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Multi-residue analysis of pharmaceuticals in wastewater by liquid chromatography–magnetic sector mass spectrometry: Method quality assessment and application in a Belgian case study

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ABSTRACT

Through systematic research a novel analytical method using solid-phase extraction (SPE) and liquid chromatography magnetic sector mass spectrometry was developed for the measurement of 43 pharmaceuticals in wastewater. A thorough method validation quantified the contribution of both the extraction recovery and matrix effects in the overall method process efficiency, and a detailed uncertainty analysis was performed to elaborate a quality labelling strategy to be used in data interpretation. Compounds for which a precise (relative standard deviation <20%) process efficiency between 60% and 140% was determined, were labelled as 'quantitative' whereas the results for other compounds should be interpreted as 'indicative'.

Method application on influent and effluent samples of (i) a conventional active sludge system and (ii) a parallel membrane bioreactor/conventional active sludge wastewater treatment plant in Belgium revealed the occurrence of 22 pharmaceuticals. The anti-inflammatory drug diclofenac and the antidepressant venlafaxine were measured in the effluents at concentrations ranging from 0.5 to 1.8 $\mu g\,L^{-1}$ and 0.2 to 0.5 $\mu g\,L^{-1}$, respectively, which indicated to be of high potential environmental risk for the receiving river Dender, Belgium.

1. Introduction

Wastewater treatment plants (WWTP) have been pointed out as the main contamination pathway for pharmaceuticals from human and veterinary applications to the environment. For a broad range of pharmaceuticals often only partial removal is achieved in biological treatment processes (Michael et al., 2013; Petrie et al., 2013). As a result, bio-recalcitrant micropollutants are continuously introduced in the environment. At least for some pharmaceuticals the risk for ecotoxicological effects in the receiving environment is assessed to be high (Stuer-Lauridsen et al., 2000; Hernando et al., 2006; de Souza et al., 2009). Since the European Commission included diclofenac in the watch list for emerging substances in the aquatic environment (directive 2013/39/EU), increasing efforts will focus on monitoring the occurrence and removal of pharmaceuticals in wastewater treatment.

High-resolution mass spectrometry (HRMS) is making advances the last decade as quantitative MS benefiting from an increased selectivity and sensitivity. An increasing number of papers report the ability of time-of-flight (Ibanez et al., 2008; Diaz et al., 2013; Vergeynst et al., 2013) and Orbitrap (Chitescu et al., 2012; Gómez-Canela et al., 2013) HRMS to quantify environmental residue levels of pharmaceuticals. Although double focusing magnetic sector HRMS has shown its merits in the analysis of micropollutants like dioxins and furans (Hernández et al., 2012), and of oxygenated polycyclic aromatic hydrocarbons in airborne matrices (Walgraeve et al., 2012), its use in multi-residue water analysis is very scarce. Recently, magnetic sector HRMS hyphenated to HPLC by electrospray ionization (ESI) has been used a first time for qualitative screening towards pharmaceuticals in surface water (K'oreje et al., 2012); but so far, no reports are available describing its application in quantitative water analysis.

The objectives of this study are twofold. First, the goal is to investigate the analytical performance, method validation, quality assessment and uncertainty analysis – the latter being only scarcely discussed in multi-residue analysis – of a newly developed and optimised double focusing magnetic sector ESI-HRMS detection method hyphenated with HPLC and preceded by solid-phase extraction (SPE) for quantitative trace analysis of 43 selected

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pharmaceuticals in influent and effluent water of WWTPs. Twenty-five pharmaceuticals were selected from three studies (Cooper et al., 2008; Kumar and Xagoraraki, 2010; Coutu et al., 2012) dealing with prioritization of emerging contaminants. This selection was extended with 8 quinolone antibiotics because of their biorecalcitrance in WWTPs (Jia et al., 2012), and with 10 antiviral drugs belonging to the most hazardous pharmaceuticals based on predicted toxicity towards fish, daphnia and algae (Sanderson et al., 2004) but only measured in the environment in a limited number of studies (Ghosh et al., 2010; Prasse et al., 2010; K'oreje et al., 2012).

The second goal is to study the occurrence of pharmaceuticals in influent and effluent waters of a parallel conventional active sludge system (CAS) – membrane bioreactor (MBR) and a second CAS WWTP in Belgium. This study brings forward one of the first concentration data of pharmaceuticals in wastewater in Belgium, and allows to perform a first trier environmental risk assessment for the Belgian river affected by the second CAS-WWTP. Finally, loads are calculated to determine removal efficiencies in both WWTP types.

