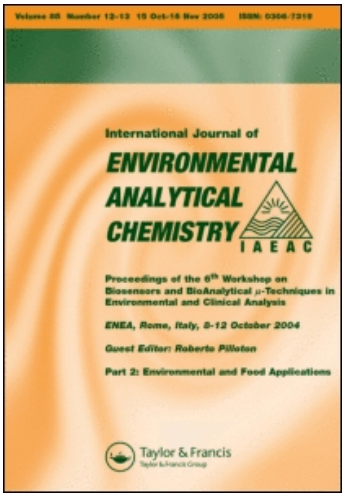

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Use of solid phase extraction and liquid chromatography-tandem mass spectrometry for simultaneous determination of various pharmaceuticals in surface water

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Use of solid phase extraction and liquid chromatography-tandem mass spectrometry for simultaneous determination of various pharmaceuticals in surface water

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This article presents the development, validation and application of a new multi-residue method for simultaneous determination of 36 pharmaceuticals (histamine receptor antagonists, psychoactive stimulant, antiepileptics, antihypertensive, non-steroidal anti-inflammatory, analgesic and antipyretic, lipid regulator, antibiotics, antibacterial, skin care ingredient and metabolites of nicotine and lipid regulators) in surface water using solid phase extraction (Strata-X at pH 5) and liquid chromatography-tandem mass spectrometry (LC–MS/MS). Recoveries were greater than 70% with less than 20% SD for the majority of analytes. The instrumental quantification limit was between 2 and 181 pg, and method quantification limit varied from 0.5 to 98 ng L^{-1} in spiked stream water. The pH and sorbent dependence of matrix effects is discussed. The optimised method was used to determine the occurrence of target analytes in surface water from the coastal Lake Erie in Oregon, northwest Ohio. Seventeen analytes were detected with concentrations up to hundreds of nanogram per litre in stream and lake water samples.

Keywords: PPCPs; solid phase extraction; LC–MS/MS; surface water; multi-residue analysis

1. Introduction

The growing use of pharmaceuticals and personal care products (PPCPs) in households, medicine and animal husbandry has received increasing concern over the past decade [1,2]. After intake by humans or animals, pharmaceutical compounds undergo metabolic processes such as hydroxylation, cleavage (breaking of chemical bonds) or glucuronation. However, high percentages of the original substance are excreted unchanged via urine or faeces and ultimately enter the wastewater stream [3]. Research on the occurrence and fate during the wastewater treatment process indicates that current techniques are not efficient at eliminating certain pharmaceuticals and detectable residues have been found in effluent waters and biosolids [4,5]. As a result, pharmaceuticals can enter the environment via effluent discharge and land application of treated or untreated sludge. A nationwide reconnaissance carried out in United States during 1999–2000 of 95 organic wastewater contaminants indicated that pharmaceuticals are among the most frequently detected organic contaminants in streams [6].

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To study the occurrence and fate of pharmaceuticals in the environment, sophisticated analytical methods are indispensable. Several methods for analysing pharmaceuticals in the aquatic environment have been reported [7–9]. Methods with a solid phase extraction (SPE) followed by high performance liquid chromatography (HPLC) or gas chromatography (GC) separation and mass spectrometry detection are most widely used. The types of mass spectrometry include triple quadrupole (QqQ), ion trap (IT) and less frequently time-of-flight (ToF). Triple quadrupole is the primary choice because of the selectivity and sensitivity, as well as the wide dynamic range [10]. However, early developed methods are either limited in the range of pharmaceutical classes analysed or use additional extraction steps and different LC methods for multiple class analysis. Recently, developing analytical methodology for pharmaceuticals from various therapeutic classes has become a trend [11–13]. The multi-residue methods provide more information than single group analysis, while reducing the time and cost for the analysis, therefore have become not only preferred but required tools [14].

The purpose of this work is to develop a multi-residue method for the simultaneous determination of pharmaceuticals from various classes using solid-phase extraction and liquid chromatography-tandem mass spectrometry (LC–MS/MS). A list of 36 analytes was generated to represent different pharmaceutical classes, physicochemical properties and prevalence in the aquatic environment (Table 1). The performance of three polymeric sorbents was tested. Sorbent and pH dependence of matrix effects was investigated. The optimum method was applied to environment samples for the determination of target analytes.

