (OTC) from Riedel De Haën (Seelze, Germany), diclofenac (DCL) from Sigma-Aldrich (Steinheim, Germany), estrone (E_1), β-estradiol (E_2), 17-α-ethinylestradiol (EE_2), sulfamethoxazole (SMX) and ibuprofen (IBU) from LGC Promochem (Molsheim, France). The chemical structures of the pharmaceuticals included in this study are shown in Fig. 1. Solvents and additives were purchased from Biosolve (Valkenswaard, The Netherlands): acetonitrile, methanol, ammonium acetate, formic acid (UPLC/MS-grade), Na_2–EDTA (ethylene diamine tetra acetic acid) from Biowhittaker (Maryland, USA) and sulfuric acid from Merck (Darmstadt, Germany). Ultra-pure water was prepared with a Milli-Q water purification system (Millipore, Bedford, USA).

2.2. Sample preparation and extraction

Different water samples were collected along a 15 km stretch of the Alzette river (basin size: approx. 350 km^2), including the outflow of the Beggen wastewater treatment plant (WWTP) with a capacity of 300,000 inhabitant equivalents. 5 samples were collected as follows: one sample (point 1) upstream of the Beggen WWTP (less than 1 km from the Beggen WWTP to avoid other sources of pollution), another one at the output of the Beggen WWTP (Outflow WWTP) and 3 samples (points 2 to 4) downstream of the Beggen WWTP (please refer to the map on the left part of Fig. 2a). Furthermore, floodwater concentrations were investigated with a high temporal resolution in a tributary of the Alzette, the Mess river (basin size: 35 km^2). The flood events of the Mess were automatically sampled in clean glass bottles by an automatic liquid sampler (ISCO). The time steps for water-level measurements average 15 min, as the runoff events are variable in very short time periods. Discharge is obtained with level-to-flow conversions applying the Manning equation. The ISCO autosampler with one-liter glass bottles (24 bottles) was connected to the flow loggers in order to trigger the sampling after a fixed water level is reached. Subsequently, sampling is performed at different intervals throughout the whole duration of the investigated events. Every sample is a spot sample and not a composite one, collected during a certain time span. A representative selection of samples has been chosen for analysis selected according to electrical conductivity (WTW 197i conductivity meter), water colour or smell. Conductivity highlights the presence of new (rainfall) and old water during flood events. Low conductivity values are an indication of runoff from paved areas (e.g. roofs, streets, sewer systems, general surface runoff) in the Mess basin. Furthermore, a conductivity peak in the rising limb of the flood events indicates a first flush from the catchment area supposed to be contaminated by pollutants. In total, between October 2006 and May 2008 11 flood events were investigated. We selected 6 bottles to analyse the compounds under investigation. Furthermore, at base flow grab samples were taken by hand to investigate low flow conditions before and after the flood events under investigation. All samples were stored at 4 °C in the dark and treated immediately as described below.

Surface water and wastewater were filtered through 3 μm glass fibre filters (Pall Corporation, Ann Arbor, USA) to eliminate the suspended matter and then filtered through 0.45 μm cellulose acetate filters (Sartorius, Göttingen, Germany). The 1 L samples were acidified to pH 4 with diluted sulfuric acid solution (25%), 5 mL of 5% diluted Na_2–EDTA were added (Choi et al., 2007; Verma et al., 2007) and directly extracted to minimize degradation.

All target compounds were extracted using an automated SPE performed by an Autotrace SPE workstation (Caliper, Teralfene, Belgium). For this extraction step, several sorbents (Oasis® HLB, Chromabond® C-18EC, Chromabond® EASY and Bond Elut® PLEXA) and pH conditions (4 and 7) were tested. Oasis® HLB (Waters, Milford, USA) was compared to Chromabond® C-18EC (Macherey-Nagel, Düren, Germany) with methanol as eluent at neutral pH. In addition, different polymeric cartridges, Oasis® HLB, Chromabond® EASY (Macherey-Nagel, Düren, Germany), Bond Elut® PLEXA (Varian, Middelburg, The Netherlands) were tested with a methanolic elutant at pH 4. Finally, EDTA was evaluated as an additive directly after filtration of the five river samples. Simultaneous extraction strategies have been tested by several authors but under different conditions. For example the extraction solvent used was a mixture of methyl *t*-butyl ether and methanol (Kim et al., 2007; Vanderford et al., 2003), and the pH was 2. This approach is inappropriate for the analysis of the tetracycline group because of their susceptibility to conformational degradation to their 4-epimers (Díaz-Cruz and Barceló, 2006). Gros et al. (2009) analysed a broad group of compounds including tetracyclines. They tested a combination of cartridges (Oasis® HLB and Oasis® MCX) at different pH conditions and with the effect of EDTA on relevant recoveries.

The following protocol was applied to filtered surface water or filtered Milli-Q spiked water. One liter of the samples has been loaded on 200 mg — 6 mL HLB, 500 mg — 6 mL C-18EC, 200 mg — 6 mL EASY, and 200 mg — 6 mL PLEXA solid phase extraction cartridges at 10 mL min^{-1}. The sorbents have been previously conditioned using 5 mL of methanol and 5 mL of water at pH 4. After sample loading, the cartridges were rinsed with 5% of methanol in water (5 mL) and dried with a stream of N_2 for 15 min. The selected compounds were eluted using methanol (2×5 mL). Extracts were concentrated with a gentle stream of N_2 and redissolved in 1 mL of a water/acetonitrile 95/5 (v/v) mixture before HPLC injection.