2. Experimental section

2.1. Chemicals and materials

The 43 pharmaceuticals selected in this study, their therapeutic usage, and their analytical standard suppliers are listed in Table S1. Preparation of the standards and matrix-matched calibration curves, and the specifications of other chemicals used in this study, are described in Supplementary content.

2.2. Sampling and WWTPs description

For the method development and validation (Section 3.1), influent and effluent grab samples were collected in prerinsed amber glass bottles at the WWTP of Lede, Belgium.

For the method application (Section 3.2), influent and effluent 24 h time integrated samples were collected in a parallel CAS-MBR WWTP (WWTP1) and a CAS WWTP (WWTP2) using an automatic sampler (50 mL sample each 20 min, Sigma 900 and ISCO 4700, Elscolab, Belgium) during 4 and 6 days, respectively, resulting in a total of 10 influent and 18 effluent samples. Effluent samples were collected 24 h after their corresponding influent sample. Fig. S1 presents a schematic overview with indication of the sampling points, and Table S2 summarises the sampling period, number of samples, precipitation data, and the main physical and chemical characteristics of both WWTPs.

Formic acid was added to the samples (pH 3) to prevent microbial activity during sample storage (at 4 °C in the dark for $\leqslant$4 days) prior to extraction.

2.3. Sample pretreatment and solid-phase extraction (SPE)

The optimised sample pretreatment and SPE protocol was as follows (details in Supplementary content). The samples (pH 7.0) were filtered and Na_2EDTA was added (1 g L^{-1}). Oasis HLB SPE cartridges (6 mL, 200 mg sorbent) were activated with methanol and water. Then, 100 mL effluent or 50 mL influent sample were loaded on the cartridge. After washing with 4 times 6 mL of deionized water, elution was performed using 5 mL of methanol. The eluent was collected in silanized glass tubes and evaporated under nitrogen stream until complete dryness. Reconstitution was performed with 1 mL of 10:90 methanol/water. The extract was finally distributed over 2 vials and used for ESI positive (+0.1% formic acid) and negative analysis.

2.4. Instrumental analysis

2.4.1. HPLC separation

Chromatographic separation of the analytes was achieved using a Surveyor HPLC system (Thermo Finnigan) equipped with a Phenomenex Luna C18(2) 150 × 2.0 mm column (3 μm particle size) and operating at 35 °C. The sample injection volume was 10 μL. The mobile phase was a mixture of (A) methanol and (B) water, both with 0.1% formic acid, and a mixture of (C) acetonitrile and (D) water for analysis in ESI positive and negative ion mode, respectively (details in Supplementary content).

2.4.2. Selective ion detection

The double focusing magnetic sector HRMS (Thermo Finnigan) was operated in multiple ion detection (MID) mode for selective target analysis at a resolving power of 10000 (10% valley definition). In MID mode, the chromatographic analysis is divided in multiple retention time windows. In each of them, the appropriate MID mass window is defined (in which the highest mass is maximum 1.2 times the lowest mass) and the masses of the target ions and the ions for internal mass calibration are consecutively measured. To assure the longest measuring time for each ion, and given the restriction of the MID window width, 3 and 1 distinct runs in ESI positive and negative ion mode, respectively, were required to measure the 43 compounds. The optimised ESI parameters, more details on the MID-operation of the instrument, and the instrumental validation protocol, are given in Supplementary content.

2.5. Method validation and determination of the SPE recovery and matrix effects

Influent and effluent water spiked with standards before (pre) and after (post) SPE extraction (standard addition method) as well as non-spiked samples were analysed as schematically represented in Fig. S2. For the pre-spiked samples, the validation range was 40–5000 ng L^{-1} or 200–25000 ng L^{-1} for influent, and 20–2500 ng L^{-1} or 100–12500 ng L^{-1} for effluent water, depending on the instrumental detection limit for the considered compound. The procedure was repeated on three different days and matrix-matched calibration curves were constructed from the pre-spiked samples (n = 4 concentration levels × 3 repetitions).

The method interday reproducibility (RSD, %) was determined from the triplicate SPE extraction and analysis of the pre-spiked samples, and method detection and quantification limits (MDL/MQL) were estimated from an average (n = 3) signal-to-noise ratio (S/N) of 3 and 10, respectively.