2. Experimental

2.1 *Chemicals and reagents*

All pharmaceutical standards (purity, 90% ~ 99%) were purchased from Sigma-Aldrich (St. Louis, MO) except clarithromycin (purity, 98%), obtained from Abbott (Chicago, IL). Internal standards $^{13}C_3$-caffeine (purity, 99%), josamycin (purity, 98%), and 2-(3-chlorophenoxy) propionic acid (purity, 99%) were also obtained from Sigma-Aldrich (St Louis, MO) and simatone was obtained from AccuStandard (New Haven, CT). All other chemicals and solvents were ACS or HPLC grade and supplied by Fisher Chemicals (Fair Lawn, NJ). Deionised water (18.3 MΩ) was provided by a Barnstead NANOpure® Infinity Ultrapure Water System (Dubuque, IA).

Individual stock standard solution was made at 50 mg L^{-1} and stored at −20°C. Penicillin G and oxacillin were dissolved in deionised water. Ciprofloxacin, norfloxacin, gatifloxacin and ofloxacin were dissolved in glacial acetic acid. All the other standards were dissolved in methanol. Working standard solution was prepared by mixing and diluting individual stock standards to the desired concentrations with methanol.

2.2 *Sample collection*

Groundwater was collected from a confined aquifer (Swanton, OH) and was used as a clean matrix for method development. Surface waters, including stream water and lake water samples, were collected in 1 L HDPE bottles pre-rinsed with methanol and deionised water. When transported back to the lab, samples were filtered through 0.7 μm TCLP glass fibre filters (Fisher Scientific, Fair Lawn, NJ), stored at 4°C, and extracted within 48 h.

Table 1. Target analytes and their physico-chemical properties.

Therapeutic classes	Compound	CAS number	MW	Log K_{ow}	pK_a
Nicotine metabolite	Cotinine	486-56-6	176.22	−1.74	4.7
Histamine H_2-receptor antagonist	Cimetidine	51481-61-9	252.34	0.4	6.8
	Ranitidine	66357-35-5	314.4	0.27	8.2
Tetracyclines	Doxycycline	564-25-0	444.44	−1.36	3.3, 7.7
	Tetracycline	60-54-8	444.44	−1.19	3.3, 7.7
	Oxytetracycline	79-57-2	460.43	−0.89	3.27, 7.32
Sulphonamides	Sulphamethoxazole	723-46-6	253.28	0.89	1.85, 5.7
	Sulphathiazole	72-14-0	255.32	0.05	2, 7.1
	Sulphamethazine	57-68-1	278.36	0.25	2.65, 7.65
	Sulphadimethoxine	122-11-2	310.33	1.4	2.13, 6.08
Bacteriostatic	Trimethoprim	738-70-5	290.32	0.91	7.12
Psychoactive stimulant	Paraxanthine	611-59-6	180.16	n.d.	8.5
	Caffeine	58-08-2	194.19	0.06	10.4
Antiepileptic	Carbamazepine	298-46-4	236.27	2.47	13.9
Lincosamides	Lincomycin	154-21-2	406.54	n.d.	7.77
	Clindamycin	18323-44-9	424.98	2.01	7.6
Antihypertensive	Diltiazem	42399-41-7	414.52	n.d.	7.7
Macrolides	Erythromycin	114-07-8	733.93	3.06	8.8
	Clarithromycin	81103-11-9	747.95	3.18	8.9
	Roxithromycin	80214-83-1	837.05	2.75	8.8
	Tylosin	1401-69-0	915.5	3.5	7.1
β-lactam	Penicillin G	61-33-6	334.4	1.87	2.79
	Oxacillin	66-79-5	401.44	n.d.	2.8
Antibacterial	Triclosan	3380-34-5	289.54	4.48	7.9
Fluoroquinolones	Norfloxacin	70458-96-7	319.33	−1.03	6.4, 8.7
	Ciprofloxacin	85721-33-1	331.35	0.28	5.9, 8.9
	Ofloxacin	82419-36-1	361.37	−0.39	5.7, 7.9
	Gatifloxacin	112811-59-3	375.39	n.d.	5.5, 9.1
Lipid regulator and lipid regulator metabolite	Gemfibrozil	25812-30-0	250.33	4.77	4.7
	Clofibric acid	882-09-7	214.65	2.57	3.2
Analgesic and antipyretic	Acetaminophen	103-90-2	151.17	0.46	9.38
Non-steroidal anti-inflammatory drugs (NSAIDs)	Ibuprofen	15687-27-1	206.3	3.97	4.91
	Ketoprofen	22071-15-4	254.28	3.12	4.45
	Diclofenac	15307-86-5	296.15	4.51	4.14
	Indomethacin	53-86-1	357.79	4.27	4.5
Skin care product ingredient	Salicylic acid	69-72-7	138.12	2.26	3.5