2.3. LC/MS–MS analysis

The chromatographic system consisted of an Ultimate 3000 Intelligent LC system (Dionex, Sunnyvale, USA) with a binary high-pressure gradient pump HPG-3200, an automatic injector WPS-3000 and a column oven TCC-3100. The chromatographic column was a NUCLEODUR C-18 ISIS column, 125×2 mm internal diameter, 3 μm particle size (Macherey Nagel, Düren, Germany). The MS–MS analyser consisted of a triple quadrupole mass spectrometer API 3200 (Applied Biosystem/MDS Sciex, Rotterdam, The Netherlands) equipped with a Turbo Ion Spray interface (Electrospray). N_2 was used as nebuliser, drying, curtain and collision gas.

Sulfonamides, tetracyclines and diclofenac were analysed in positive electrospray ionisation mode (+ESI) while estrogens and ibuprofen were analysed separately in negative electrospray ionisation mode (−ESI). For chromatography, solvent A and solvent B consisted of water and acetonitrile respectively, with addition of 0.1% of formic acid in +ESI and 10 mM ammonium acetate in water in −ESI. In +ESI the gradient started with 5% B, increased to 30% within 10 min, to 95% within 5 min, kept at 95% for 5 min, returned to initial composition within 1 min and equilibrated within 3 min for a total run time of 25 min. The mobile phase flow rate was 0.25 mL min^{-1} and the column was kept at 35 °C. The injection volume was 25 μL and all compounds