The methodology proposed by Matuszewski et al. (2003) was applied for the determination of the recovery (RE), matrix effects (ME), and the overall 'process efficiency' (PE). Recovery refers to the extraction efficiency of the SPE procedure (SPE extraction, drying and reconstitution) and has theoretically a value between 0% and 100%. A ME of 1 (i.e. 100%) is obtained when no matrix effects are present; and ME >100% and <100% represent signal enhancement and suppression, respectively. The overall 'process efficiency' refers to the combined effect of the recovery and matrix effects. For the determination of PE, the experimentally determined concentrations of the pre-spiked samples ($C_{exp,pre}$), calculated using external calibration, were plotted as a function of the theoretical pre-spiked concentrations ($C_{th,pre}$). The slope of this curve, determined by $1/x^2$ weighted least squares regression, equals the PE (Eq. (1)). ME and RE were determined using Eqs. (2) and (3) ($C_{exp,sample}$ and $C_{exp,post}$ represent the measured concentrations in the non-spiked and post-spiked samples, respectively). Finally, Eq. (4) shows how the concentration present in the non-spiked sample (C_{sample}) is calculated from $C_{exp,sample}$ by applying the PE.

$$C_{exp,pre} = PE \cdot C_{th,pre} + C_{exp,sample} \quad (1)$$

$$C_{exp,post} = ME \cdot C_{th,post} + C_{exp,sample} \quad (2)$$

$$PE = RE \cdot ME \quad (3)$$

$$C_{exp,sample} = PE \cdot C_{sample} \quad (4)$$

The equations for calculating the standard deviations on PE and ME are given in Supplementary content.

2.6. First trier environmental risk assessment

The effluent of WWTP2 is discharged into a creek and subsequently into the river Dender, Belgium, for which flow data are available. The environmental risk posed by the discharged pharmaceuticals was assessed by means of the risk quotient (RQ), being the ratio between the measured environmental concentration (MEC) and the predicted no effect concentration (PNEC). Since no river water was analysed, quasi-MECs (Grung et al., 2008) of the compounds quantified in the effluent were estimated by considering a dilution factor of 41, calculated from the average effluent flow of WWTP2 (Table S2) and the dry weather flow of the river Dender (about 10 $m^3\ s^{-1}$, www.waterstanden.be). PNEC values were based on ecotoxicity data and calculated according to the European Medicine Agency guideline (EMEA/CHMP/SWP/4447/00).

3. Results and discussion

3.1. Method validation and quality assessment

The results of the method (instrumental analysis and SPE) development and optimisation, and the instrumental validation parameters, are given in Supplementary content. MDLs were lower than 100 ng L^{-1} for 27 and 34 out of the 43 target pharmaceuticals for influent and effluent, respectively. Even at the lowest detectable concentration, the RSD on the peak area was better than 20% for 90% of the compounds in both types of wastewater (Tables S6 and S7). The PE for most of the compounds ranged from 60% to 140% (guideline according to SANCO/10684/2009), except for acyclovir, amoxicillin, chloramphenicol, fluoxetine, lamivudine, oxytetracycline, paracetamol, paroxetine, pleconaril, temazepam and triclosan in one of both matrices.

3.1.1. The process efficiency unravelled: extraction recovery and matrix effects

The decomposition of PE into RE and ME (Eqs. (1)–(4) and Fig. 1) allows to differentiate whether PE values differing from 100% result from low SPE recovery and/or matrix signal suppression/enhancement. For influent and effluent water, the recovery was higher than 80% for 37 and 34 out of the 43 compounds, respectively. Fig. S3 shows that low recoveries can occur for very polar or hydrophilic compounds such as acyclovir, amoxicillin and lamivudine ($\log K_{ow}$ from −1.59 to 0.06) and for very lipophilic compounds such as pleconaril ($\log K_{ow} > 5$). On the other hand, tetracycline, oxytetracycline, zidovudine and metronidazole have $\log K_{ow} < 0$ but SPE recoveries >95% indicating that also other parameters affect the analyte behaviour during SPE. Matrix effects were <80% and >120%, respectively, for 20 and 3 compounds in effluent, and for 11 and 2 compounds in influent, showing their importance in PE for ESI analysis of complex waters.

It should be denoted that the precision on the estimated RE and ME for 18 and 6 compounds, respectively, was higher than 20% RSD in effluent, whereas this was only the case for 1 compound in influent. The reason can mainly be accounted to the lower post-spiking concentration applied for the validation of the effluent. A more in-depth uncertainty analysis is performed in Section 3.1.2.