Note: n.d.: no data.

2.3 *Sample preparation*

Since PPCPs are ubiquitous in surface waters, the following method was optimised using groundwater as a clean resemble matrix, while further method validation was conducted on surface water samples. The sorbents selected for investigation were Oasis® HLB, Strata-X® and Bond Elut Plexa®. Their performance was tested by comparison of recoveries and matrix effects at pH 3, 5 and 7. Recoveries were evaluated by analysing 250 mL groundwater samples spiked with 50 ng standards before and after SPE. Matrix effects were estimated by comparing groundwater samples spiked after sample preparation with standards. The loss of analytes during solvent reduction was also evaluated by comparing groundwater samples spiked after SPE and after sample preparation.

The SPE was accomplished using a Supelco 12-position vacuum manifold (Bellefonte, PA). Prior to extraction, 0.2 g Na_2-EDTA was dissolved in 250 mL sample to prevent tetracyclines and fluoroquinolones from complexing with metal ions and sample pH was adjusted with H_2SO_4 and/or 5% NH_4OH. The cartridges were conditioned with 2×3 mL methanol and 2×3 mL deionised water containing 1% (w/v) Na_2-EDTA. Samples were then loaded onto the sorbent bed at a rate of 10 mL min^{-1}. After loading, cartridges were washed with 2 mL 5% (v/v) methanol and vacuum dried for 2 min. The analytes were then eluted with 2×3 mL methanol. The eluate was collected in a glass conical vial and evaporated to around 200 μL under a gentle nitrogen stream. After spiking with 100 ng internal standards, samples were reconstituted to 1 mL using 50% (v/v) methanol and transferred to 2 mL amber glass vials for LC–MS/MS analysis.

2.4 *LC–MS/MS analysis*

The LC–MS/MS system consists of a ProStar® 210 solvent delivery module with a ProStar 430 autosampler and a 1200 L triple-stage quadrupole mass spectrometer with a dual off-axis ESI interface (Varian Inc., Walnut Creek, CA). Analytes were separated using a Supelco Discovery® HS C18 column ($150 \times 4.6\,mm^2$, 3 μm). Mobile phase A was 0.1% (v/v) formic acid (pH = 2.7), phase B was 100% acetonitrile and the total flow rate was 0.2 mL min^{-1}. The injection volume was 20 μL. The gradient started with 5% B, held for 2 min, increased to 25% in 2 min, ramped to 100% in 24 min, held for 16 min, then returned to the initial condition in 2 min, and held for 10 min for equilibration of column and pumps.

The electrospray settings (nebuliser needle voltage, spray shield voltage, drying gas temperature, ion-transfer capillary voltage, etc.) and parameters of precursor and transition ions were optimised by infusing 5 mg L^{-1} individual standard solutions into the ESI source at a flow rate of 20 μL min^{-1} using a mechanical syringe pump (Harvard, Cambridge, MA). Precursor ions, ion-transfer capillary voltage, two most abundant transition ions, collision energy and retention time of the analytes are provided in Table 2. The instrument was operated in positive and negative voltage switching mode. Nebuliser needle and shield voltages were set at 3800 and 275 V for positive ion mode and −4500 and −600 V for negative mode, respectively. Drying gas (air) temperature was 300°C. ESI housing temperature was 50°C, the collision gas (argon) pressure was 2.0 mTorr and the dwell time was 50 ms.