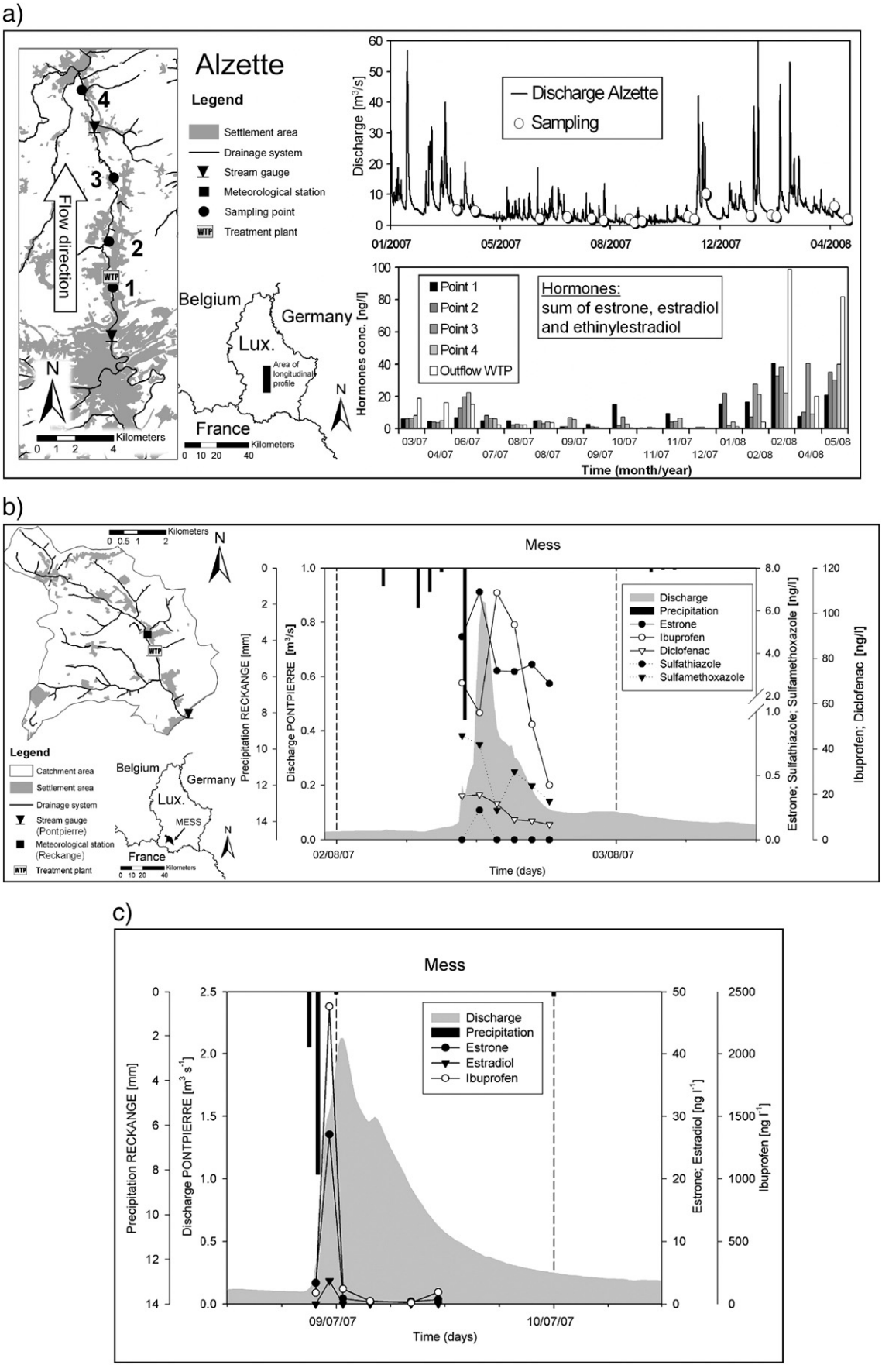

Fig. 2. a) Longitudinal profile of the Alzette river during a complete hydrological year (2007): the case of hormones at different sample points. b) Concentrations of selected pharmaceuticals (ng L^{-1}) during a flood event (August 2nd, 2007) of the Mess river. Rainfall measured at the meteorological station of Reckange. Discharge measurements were obtained at the streamgauge station of Pontpierre. c) Selected pharmaceutical concentrations (ng L^{-1}) during a flood event (July 9th, 2007) of the Mess river at the Pontpierre streamgauge station.

Table 2a
Pharmaceutical recoveries (% ± R.S.D.) of spiked water samples (40 ng L^{-1}) using HLB/EASY/PLEXA/C-18EC at pH 7 and 4.

	pH neutral			pH 4		pH 4	
Column	HLB	C-18EC	HLB/	PLEXA	EASY	HLB	HLB/EDTA
Compound	($n=3$)	($n=3$)	EDTA ($n=4$)	($n=5$)	($n=3$)	($n=3$)	($n=4$)
IBU	100 (±4)	86 (±2)	122 (±17)	98 (±7)	85 (±2)	95 (±2)	95 (±6)
DCL	61 (±1)	66 (±4)	78 (±13)	77 (±6)	20 (±15)	67 (±1)	91 (±6)
E1	72 (±3)	63 (±4)	101 (±16)	78 (±7)	61 (±3)	81 (±6)	83 (±7)
E2	83 (±4)	76 (±4)	92 (±18)	77 (±9)	70 (±6)	79 (±2)	81 (±4)
EE2	71 (±4)	69 (±8)	110 (±19)	69 (±4)	63 (±9)	62 (±7)	82 (±7)
STZ	52 (±4)	18 (±28)	136 (±12)	82 (±7)	67 (±7)	72 (±9)	79 (±8)
SMT	49 (±2)	41 (±21)	77 (±13)	98 (±5)	82 (±45)	76 (±4)	85 (±4)
SMX	73 (±1)	28 (±14)	91 (±14)	87 (±9)	90 (±6)	85 (±4)	86 (±3)
SDM	61 (±1)	49 (±17)	100 (±13)	110 (±2)	70 (±3)	75 (±3)	94 (±4)
T	4 (±24)	1	99 (±11)	12 (±18)	4 (±25)	32 (±8)	73 (±5)
OTC	4 (±27)	1	49 (±20)	15 (±19)	5 (±30)	35 (±6)	74 (±9)
Average	57	45	96	73	56	69	81
Wilcoxon *p*	0.01			0.004		0.005	

n = number of replicates.

were eluted within 15 min. In − ESI the gradient started with 20% of B during the first minute, increased to 70% at 14 min, remained at 70% for 1 min, returned to its initial composition within 1 min and equilibrated within 3 min for a total run time of 20 min. The mobile phase flow rate was 0.25 mL min^{-1} and the column was kept at 30 °C. The injection volume was 25 μL and all molecules were eluted within 11 min.

The API 3200 triple quadrupole mass spectrometer was running under selected reaction-monitoring mode (SRM) for increased sensitivity, with two SRM transitions for each molecule for improved selectivity. Optimal conditions were chosen in each mode as shown in the Table 3b.

2.4. Method validation

The performance characteristics of the SPE-LC/MS–MS method were investigated through a validation procedure with spiked deionised water. Selectivity, sensitivity (limits of detection, LOD, and limits of quantification, LOQ), linearity, precision and recovery were studied.

Table 2b
Repeatability of the methodology for analysis of common pharmaceuticals in water samples (40 ng L^{-1}) over a 5 months period ($n=5$ in triplicate).

Analyte	Recovery (±R.S.D.)	Month one	Month two	Month three	Month four	Month five
IBU	94 (±4)	95	93	89	100	94
DCL	83 (±15)	91	100	76	77	71
E_1	88 (±10)	83	76	88	93	99
E_2	85 (±12)	81	69	90	90	96
EE_2	82 (±15)	82	63	83	93	92
STZ	79 (±19)	79	103	67	79	67
SMT	76 (±16)	85	91	64	72	66
SMX	75 (±10)	86	66	78	72	75
SDM	87 (±15)	94	105	88	73	77
T	70 (±13)	80	77	70	61	60
OTC	71 (±13)	64	85	77	65	66

Table 2c
Within-run and between-run accuracy (mean values) and precision (R.S.D.) using water samples spiked with 50 ng L^{-1} ($n=3$).