3.1.2. Uncertainty analysis

Validating an analytical method for compounds being ubiquitously present in environmental matrices such as wastewater can be problematic regarding at least two aspects.

The first aspect is related to the determination of the MDL/MQL values. Some compounds were clearly present (S/N ratios $\gg 10$) in the non-spiked validation samples at elevated concentrations (e.g. >1 μg L^{-1} for ibuprofen, naproxen, paracetamol and tetracycline in influent). Validating the method for such compounds at lower concentrations is not possible using the standard addition (pre-spiking) technique because truly blank matrix samples are hard to find. As such, extrapolation to a S/N level of 3 and 10 is required for the estimation of the MDL and MQL, respectively, and therefore the uncertainty on these values is expected to be high.

The second aspect is related to the precise determination of PE. The concentration of the spiking level must be high enough (see Section 3.1.1) to obtain a PE with precision better than 20% RSD. For example, although the method was reproducible for paracetamol in influent and for carbamazepine in effluent water (precision on peak area <14% and 7% RSD, respectively), very elevated uncertainties were obtained for the determination of the PE (105% and 36% RSD, respectively) due to their high concentration in the non-spiked validation sample, being much higher than the highest spiking level. This uncertainty propagates and results in high uncertainties for the calculated concentration in the sample (e.g. 386 ± 409 for paracetamol in influent and 3 ± 1 μg L^{-1} for carbamazepine in effluent). Considering the observed RSDs, the highest spiking concentration should at least be 3–5 times higher than the concentration in the non-spiked sample – which is, however, not a priori known – to enable a precise (RSD < 20%) PE determination. For example, the PE of venlafaxine in effluent was precisely (98 ± 4%) determined and the highest spiking level (2.5 μg L^{-1}) was 3.6 times higher than the concentration in the non-spiked sample (0.7 ± 0.1 μg L^{-1}; RSD < 15%).

These bottlenecks in method validation have to be taken into account when interpreting data and their uncertainty. When applying the validated method to measure and calculate concentrations in influent and effluent samples (Section 3.2), three sources of uncertainty have to be considered: (i) the HPLC-MS instrumental variability, (ii) the variability during SPE and (iii) the uncertainty on the PE. The HPLC-MS and SPE variability are included and documented by the reproducibility (%RSD on peak area) as presented in Tables S6 and S7. When calculating concentrations from the obtained peak areas by external calibration, the PE values are used as correction factor and therefore, also their uncertainty is important for data interpretation. To account for this, we propose the use of a quality labelling system. Compounds having a RSD < 20% on a PE value ranging between 60% and 140% (SANCO/10684/2009) are labelled as class A, being referred to as 'quantitative compounds'. Other compounds, whose results have larger uncertainty and should be interpreted as 'indicative', are labelled as class B. A total of 37 (6) and 33 (10) of the 43 compounds were labelled as class A (B) for influent and effluent water, respectively.

3.2. Application in 2 WWTPs

3.2.1. Concentrations and potential associated environmental risks

A total of 22 pharmaceuticals, belonging to the anti-inflammatory drugs, antiviral drugs, psychoactive drugs (antidepressants, tranquilizers, anti-epileptics), and antibiotics were detected at least once in the influent or effluent of one of both WWTPs. Measured concentrations and detection/quantification frequencies in