2.5 *Quantification and method validation*

Instrument control, peak detection and integration were carried out using Varian MS Workstation (Version 6.8). Data acquisition was performed under multiple reaction monitoring (MRM) mode. Identification of target analyte was based on the presence of two MRM transitions and match of retention time with the reference standard. The ratio of two MRM transitions was used for the confirmation. The most intense MRM transition was selected for the quantification of each analyte. The standard addition method was used for quantification to compensate for matrix effects. Briefly, each reconstituted sample was separated into three equal aliquots (300 μL). The first vial (aliquot 1) was amended with 10 μL methanol, the second vial (aliquot 2) was amended with 10 μL 200 μg L^{-1} standard solution of each analyte, and the third vial (aliquot 3) was amended with

Table 2. Parameters for the determination of target analytes.

Compound	R_t (min)	CV[a]	Precursor ion (m/z)	MRM1 (CE[b])	MRM2 (CE)	MRM ratio
Cotinine	11.5	55	177.1	80.0 (−13.5)	98.0 (−11)	3.7
Cimetidine	14.5	43	253.1	159.0 (−12)	117.0 (−13.5)	1.2
Ranitidine	14.6	40	315.1	176.0 (−14)	130.0 (−23)	1.7
Lincomycin	14.7	32	407.1	126.0 (−20.5)	359.1 (−15.5)	14.2
Paraxanthine	14.8	35	181.2	124.0 (−13)	96.0 (−17.5)	11.6
Norfloxacin	14.8	46	320.1	302.0 (−13.5)	230.9 (−36)	4.0
Ofloxacin	14.9	50	362.1	318.0 (−9.5)	344.0 (−13)	6.8
Trimethoprim	15.0	60	291.1	123.0 (−14.5)	230.0 (−14.5)	1.3
Ciprofloxacin	15.1	40	332.1	314.0 (−13)	230.9 (−34.5)	1.2
Oxytetracycline	15.2	40	461.2	426.0 (−13.5)	443.1 (−6)	3.2
Acetaminophen	15.3	40	152.2	110.0 (−9.5)	93.0 (−17)	3.5
Tetracycline	15.4	45	445.1	410.0 (−12)	153.9 (−22)	2.4
Caffeine	15.5	47	195.2	138.0 (−10)	110.0 (−14)	3.0
Penicillin G	15.6	30	335.1	127.9 (−22)	160.0 (−14.5)	2.7
Gatifloxacin	15.7	50	376.1	260.9 (−26.5)	358.0 (−12.5)	2.9
Clindamycin	16.5	45.8	425.2	126.0 (−19.5)		
Sulphathiazole	16.6	33	256.1	156.0 (−9.5)	92.0 (−19)	1.8
Doxycycline	17.2	45	445.1	428.0 (−10.5)	320.8 (−24)	10.8
Tylosin	18.2	80	916.4	174.0 (−17)	156.0 (−21.5)	10.4
Sulphamethazine	18.6	40	279.1	186.0 (−12)	124.0 (−17.5)	1.7
Diltiazem	18.8	42.3	415.1	178.0 (−18.5)	149.9 (−34)	3.2
Erythromycin	19.1	50	716.4	158.0 (−19)	558.0 (−9)	9.0
Clarithromycin	19.7	41	748.5	158.0 (−19.5)	590.3 (−9)	6.2
Roxithromycin	19.9	50	837.6	158.0 (−20.5)	679.3 (−8)	3.3
Sulphamethoxazole	21.2	34	254.1	156.0 (−10.5)	92.0 (−19)	1.1
Sulphadimethoxine	22.5	50	311.1	156.0 (−14)	108.0 (−21.5)	2.3
Salicylic acid	23.2	−31	136.8	92.8 (15)		
Carbamazepine	23.5	41	237.1	194.0 (−16)	192.0 (−20.5)	4.8
Oxacillin	25.6	40	402.1	143.9 (−19.5)	186.0 (−13.5)	3.5
Ketoprofen	27.5	45	255.1	209.0 (−9)	105.0 (−17)	1.3
Clofibric acid	28.4	−30	213.1	126.8 (12.5)	85.0 (9.5)	4.0
Indomethacin	31.7	35	358.0	139.0 (−13.5)	174.0 (−8)	4.9
Diclofenac	32.0	−36	293.8	249.8 (10)		
Ibuprofen	33.2	−25	204.9	161.0 (7)	159.0 (5.5)	4.9
Gemfibrozil	35.7	−30	249.2	121.0 (12.5)		
Triclosan	36.8	−25	286.8/289.0	35.1 (7)		2.8
Internal standard						
Simatone	16.3	55	198.1	113.9 (−9)		
$^{13}C_3$-Caffeine	15.5	45	198.1	140.0 (−9)		
Josamycin	20.7	84	828.5	174.0 (−13.5)		
2-(3-chlorophenoxy) propionic acid	26.6	−40	198.9	126.6 (11)		