	Intra-day		Inter-day	
Analyte	Accuracy	Precision	Accuracy	Precision
IBU	85	11	97	15
DCL	82	1	95	16
E_1	74	11	92	20
E_2	79	3	94	17
EE_2	82	19	95	18
STZ	87	8	97	14
SMT	98	4	102	11
SMX	84	3	96	15
SDM	89	7	98	13
T	82	20	95	22
OTC	73	23	92	23

For qualitative purposes precision of the retention time of the selected pharmaceuticals is evaluated. For quantitative purposes, sensitivity (LOD and LOQ), linearity, precision and recovery were studied. Our limits of detection (LOD) and quantification (LOQ) were estimated experimentally with the lowest concentration level able to reach a signal-to-noise ratio of 3 for LOD and 10 for LOQ (Table 1). The linearity was evaluated for each molecule from standard mixtures using 7 concentrations in the range of 1–100 ng mL^{-1} (except for IBU where the range was 1–5000 ng mL^{-1}). For our recovery experiments, 100 mL of deionised water were spiked with 10, 40 and 80 ng L^{-1} of standard molecules, filtered as described above, extracted on HLB sorbents, and analysed according to the proposed methodology (Table 2a). Repeatability of the method in terms of reliability and performance was realised periodically over 5 months (Table 2b). To assess the accuracy and day-to-day variation of the method, aliquots of three surface water samples were spiked with 50 ng L^{-1} of the target compounds and extracted to obtain independent replicates (Table 2c).

To evaluate the matrix effect, surface water extracts were spiked with 50 ng L^{-1} of pharmaceuticals. The effect of the matrix on the corresponding signal (Table 2d) was calculated by comparing the peak areas of spiked surface water extracts (after subtracting the peak areas corresponding to the native analytes present in the sample) with peak areas of spiked deionised water.

3. Results and discussion

3.1. Optimization of SPE

Extraction is an important step in the method development, especially since the studied pharmaceuticals have amphoteric properties (Table 1, Fig. 1). The extraction efficiency of different sorbents was determined by recovery experiments (Table 2a). At neutral pH, apolar molecules such as estrogens are retained to a high extent, either on HLB or C-18EC. Polymeric cartridges as HLB tended to be more

Table 2d
Percentage of matrix effect (mean values and R.S.D.) using surface water samples spiked with 50 ng L^{-1} ($n=5$).

Analyte	Recovery (R.S.D.)	% Matrix effect
IBU	123 (+/−19)	23
DCL	87 (+/−12)	−13
E_1	80 (+/−8)	−20
E_2	84 (+/−14)	−16
EE_2	97 (+/−11)	−3
STZ	44 (+/−15)	−56
SMT	26 (+/−14)	−74
SMX	47 (+/−13)	−53
SDM	28 (+/−19)	−72
T	99 (+/−18)	−1
OTC	75 (+/−18)	−25

Table 3a
Selected Reaction Monitoring (SRM) transitions of target compounds: precursor, quantifier product, qualifier product, collision energy in eV (between brackets) and retention time (RT).

Analyte	m/z Precursor	m/z Quantifier	m/z Qualifier	RT (min)
IBU	205.1	161.1 (−8)	159.2 (−10)	6.01
DCL	296.1	214.2 (47)	215.3 (23)	14.45
E_1	269.1	145.1 (−52)	143.1 (−74)	10.79
E_2	271.1	145.2 (−54)	143.1 (−74)	9.69
EE_2	295.1	145.0 (−58)	143.1 (−70)	10.55
STZ	256.1	156.0 (19)	92.1 (35)	4.82
SMT	279.2	92.1 (39)	124.2 (35)	6.44
SMX	254.1	92.0 (37)	156.0 (21)	9.01
SDM	311.2	156.1 (29)	92.1 (41)	11.14
T	479.2	154.2 (37)	98.2 (57)	5.29
OTC	445.3	410.1 (27)	154.2 (33)	5.01

polyvalent for amphoteric compounds, rather than the reverse phase C-18EC sorbent (Wilcoxon p 0.01). In this study the mean recoveries of sulfonamides as sulfathiazole or sulfamethoxazole decreased from 52% and 73% using HLB to 18% and 28% using C-18EC. Thus, HLB cartridge was chosen as a reference for the following comparisons. At pH 4, two other polymeric cartridges, EASY (polar modified polystyrene-divinylbenzene copolymer with a weak anion exchanger) and PLEXA (another polymer sorbent) were compared to HLB. An acceptable recovery was observed for estrogens, sulfonamides and anti-inflammatory drugs both for PLEXA and HLB at pH 4 (EASY was less successful for DCL). Nevertheless, HLB appeared to be better suited for the tetracycline group at pH 4 (32–35% recovery range using HLB instead of 12–15% recovery range with the other polymeric cartridges). To improve the extraction efficiencies of tetracycline group, EDTA was added to chelate cations. Relative good results were now obtained with a significant enhancement of recoveries for tetracycline and oxytetracycline using EDTA at acidic and neutral pH.

The pH of the samples determines the chemical form (speciation) of the analytes in the matrices and the interaction between analyte and sorbent during SPE (Babic et al., 2006; Díaz-Cruz and Barceló, 2005; Yang and Carlson, 2003). With respect to the pKa data shown in Table 1 (Carpinteiro et al., 2004; Choi et al., 2007; Gros et al., 2006, Lewis and Archer, 1979; Qian and Adams, 2004), pH 4 was chosen where all compounds existed in neutral form (pH 2, 3 and 5 were already tested but without significant improvement compared to pH 4). Furthermore, tetracyclines are molecules, which have the capability to bind with cations like Ca^{2+} present in the medium and to silanol groups of the solid-phase sorbents (Hernández et al., 2007). As a consequence, the addition of a chelatant cation as EDTA (Yang et al., 2005) increased the recovery of the tetracycline group. Finally, the best recoveries were obtained for all compounds on HLB cartridges with EDTA at pH 4 (70–95%) and at pH 7 (49–136%). Nevertheless, pH 4 was chosen for the better recovery of OTC and the better R.S.D. Authors using Oasis® HLB cartridges at pH 2 (Kim et al., 2007; Vanderford et al., 2003) without EDTA were not interested in analysing tetracyclines. Gros et al. (2009) analysed a broad group of compounds including tetracyclines. They used Oasis® HLB cartridges with EDTA at the natural pH of the sample (instead of Oasis® MCX cartridges or tandem HLB–MCX).