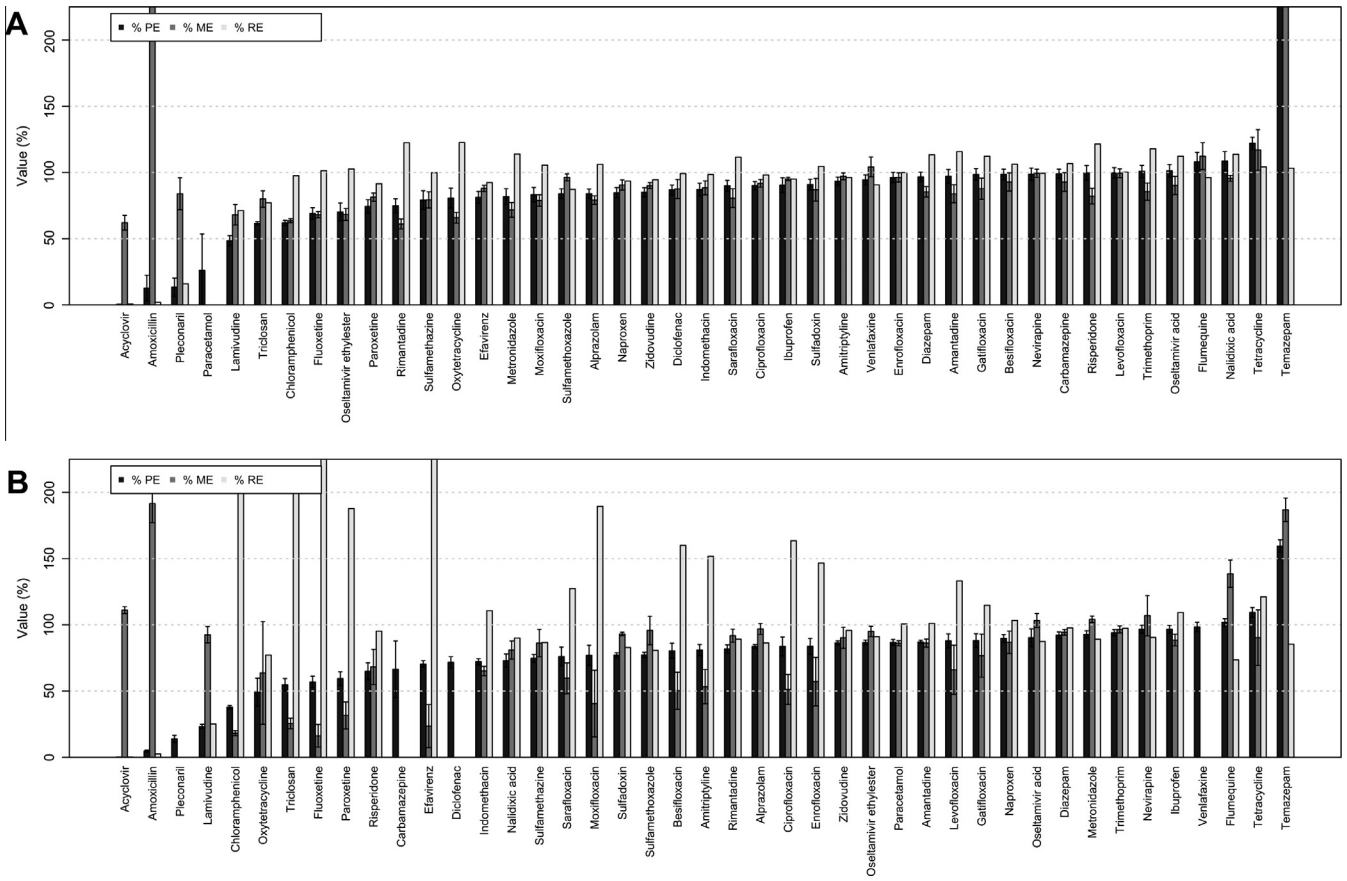

Fig. 1. Decomposition of the process efficiency (PE) in matrix effects (ME) and SPE recovery (RE) for (A) WWTP influent and (B) effluent water. The error bars represent 1 standard deviation. The compounds are ordered by increasing PE.