Notes: [a]Capillary voltage; [b]Collision gas energy.

10 μL 1000 μg L^{-1} standard solution. Samples were then analysed consecutively. The concentration of target analyte was calculated using following equation:

$$X = \frac{R_X \times m}{(R_{A+X} - R_X) \times V},$$

where X is the concentration ($ng\,L^{-1}$) of analyte in the sample, m is the mass of added standard for each analyte in each vial (2 or 10 ng in this situation), V is the volume (0.25 L) of extracted sample and R_X and R_{A+X} are the ratio of the area of analyte signal to that of the internal standard signal for methanol amended sample and standard amended sample, respectively. The mass of added standard used for the calculation was determined by the relative signal intensity of target analyte between aliquot 1 and aliquots 2 and 3. Usually, the one with signal intensity closer to that of aliquot 1 was used.

After optimised using groundwater, the method was validated using surface water. Linearity and dynamic range of the method were evaluated using stream water samples spiked at seven different concentrations ranging from 10 to 2000 $ng\,L^{-1}$. Instrumental detection limits (IDL) and instrumental quantification limits (IQL) were set as the minimum amount of analyte in standard solution with a signal-to-noise ratio of 3 and 10, respectively. Signal-to-noise ratio was calculated using the software and was extrapolated to the previously defined signal-to-noise ratio. The method detection limits (MDL) and method quantification limits (MQL) were evaluated by analysing extracted stream water samples spiked at known concentrations (10 or 100 $ng\,L^{-1}$) and were calculated similarly to the previous definition. The within- and between-day variation of methods were evaluated by calculating the relative standard deviation (RSD%) of three replicates of spiked stream water at 200 $ng\,L^{-1}$, within the range of concentrations commonly detected in surface waters, analysed within a day and on different days.

3. Result and discussion

3.1 *Solid phase extraction*

One of the greatest difficulties in developing a multi-residue analysis method is finding the appropriate sorbent and extraction conditions that produce an acceptable recovery for most analytes. This necessitates compromise at times. In this study three potential polymeric sorbents were tested, including the widely used Oasis HLB, a copolymer of divinylbenzene and vinylpyrrolidone; Strata-X, a polydivinylbenzene resin chemically modified with piperidone groups; and Bond Elut Plexa, a polymeric resin with hydroxylated exterior and pore structure. Methods of pharmaceutical analysis in wastewater and surface water using Oasis HLB and Strata-X have been reported previously [15–17], and indicate that both these sorbents provide good recoveries for pharmaceuticals of diverse classes. Bond Elut Plexa is only commercially available recently and no application of this sorbent for extracting pharmaceuticals from environmental water samples has been reported. It is advertised as a new generation of polymeric SPE product, which has a unique polymeric architecture to improve performance and reduce matrix [18].