To confirm all these data, a statistical paired test was realised with the software package SPSS (version 15). We used the Wilcoxon test for paired samples as the non-parametric equivalent of the paired samples *t*-test because our values are not normally distributed. We realised a two-tailed test with a 0.05 level of significance. The paired sample Wilcoxon test procedure compared the mean of 2 recovery series (different sorbents) for a single pH. The conclusion was HLB is the best sorbent. Furthermore we can show that EDTA enhanced significantly the recoveries (Table 2a). In addition, we tested HLB/EDTA at neutral pH and at pH 4. The Wilcoxon test indicated no significant difference between both conditions ($p = 0.11$).

3.2. LC/MS–MS analysis

Mass spectrometry has become an efficient tool for analysing pharmaceuticals at nanomole concentration due to its high sensitivity and selectivity (Chang et al., 2008; Díaz-Cruz et al., 2008; Granelli et al., 2008). Table 3a shows the precursor ion m/z of each analyte, the different fragments (one chosen as quantifier, the other as qualifier for confirmation) and the collision energy (CE) required to produce them. Precursor ions of sulfonamides correspond to $[M+H]^+$ ion and fragmentation gives a major fragment at $m/z = 156$, corresponding to the sulfanilyl ring $[NH_2–C_6H_4–SO_2]^+$ and at $m/z = 92$, corresponding to further loss of the sulfonyl group SO_2. For tetracyclines, the precursor ion is $[M+H]^+$ and the major fragment ions are $[M+H–H_2O]^+$, $[M+H–NH_3]^+$ or $[M+H–H_2O–NH_3]^+$. For IBU the $[M–H]^-$ parent ion gives fragments due to the loss of a carboxyl group. For DCL the $[M+H]^+$ parent ion gives fragments by decarboxylation, dehydratation and the loss of a chlorine ion. For estrogens the $[M–H]^-$ parent ion gives two main fragments corresponding to the cleavage between the rings 2 and 3 (Table 3b).

3.3. Method validation

For qualitative purposes, the combination of the retention factor and the choice of two specific transition fragments resulted in successful signatures of each substance. The blank samples did not show any false positive results.

For quantitative purposes, linearity was analysed by linear regression. The correlation coefficients (r^2), with values higher than 0.997, were considered satisfactory (Table 1). Recoveries at three different concentrations (10, 40, 80 ng L^{-1}) were analysed in triplicates and summarised in Table 2b. The 40 ng L^{-1} values are representative because no concentration dependence was observed. Results were achieved for all substances with R.S.D. in the range of 4–19% over the 5 months period.

Accuracy and precision within run and between runs were determined using spiked deionised water. The results are summarised in the Table 2c. Within the run, the accuracy range was 73–98%, well within the acceptable values of 70–120%. The precision, as the relative standard deviation calculated from these experiments, ranged from 1 to 23%. Between two runs, the accuracy range was 92–102% and the precision ranged from 11 to 23%.

The matrix effects were evaluated for each compound in surface water extracts. Matrix effects are the main problem encountered in the quantitative LC/MS–MS analysis. They occur because the ESI source is highly susceptible to organic matter, salts, ion-pairing agents, and non-target contaminants present in the matrix. Nevertheless, when the matrix effects occur the signal intensity for the analytes decrease (ion suppression) or increase (enhancement). The percentage of ion suppression or enhancement is summarised for our experiments in Table 2d and ranged from −74 up to +23%. The results show moderate signal suppression for DCL (−13%), hormones (−3 to −16%), or tetracyclines (−1 to −25%), to more important signal suppression for sulfonamides (−53 to −74%). This matrix effect, which can be considered as reasonable for a great part of our analytes, can be even lowered by diluting the extracts, but with a slight loss in sensitivity (work in progress, data not shown). Additionally, the present method will be further developed by the

Table 3b
Optimal parameters for MS–MS.

	Curtain gas (psi)	Nebulizer gas (psi)	Collision gas (psi)	Drying gas (psi)	T°C	Ion spray voltage (V)
+ESI	30	40	5	55	650	5500
−ESI	20	45	5	45	650	−4500

Table 4
Range of minimum-maximum pharmaceutical concentrations measured in the WWTP of Beggen, as well as in the Alzette and Mess rivers (24 campaigns in total from March 2007 to May 2008).