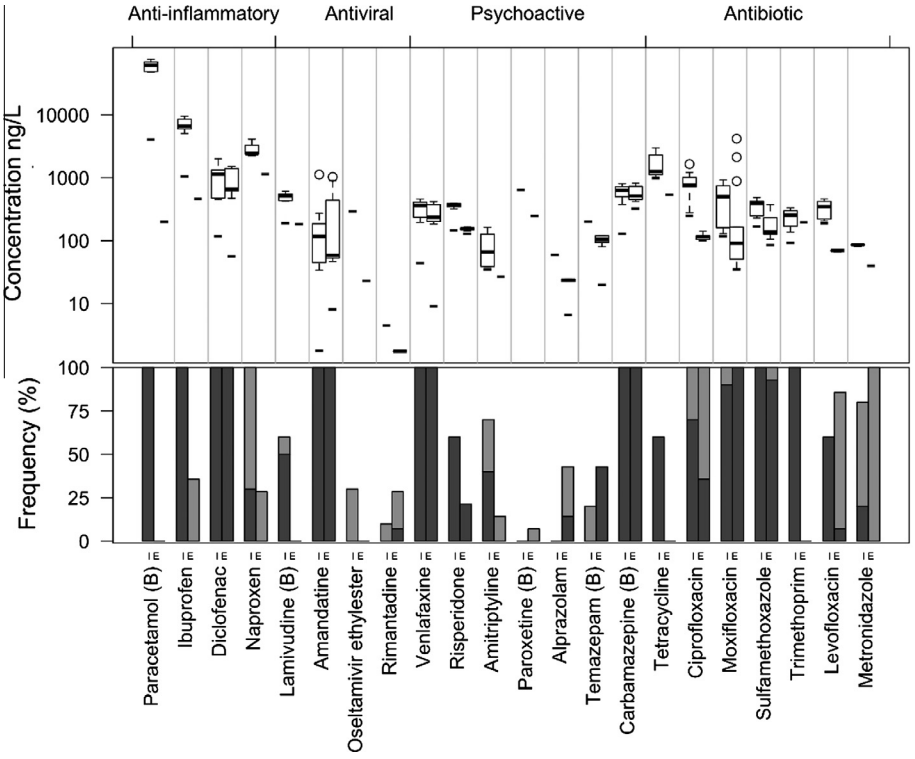

Fig. 2. Boxplots of the measured influent (*I*) and effluent (*E*) concentrations and the detection (grey)/quantification (black) frequencies based on all measured data for both WWTPs. The compounds are ordered by therapeutic class and the MQL values are indicated (–) in the box plots.

Table 1
Average influent and effluent concentrations (ng L^{-1}), mass balance and removal efficiencies for the pharmaceuticals detected in both WWTPs.

Substance	WWTP1							WWTP2		
	Influent CAS + MBR[a] (n = 4)	Effluent CAS[a] (n = 4)	Effluent MBR[a] (n = 4)	Effluent TOT[a] (n = 4)	Mass balance: TOT/(CAS + MBR)[b]	% removal CAS[c]	% removal MBR[c]	Influent[a] (n = 6)	Effluent[a] (n = 6)	% removal[c]
Alprazolam	n.d.	n.d.	n.d.	n.d.				n.d.	23 ± 1(2)	
Amantadine	44 ± 8(4)	54 ± 5(4)	53 ± 4(4)	55 ± 6(4)	1.0 ± 0.1(4)	−22	−20	326 ± 394(6)	592 ± 325(6)	−109
Amitriptyline	b.l.q.	n.d.	b.l.q.	n.d.				83 ± 59(4)	n.d.	>90
Carbamazepine (B)	462 ± 72(4)	460 ± 32(4)	468 ± 42(4)	481 ± 40(4)	1.0 ± 0.1(4)	2	−1	708 ± 69(6)	741 ± 83(6)	−17
Ciprofloxacin	278(1)	120(1)	121 ± 19(3)	107 ± 4(3)	0.8(1)			978 ± 380(6)	104(1)	>97
Diclofenac	507 ± 82(4)	623 ± 59(4)	559 ± 78(4)	542 ± 73(4)	0.9 ± 0.1(4)	−24	−10	1450 ± 366(6)	1391 ± 163(6)	−2
Ibuprofen	5711 ± 513(4)	b.l.q.	n.d.	n.d.		>98	>98	7847 ± 1239(6)	b.l.q.	>98
Lamivudine (B)	n.d.	n.d.	n.d.	n.d.				507 ± 80(5)	n.d.	>89
Levofloxacin	n.d.	b.l.q.	b.l.q.	b.l.q.				335 ± 112(6)	70(1)	>94
Metronidazole	b.l.q.	b.l.q.	b.l.q.	b.l.q.				86 ± 1(2)	b.l.q.	
Moxifloxacin	149 ± 17(3)	62 ± 26(4)	66 ± 29(4)	62 ± 39(4)	1.0 ± 0.4(4)	62	56	688 ± 197(6)	1253 ± 1628(6)	75(day 1–3); −161(day 4–6)
Naproxen	4110(1)	b.l.q.	n.d.	n.d.				2374 ± 76(2)	n.d.	
Oseltamivir ethylester	n.d.	n.d.	n.d.	n.d.				b.l.q.	n.d.	
Paracetamol (B)	67 107 ± 8026(4)	n.d.	n.d.	b.l.q.		>99.9	>99.9	56 172 ± 9430(6)	n.d.	>99.9
Paroxetine (B)	n.d.	b.l.q.	n.d.	b.l.q.				n.d.	n.d.	
Rimantadine	b.l.q.	b.l.q.	2(1)	b.l.q.				n.d.	n.d.	
Risperidone	n.d.	n.d.	n.d.	n.d.				364 ± 28(6)	154 ± 12(3)	64
Sulfamethoxazole	245 ± 15(4)	133 ± 5(3)	124 ± 16(4)	121 ± 2(3)	1.0 ± 0.1(3)	50	49	429 ± 39(6)	250 ± 90(6)	32
Temazepam (B)	n.d.	n.d.	n.d.	n.d.				b.l.q.	104 ± 16(6)	
Tetracycline	n.d.	n.d.	n.d.	n.d.				1658 ± 807(6)	n.d.	>90
Trimethoprim	158 ± 17(4)	n.d.	n.d.	n.d.		>62	>62	228 ± 32(6)	n.d.	>79
Venlafaxine	219 ± 22(4)	208 ± 22(4)	213 ± 21(4)	205 ± 17(4)	1.0 ± 0.1(4)	5	3	403 ± 38(6)	365 ± 58(6)	−3