Recoveries of three sorbents at tested conditions are summarised in Table 3. The Oasis HLB at pH 3 and 5 and Strata-X at pH 5 yielded over 70% recoveries for most of the analytes. Even at neutral condition, nearly 90% analytes had a recovery better than 50% using these two sorbents. Generally, pH controls the ionisation of polar compounds and thus affects its hydrophobicity and interaction with the sorbent. For traditional reverse phase sorbents, retention of a compound tends to increase at the pH condition favouring the unionised form. Few analytes (e.g. Cotinine, Cimetidine, Caffeine and Penicillin G) show noticeable pH dependence, indicating that Oasis HLB and Strata-X have multiple retention mechanisms other than hydrophobic interaction. This can be attributed to the functional groups combined to the polymeric skeleton, which have hydrophilic,

Table 3. Recoveries (mean and SD of three replicates) of analytes in spiked groundwater.

	Oasis HLB			Strata-X			Bond Elut Plexa		
Compound	pH 3	pH 5	pH 7	pH 3	pH 5	pH 7	pH 3	pH 5	pH 7
Cotinine	67 (6)	125 (4)	87 (6)	5 (3)	63 (5)	93 (5)	1 (1)	17 (2)	33 (4)
Cimetidine	71 (15)	82 (1)	129 (9)	0	61 (17)	74 (16)	0	0	23 (8)
Ranitidine	76 (8)	52 (9)	62 (9)	60 (6)	50 (6)	55 (3)	1 (1)	4 (1)	46 (17)
Trimethoprim	117 (7)	90 (5)	78 (8)	105 (4)	108 (9)	87 (1)	73 (5)	101 (5)	104 (13)
Doxycycline	90 (3)	81 (7)	92 (4)	83 (7)	100 (3)	99 (7)	84 (13)	81 (5)	74 (14)
Tetracycline	105 (6)	126 (11)	121 (20)	94 (13)	84 (5)	95 (9)	98 (18)	96 (14)	89 (5)
Oxytetracycline	124 (6)	131 (4)	144 (7)	100 (9)	100 (9)	134 (12)	102 (11)	92 (20)	68 (1)
Sulphamethoxazole	122 (2)	77 (3)	96 (3)	80 (14)	76 (6)	99 (4)	123 (9)	164 (11)	112 (15)
Sulphathiazole	78 (1)	68 (5)	65 (3)	117 (4)	124 (0)	89 (6)	99 (6)	101 (10)	95 (10)
Sulphamethazine	81 (10)	68 (2)	74 (2)	88 (6)	85 (8)	90 (3)	85 (4)	81 (14)	89 (8)
Sulphadimethoxine	102 (3)	90 (7)	99 (4)	90 (13)	95 (1)	103 (3)	77 (5)	107 (13)	118 (11)
Paraxanthine	67 (8)	58 (7)	91 (9)	61 (10)	67 (11)	92 (13)	57 (5)	48 (5)	53 (6)
Caffeine	59 (2)	78 (8)	100 (3)	76 (11)	76 (6)	83 (11)	87 (8)	81 (15)	87 (15)
Carbamazepine	68 (1)	72 (6)	75 (2)	75 (8)	67 (4)	62 (4)	48 (0)	56 (6)	41 (2)
Lincomycin	56 (2)	55 (3)	64 (3)	49 (7)	69 (2)	70 (3)	0	6 (2)	75 (7)
Clindamycin	84 (4)	81 (7)	76 (1)	84 (12)	74 (3)	71 (7)	44 (2)	71 (4)	51 (2)
Diltiazem	75 (2)	74 (4)	62 (1)	79 (8)	67 (3)	51 (4)	46 (2)	51 (5)	37 (4)
Erythromycin	71 (9)	49 (6)	67 (4)	61 (5)	61 (8)	50 (4)	55 (13)	81 (12)	57 (8)
Clarithromycin	121 (7)	84 (24)	46 (7)	106 (27)	84 (28)	90 (10)	49 (5)	52 (5)	53 (4)
Roxithromycin	100 (2)	91 (4)	50 (1)	101 (15)	69 (7)	43 (5)	78 (11)	76 (8)	78 (9)
Tylosin	46 (4)	56 (10)	47 (21)	66 (11)	100 (19)	60 (16)	64 (21)	99 (13)	103 (2)
Acetaminophen	113 (9)	125 (11)	96 (2)	78 (8)	53 (7)	64 (4)	0	0	0
Triclosan	89 (8)	82 (20)	76 (8)	109 (20)	114 (11)	109 (15)	77 (11)	101 (13)	71 (6)
Penicillin G	63 (2)	62 (3)	23 (3)	78 (6)	50 (3)	22 (1)	10 (3)	1 (2)	0
Oxacillin	54 (4)	54 (2)	49 (5)	96 (10)	101 (6)	65 (5)	55 (1)	68 (6)	71 (13)
Norfloxacin	109 (6)	132 (7)	66 (8)	59 (6)	101 (4)	57 (10)	103 (7)	106 (9)	101 (19)
Ciprofloxacin	102 (6)	120 (6)	70 (5)	109 (10)	83 (13)	38 (6)	53 (4)	43 (9)	28 (4)
Ofloxacin	64 (7)	64 (2)	65 (4)	108 (9)	81 (10)	35 (2)	24 (29)	26 (20)	24 (31)
Gatifloxacin	94 (1)	72 (2)	71 (3)	109 (2)	92 (19)	75 (3)	94 (6)	76 (17)	86 (10)
Gemfibrozil	99 (5)	104 (4)	123 (4)	109 (9)	102 (7)	118 (12)	103 (7)	106 (9)	93 (6)
Clofibric acid	94 (3)	95 (2)	101 (3)	120 (4)	116 (4)	78 (7)	102 (5)	109 (12)	54 (7)
Ibuprofen	114 (2)	93 (15)	118 (12)	107 (10)	78 (11)	79 (6)	109 (12)	74 (7)	103 (50)
Ketoprofen	110 (6)	90 (4)	100 (14)	119 (5)	124 (1)	67 (6)	91 (3)	97 (3)	86 (10)
Diclofenac	99 (5)	89 (2)	100 (7)	133 (12)	124 (10)	81 (4)	115 (14)	130 (14)	107 (7)
Indomethacin	61 (3)	74 (5)	77 (7)	138 (21)	158 (20)	142 (19)	103 (24)	138 (14)	127 (15)
Salicylic acid	95 (2)	96 (1)	63 (5)	125 (5)	117 (1)	97 (3)	103 (3)	62 (7)	20 (1)