Substances [ng L^{-1}]	Values in WWTP-inlet	Values in WWTP-outlet	Values in Alzette	Values in mess	Values of surface water studies in other western countries
IBU	[82–3080]	[3–359]	[10–295]	[9–2383]	[150–2010]
DCL	[2–43]	[LOQ-78]	[LOQ-55]	[LOQ-19]	[8–380]
E1	[LOQ-9]	[LOQ-14]	[LOQ-6]	[LOQ-27]	[0–12]
E2	[LOQ-102]	[LOQ-85]	[LOQ-35]	[LOQ-6]	[0–6]
EE2	[LOQ-24]	<LOQ	<LOQ	<LOQ	[0–1]
STZ	[LOQ-2]	<LOQ	<LOQ	[LOQ-2]	[0–10]
SMT	[LOQ-2]	<LOQ	<LOQ	<LOQ	0
SMX	[13–155]	[4–39]	[1–22]	[LOQ-5]	[2–80]
SDM	[LOQ-26]	[LOQ-9]	[LOQ-3]	<LOQ	[0–40]
T	[LOQ-85]	[LOQ-24]	[LOQ-8]	[LOQ-7]	[0–20]
OTC	[LOQ-7]	[LOQ-5]	[LOQ-2]	[LOQ-7]	[0–10]

Comparison with a range of concentrations determined for European and American rivers.
LOQ: limit of quantification.
WWTP: wastewater treatment plant.

addition of several deuterated internal standards (sulfadimethoxine-d_6, ibuprofen-d_3, 17-β-estradiol-d_3, tetracycline-d6). This will significantly improve the robustness of the method, by correcting the ion suppression independently of the sample matrix.

4. Application to surface water and wastewater samples

The validated method was applied to surface and wastewater samples along the Alzette River, including the Beggen WWTP outflow.

Fig. 2a shows the total of the measured hormone concentrations (E_1, E_2, EE_2) in the Alzette River. 17 profiles were realised between March 2007 and May 2008. The outflow of the WWTP of Luxembourg City is located between measurement points 1 and 2. In the warm season (July to September), when degradation processes are accelerated, only minor concentrations were measured at this outflow point, as well as in the river itself (samplings 4 to 11). In this period, one could expect higher concentrations in the Alzette due to the decline of the discharge and the more important contribution of wastewaters to the total discharge. Nevertheless, the Alzette shows a lower degree of contamination. In the warm period, this particular situation could be explained because the Alzette is mainly fed and diluted by groundwater. As a consequence, the hormone concentrations were comparatively low. With weaker degradation processes and increased pollution from separated sewer systems characterising the cold season, concentrations rise.

Furthermore, we analysed water from the Mess River (a left-bank tributary of the Alzette river, upstream of Luxembourg-city) during flood events. The pharmaceutical concentrations are variable during a flood event. The highest concentrations can be reached during the first flush effects, mainly during the rising limb of the flood hydrographs. In the Mess basin different pharmaceuticals show their concentration peaks during different times of a flood event. This suggests for more sources than the storm drainage through the spillway of the single sewage water treatment plant. Especially discharge from numerous sewer overflows of the combined sewer system varies in space and time. Separated sewer overflows are well known for their discharge of untreated water during storm events (Hatt et al., 2004).

Two figures (Fig. 2b and c) highlight the dissolved pharmaceutical concentrations during two characteristic flood events in the Mess River. In the case of Fig. 2b, representing the flood event of August 2nd 2007, the rainfall event was relatively long (6 h with a total precipitation of 13.1 mm) and produced a comparatively low peak discharge (0.8 m^3 s^{-1}). Different pharmaceuticals showed peak concentrations at different times of the flood event. An example is the estrone peak that occurred 1 to 2 h before the highest concentration of IBU. In the case of Fig. 2c, representing the flood event of July 9th 2007, the rainfall duration was shorter (1 h for a total precipitation of 10.8 mm), and a higher peak discharge was produced (2 m^3 s^{-1}). This flood event followed an extended dry period, resulting in a distinct first flush of highly concentrated dissolved pharmaceuticals released by the sewer system. Calculated total discharge associated with the flood event of July 9th were 24 g of IBU, 274 mg of E_1 and 32 mg of E_2 compared to the flood event of August 2nd with 713 mg of IBU, 46 mg of E_1, 148 mg of DCL, 0.9 mg of STZ and 5 mg of SMX.

In floodwaters (11 floods, 66 samples), the highest concentrations were measured for IBU (9–2382 ng L^{-1}), E_1 (4–27 ng L^{-1}) and DCL (3–20 ng L^{-1}). From the tetracycline group, especially T itself was of relevance (0–9 ng L^{-1}), while the sulfonamides were mainly represented by SMX (1–5 ng L^{-1}).

Table 4 compares the minimum–maximum pharmaceutical's concentrations found in the Mess and the Alzette rivers, including the inlet and outlet of the WWTP, to those measured in some other rivers in Western countries (Yang et al., 2005; Zuccato et al., 2005). IBU and DCL are the main compounds measured as well in our study as in various publications (Gros et al., 2006; Heberer, 2002; Kasprzyk-Hordern et al., 2008; Roberts and Thomas, 2006). In the case of hormones, E_1 and E_2, the natural ones were the most abundant (Laganà et al., 2004). For the sulfonamides, SMX was the more commonly found and for the tetracycline group, T itself was the most significant (Kim and Carlson, 2007). In general, selected pharmaceuticals were concentrated in the inlet of the WWTP and diluted in the river. For example IBU had a concentration up to 3080 ng L^{-1} in the inlet of the WWTP and diluted to 295 ng L^{-1} in the Alzette or SMX had a concentration of 155 ng L^{-1} in the inlet of the WWTP and diluted to 22 ng L^{-1} in the Alzette. Generally, the selected pharmaceuticals were concentrated in the sewage treatment plant, eliminated with more or less efficiency and returned back in the river with a smaller concentration. It was observed that the efficiency of the WWTP depended on the compound; 90% degradation for IBU, 75% for SMX, 70% for T but only 15% for E_2 whereas DCL was more resistant. In fact DCL accumulated in the outlet of the WWTP (the process of elimination was probably not adapted to this compound) and returned back to the river in non-negligible concentrations (up to 55 ng L^{-1}). The river concentration of E_2 was relatively high (35 ng L^{-1}), especially when taking into account its presumed endocrine disrupting effect on animals at this level. For the Mess River, the most abundant compound has been IBU (with E_1 and DCL). In the particular case of flood events, high concentrations of IBU or other pharmaceuticals were related to the flushing phenomenon of combined sewage water systems.