n.d. = not detected (<MDL); b.l.q. = below limit of quantification (between MDL and MQL).
[a] Concentration (ng L^{-1}) ± standard deviation (number of quantifications).
[b] The mass balance over the effluent loads is calculated for each day, and the average of the TOT/(MBR + CAS) ratios and its standard deviation (number of data points) are given.
[c] Removal efficiencies are only calculated if the quantification frequency for the influent is >50%.

Table 2
PNECs (literature data), quasi-MECs and RQs (mean and maximal values) for the quantified pharmaceuticals discharged from WWTP2 into the river Dender, Belgium.

Substance	Most sensitive taxon (acute/chronic ecotoxicity data)	PNEC (ng L^{-1})	References	Quasi-MEC (ng L^{-1})		RQ		Risk level
				Mean	Max	Mean	Max	
Carbamazepine (B)	Crustacean (chronic)	250	Ferrari et al. (2004)	18	20	7×10^{-2}	8×10^{-2}	Low
Ciprofloxacin	Algae (acute)	5	Grung et al. (2008)	–[d]	3	–[d]	5×10^{-1}	Medium
Diclofenac	Fish (subchronic)	5	Hoeger et al. (2005)	34	37	7	7	High
Levofloxacin	Bacteria (acute)	60	Kümmerer and Henninger (2003)	–[d]	2	–[d]	3×10^{-2}	Low
Moxifloxacin	Algae (acute)	780[a]	Van Doorslaer et al. (2014)	31	102	4×10^{-2}	1×10^{-1}	Medium
Sulfamethoxazole	Algae (chronic)	18	Grung et al. (2008)	6	9	5×10^{-2}	8×10^{-2}	Low
Temazepam (B)	Not reported	4300[b]	Van der Aa et al. (2013)	3	3	6×10^{-4}	7×10^{-4}	Low
Venlafaxine	Mollusc	0.313[c]	Fong and Hoy (2012)	9	10	28	33	High

[a] The concentration resulting in 50% effect (EC_{50}) for the green micro-alga *P. subcapitata*, taking into account an assessment factor of 1000 according to the EMEA guidelines.
[b] PNEC value of oxazepam is used because both are benzodiazepines having similar metabolic pathways.
[c] PNEC replaced by the lowest observed effect concentration (LOEC) for freshwater snails.
[d] One data point available (n = 1).

both matrices are presented as boxplots in Fig. 2. Average concentrations for each WWTP are presented in Table 1. The discussion is mainly based on the 17 detected class A compounds.

Four anti-inflammatory drugs were detected in all influent samples and occurred at the highest concentrations (500 ng L^{-1} to >50 μg L^{-1}) amongst all measured pharmaceuticals. On the other hand, their effluent concentrations were in most of the cases below MDL (except for diclofenac). Four antiviral drugs were detected at least once. Amantadine was quantified in all influent and effluent samples and measured concentrations ranged from 50 ng L^{-1} to 1 μg L^{-1}. Ghosh et al. (2010) reported comparable influent concentrations (200–600 ng L^{-1}) for amantadine in WWTPs in Japan. A total of 3 antidepressants (venlafaxine, risperidone, and amitriptyline) and 2 tranquilizers (alprazolam and temazepam) were found at concentrations varying from 40 to 450 ng L^{-1} in influent and from 20 to 420 ng L^{-1} in effluent. According to the authors' knowledge, no other studies quantified risperidone in WWTPs. Paroxetine was detected in effluent below MQL. Carbamazepine, an anti-epileptic drug, was found at concentrations between 430 and 820 ng L^{-1} in both influent and effluent. Finally, seven antibiotics were measured at concentrations (37–4200 ng L^{-1}) similar as recently reviewed by Verlicchi et al. (2012).