hydrophobic and π–π retention mechanisms. The Bond Elut Plexa also provided good recoveries for the majority of the analytes. However, recoveries for more analytes were affected by the extraction pH for Plexa, while recoveries for cimetidine, acetaminophen, penicillin G and ofloxacin were lower than 30% at all tested conditions. Recovery results indicate that both Oasis HLB and Strata-X can be used to extract selected analytes simultaneously.

3.2 *Matrix effects*

Matrix effects have been identified as one of the main drawbacks of ESI–MS. Interference caused by the presence of co-extracted constituents lead to a difference of response between

a standard solution and a sample extract. The matrix found in environmental samples can be highly variable and is attributed mainly to organic and/or inorganic matter co-eluting with the analytes during extraction. Analytes may adsorb to dissolved organic matter in the samples, which in turn decreases the concentration of freely dissolved analytes [19]. The chromatogram baseline could be raised so that the peak of the analyte is masked or elevated. The matrix can also compete for droplet surface with the analyte ions during gas phase emission [20]. As a result, analyte signal might be suppressed, or less frequently enhanced, reducing the sensitivities and causing quantification complexities. Several approaches have been used to reduce or compensate for matrix effects, such as post column introduction of internal standard [21], using decreased flow rates or post column splitting [22], diluting samples [19] or isotope dilution [23]. However, every method has benefits and drawbacks. In the literature, no common agreement exists on what methods should be used.

In this work, the relationship between matrix effects and extraction pH and sorbent type was investigated. The matrix effect (ME%) is calculated as $ME\% = 100 \times (B/A)$ following the procedure suggested by Matuszewski *et al*. [24], where A is the response of analyte in standard and B is the response of sample spiked after sample preparation. ME% is equal to 100 when there is no matrix effect, a value larger than 100 indicates signal enhancement and a value less than 100 indicates signal suppression. The matrix effect results for each analyte, pH and sorbent combination are presented in Table 4.