5. Conclusion

The development of a simultaneous extraction method on HLB cartridges constituted a significant improvement in the extraction of sulfonamides, tetracyclines, hormones and anti-inflammatories. Recoveries were acceptable compared to literature (70–94%), however this “multi-residue” method was easier to carry out and less time and material consuming, compared to separated extractions by classes of compounds, which also use different cartridges and more solvents. SPE-LC/MS–MS resulted in detection limits ranging from 0.3 to 2.0 ng L^{-1}, allowing the determination of pharmaceuticals in highly polluted wastewaters and storm waters. This analysis of pharmaceuticals by LC/MS–MS can be a useful tool to trace their elimination from the wastewater treatment plants to the aquatic environment.

The detailed analysis of flood events using the rainfall pattern, the hydrograph, and dissolved pharmaceutical chemographs can provide a good insight into the temporal structure of flood events. However, the corresponding anthropogenic sources show a high temporal and spatial variability that is caused by different rainfall patterns and

distributions, and the different characteristics (e.g. retention capacities) of the combined sewer systems. We can show that the combined sewer overflows deliver an important part of the dissolved pharmaceuticals into the Mess River network.

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References

Ahrer W, Scherwenk E, Buchberger W. Determination of drug residues in water by the combination of liquid chromatography or capillary electrophoresis with electrospray mass spectrometry. J Chromatogr A 2001;910(1):69–78.

Babic S, Asperger D, Mutavdzic D, Horvat AJM, Kastelan-Macan M. Solid phase extraction and HPLC determination of veterinary pharmaceuticals in wastewater. Talanta 2006;70:732–8.

Balakrishnan VK, Terry KA, Toito J. Determination of sulfonamide antibiotics in wastewater: a comparison of solid phase microextraction and solid phase extraction methods. J Chromatogr A 2006;1131:1-10.

Ben W, Quiang Z, Adams C, Zhang H, Chen L. Simultaneous determination of sulfonamides, tetracyclines and tiamulin in swine wastewater by solid-phase extraction and liquid chromatography-mass spectrometry. J Chromatogr A 2008;1202:173–80.

Carpinteiro J, Quintana JB, Rodríguez I, Carro AM, Lorenzo RA, Cela R. Applicability of solid-phase microextraction followed by on-fiber silylation for determination of estrogens in water samples by gas chromatography-tandem mass spectrometry. J Chromatogr A 2004;1056:179–85.

Chang H, Hu J, Asami M, Kunikani S. Simultaneous analysis of 16 sulfonamide and trimethoprim antibiotics in environmental waters by liquid chromatography–electrospray tandem mass spectrometry. J Chromatogr A 2008;1190:390–3.

Choi KJ, Kim SG, Kim CW, Kim SH. Determination of antibiotic compounds in water by on-line SPE-LC/MSD. Chemosphere 2007;66:977–84.

Díaz-Cruz MS, Barceló D. LC-MS^2 trace analysis of antimicrobials in water, sediment and soil. Trends Anal Chem 2005;24(7):645–57.

Díaz-Cruz MS, Barceló D. Determination of antimicrobial residues and metabolites in the aquatic environment by liquid chromatography tandem mass spectrometry. Anal Bioanal Chem 2006;386:973–85.

Díaz-Cruz MS, Garcia-Galan MJ, Barceló D. Highly sensitive simultaneous determination of sulfonamide antibiotics and one metabolite in environmental waters by liquid chromatography–quadrupole linear ion trap–mass spectrometry. J Chromatogr A 2008;1193:50–9.

Erickson BE. Analysing the ignored environmental contaminants. Environ Sci Technol 2002;36:140–4.

Gabet V, Miège C, Bados P, Coquery M. Analysis of estrogens in environmental matrices. Trends Anal Chem 2007;26(11):1113–31.

Granelli K, Elgerud C, Lundström A, Ohlson A, Sjöberg P. Rapid multi-residue analysis of antibiotics in muscle by liquid chromatography–tandem mass spectrometry. Anal Chim Acta 2008;637(1-2):87–91.

Gros M, Petrovic M, Barceló D. Development of a multi-residue analytical methodology based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters. Talanta 2006;70:678–90.

Gros M, Petrovic M, Barceló D. Tracing pharmaceutical residues of different therapeutic classes in environmental waters by using liquid chromatography/quadrupole-linear ion trap mass spectrometry and automated library searching. Anal Chem 2009;81(3):898–912.