Reported PNEC values and calculated environmental RQs of the compounds quantified in the WWTP2 effluent are presented in Table 2. No ecotoxicological data were found in open literature for alprazolam, amantadine and risperidone. According to the frequently applied risk classification (Hernando et al., 2006), diclofenac and venlafaxine showed 'high' risk (RQ > 1) to the environment, while the fluoroquinolone antibiotics ciprofloxacin and moxifloxacin indicated medium environmental risk

(0.1 < RQ < 1). Comparable 'high' risk was concluded in other studies for diclofenac (Hernando et al., 2006).

3.2.2. Loads and elimination

The measured concentrations (Table 1) and the wastewater flows (Table S2) are multiplied to calculate pharmaceutical loads (g day^{-1}) and removal efficiencies. Comparing both WWTPs and the CAS versus MBR processes in WWTP1 only marginal differences are noticed. Regardless the technology (MBR or CAS), removal efficiencies better than 98% were observed for the most prevalent compounds belonging to the anti-inflammatory drugs (ibuprofen and paracetamol). In WWTP2, removal efficiencies were also >90% for amitriptyline, ciprofloxacin, lamivudine, levofloxacin and tetracycline. On the other hand, compounds such as carbamazepine, diclofenac and venlafaxine were clearly persistent (<5% removal). Moxifloxacin, risperidone and sulfamethoxazole were removed with efficiencies between 32% and 75%. Amantadine had a total effluent load higher than the influent load ('negative' removal) in both WWTPs, which was possibly due to desorption from suspended solids present in the influent ($\log K_{ow}$ 2.44; Ghosh et al., 2010) rather than deconjugation (<3.6% human excretion as acetylamantadine; Bras et al., 1998). Similarly, for moxifloxacin ($\log K_{ow}$ 2.49; Dorival-García et al., 2012) higher effluent concentrations were measured in WWTP2 after rainfall events (day 4–6, see Table S2) resulting in 'negative' removal (−160%). Since moxifloxacin had removal efficiencies of 56–62% (WWTP1) and 75% (day 1–3, WWTP2) during dry weather conditions, desorption from the higher input of suspended solids after rainfall events probably caused the high effluent concentrations.

A mass balance was made over the loads of the combined and individual MBR and CAS effluents of WWTP1 (Table 1). No treatment processes occurred between these sampling points and therefore TOT/(CAS + MBR) is expected to be 1. This verification was possible for 7 compounds and the ratios were in the range of 0.8–1.2 for all the compounds on the different days, except for moxifloxacin (0.6–1.5). These results support the quality and applicability of the analytical method.

4. Conclusions

A novel method using SPE and HPLC coupled to magnetic sector HRMS, which has been applied here for the first time in quantitative water analysis, has been developed for the analysis of 43 pharmaceuticals in WWTP influent and effluent. Apart from prioritized pharmaceuticals, also drugs like antivirals are included, which have been considered only in a very limited number of environmental studies. Method quality assessment and uncertainty analysis showed some scarcely reported bottlenecks in validating a multi-residue method for compounds present at relatively high levels in non-spiked validation samples. True blank samples are hard to find for some pharmaceuticals, and the highest spiking concentration should be at least 3–5 times higher than that in the non-spiked sample for a precise determination of the process efficiency. In order to consider these bottlenecks when interpreting data obtained with the validated method, a quality labelling system is proposed taking into account both the process efficiency and its uncertainty.

The method application revealed – to the author's knowledge – one of the first data on the occurrence, loads and removal efficiencies of 22 pharmaceuticals in a parallel CAS-MBR and a CAS WWTP in Belgium. The presence of scarcely measured antiviral drugs, such as amantadine (50 ng L^{-1} to 1 μg L^{-1}) and lamivudine (400–600 ng L^{-1}), and the antidepressant risperidone (150–400 ng L^{-1}) has been shown, and a first trier environmental risk assessment of the discharged pharmaceuticals indicated that the anti-inflammatory drug diclofenac (450 ng L^{-1} to 1.8 μg L^{-1}) and the antidepressant venlafaxine (180–460 ng L^{-1}) posed a potential 'high' risk to the receiving river Dender, Belgium. No ecotoxicological data were found in open literature for alprazolam, amantadine and risperidone, which established the need for more research in order to better assess the risk of pharmaceutical residues in the environment.

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Appendix A. Supplementary material

Supplementary materials associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.03.069.

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