Hatt BE, Fletcher TD, Walch CJ, Taylor SL. The influence of urban density and drainage infrastructure on the concentrations and loads of pollutants in small streams. Environ Manage 2004;34:112–24.

Heberer T. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol Lett 2002;131:5-17.

Hernández F, Sancho JV, Ibáñez M, Guerrero C. Antibiotic residue determination in environmental waters by LC-MS. Trends Anal Chem 2007;26(6):466–85.

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. The effect of signal suppression and mobile phase composition on the simultaneous analysis of multiple classes of acidic/neutral pharmaceuticals and personal care products in surface water by solid-phase extraction and ultra performance liquid chromatography–negative electrospray tandem mass spectrometry. Talanta 2008;74:1299–322.

Kaufmann A, Butcher P, Maden K, Widmer M. Quantitative multiresidue method for about 100 veterinary drugs in different meat matrices by sub 2-μm particulate high-performance liquid chromatography coupled to time of flight mass spectrometry. J Chromatogr A 2008;1194:66–79.

Kim SC, Carlson K. Quantification of human and veterinary antibiotics in water and sediment using SPE/LC/MS/MS. Anal Bioanal Chem 2007;387:1301–15.

Kim SD, Cho J, Kim IS, Vanderford BJ, Snyder SA. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. Water Res 2007;41(5):1013–21.

Laganà A, Bacaloni A, De Leva I, Faberi A, Fago G, Marino A. Analytical methodologies for determining the occurrence of endocrine disrupting chemicals in sewage treatment plants and natural waters. Anal Chim Acta 2004;501:79–88.

Lewis KM, Archer RD. pK_a values of estrone, 17β-estradiol and 2-methoxyestrone. Steroids 1979;34(5):485–99.

Mauduit C, Florin A, Amara S, Bozec A, Siddeek B, Cunha S, et al. Long-term effects of environmental endocrine disruptors on male fertility. Gynecol Obstet Fertil 2006;34:978–84.

Petrovic M, Gonzalez S, Barceló D. Analysis and removal of emerging contaminants in wastewater and drinking water. Trends Anal Chem 2003;22(10):685–96.

Rodriguez-Mozaz S, López de Alda MJ, Barceló D. Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction–liquid chromatography–mass spectrometry. J Chromatogr A 2004;1045:85–92.

Qian Z, Adams C. Potentiometric determination of acid dissociation constants (pK(a)) for human and veterinary antibiotics. Water Res 2004;38:2874–90.

Quintana JB, Reemtsma T. Sensitive determination of acidic drugs and triclosan in surface and wastewater by ion-pair reverse-phase liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2004;18:765–74.

Renew JE, Huang CH. Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography–electrospray mass spectrometry. J Chromatogr A 2004;1042:113–21.

Roberts PH, Thomas KV. The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Sci Total Environ 2006;356:143–53.

Snyder SA, Westerhoff P, Yoon Y, Sedlak DL. Pharmaceuticals, personal care products and endocrine disrupters in water: implications for water industry. Environ Eng Sci 2003;20(5):449–69.

Ternes TA. Analytical methods for the determination of pharmaceuticals in aqueous environmental samples. Trends Anal Chem 2001;20:419–34.

Turnipseed SB, Andersen WC, Karbiwnyk CM, Madson MR, Miller KE. Multi-class, multi-residue liquid chromatography/tandem mass spectrometry screening and confirmation methods for drug residues in milk. Rapid Commun Mass Spectrom 2008;22:1467–80.

Vanderford BJ, Pearson RA, Rexing DJ, Snyder SA. Analysis of endocrine disruptors, pharmaceuticals, and personal care products in water using liquid chromatography/tandem mass spectrometry. Anal Chem 2003;75(22):6265–74.

Verma B, Headley JV, Robarts RD. Behaviour and fate of tetracycline in river and wetland waters on the Canadian Northern Great Plains. J Environ Sci Health A 2007;42(2):109–17.

Wang S, Huang W, Fang G, He J, Zhang Y. On-line coupling of solid-phase extraction to high-performance liquid chromatography for determination of estrogens in environment. Anal Chim Acta 2008;606:194–201.

Wissiack R, Rosenberg E, Grasserbauer M. Comparison of different sorbent materials for on-line solid-phase extraction with liquid chromatography–atmospheric pressure chemical ionization mass spectrometry of phenols. J Chromatogr A 2000;896(1-2):159–70.

Yang S, Carlson K. Evolution of antibiotic occurrence in a river through pristine, urban and agricultural landscapes. Water Res 2003;37:4645–56.

Yang S, Cha J, Carlson K. Simultaneous extraction and analysis of 11 tetracycline and sulfonamide antibiotics in influent and effluent domestic wastewater by solid-phase extraction and liquid chromatography-electrospray ionization tandem mass spectrometry. J Chromatogr A 2005;1097:40–53.

Zhu J, Snow DD, Cassada DA, Monson SJ, Spalding RF. Analysis of oxytetracycline, tetracycline, and chlortetracycline in water using solid-phase extraction and liquid chromatography–tandem mass spectrometry. J Chromatogr A 2001;928(2):177–86.

Zuccato E, Castiglioni S, Fanelli R. Identification of the pharmaceuticals for human use contaminating the Italian aquatic environment. J Hazard Mater 2005;122:205–9.