Both signal suppression and signal enhancement were observed. Matrix effects varied by compounds and were also related to the sorbents and pH conditions used for sample extraction. Frequency distributions showing overall matrix effects are presented in Figure 1. For all sorbents elevated signal interferences were observed at pH 3 compared to pH 5 and 7. This can be attributed to the factor that less humic and fulvic acids are co-extracted at neutral condition than acidic condition [25].

Matrix effect (Table 4) for each compound was assessed to further understand pH dependence. Four different trends were observed: (1) Matrix effects reduced as pH increased from 3 to 7 (either less signal enhancement or suppression); (2) Matrix effects did not change with pH; (3) Matrix effects changed from signal enhancement to signal suppression as pH decreased and (4) Matrix effects decreased when pH increased from 3 to 5 and then increased when pH was raised to 7. The differing responses are related to their physicochemical properties, elution time, interaction with matrix and how the matrix composition changes with pH. Although matrix effects are relevant to dissolved organic matter more than inorganic matter, both the quantity and quality of the organic matter influence the matrix effects [20]. Since humic and fulvic acids are not the only organic matter in the water samples, as pH changes, the constituents of co-extracted organic matter may also change. The change of co-extracted organic matter will have a different affect on observed matrix effects for each individual analyte.

Matrix effects obtained using different sorbents were also evaluated. Overall, samples extracted using Bond Elut Plexa suffered less, probably because the featured polymer architecture helps reduce the coeluted matrix. The multi-retention mechanisms of HLB sorbent introduced an increased amount of co-extracted matrix compared to the other sorbents, also observed by other researchers [26]. The Strata-X performed similarly to HLB at pH 3, whereas at pH 5 and 7 Strata-X gave relatively low matrix effects for more analytes. As a result, the Strata-X at pH 5 was selected as the optimum method based on maximum recoveries and minimal matrix effects for the majority of the target analytes. The standard addition method was chosen for quantification since four internal standards could not well compensate the matrix effects of all the analytes.

Table 4. Matrix effects (mean and SD of three replicates) in groundwater samples.

	Oasis HLB			Strata-X			Bond Elut Plexa		
Compound	pH 3	pH 5	pH 7	pH 3	pH 5	pH 7	pH 3	pH 5	pH 7
Cotinine	82 (5)	80 (4)	85 (1)	87 (5)	90 (4)	77 (4)	88 (7)	92 (2)	80 (6)
Cimetidine	43 (2)	64 (5)	58 (5)	49 (3)	66 (4)	55 (8)	64 (3)	66 (6)	59 (3)
Ranitidine	55 (3)	55 (8)	51 (0)	52 (2)	63 (2)	54 (3)	71 (3)	72 (6)	66 (3)
Trimethoprim	59 (1)	70 (4)	74 (7)	58 (1)	67 (2)	67 (2)	83 (5)	85 (5)	81 (2)
Doxycycline	76 (8)	63 (8)	64 (13)	72 (4)	90 (5)	99 (7)	93 (9)	104 (13)	102 (1)
Tetracycline	61 (11)	62 (17)	64 (14)	62 (1)	95 (1)	84 (11)	85 (4)	101 (11)	86 (5)
Oxytetracycline	73 (7)	65 (12)	68 (6)	69 (5)	85 (2)	88 (7)	91 (5)	90 (12)	88 (2)
Sulphamethoxazole	65 (2)	77 (5)	75 (2)	69 (4)	83 (3)	70 (4)	69 (2)	72 (2)	77 (3)
Sulphathiazole	65 (2)	70 (3)	76 (1)	66 (1)	74 (3)	71 (4)	70 (5)	71 (5)	72 (5)
Sulphamethazine	73 (4)	74 (3)	76 (3)	73 (2)	80 (4)	73 (2)	77 (2)	74 (5)	74 (3)
Sulphadimethoxine	86 (4)	92 (7)	86 (1)	87 (3)	102 (7)	82 (2)	90 (4)	93 (4)	77 (6)
Paraxanthine	62 (4)	88 (8)	84 (10)	59 (2)	77 (1)	82 (4)	84 (7)	94 (8)	86 (4)
Caffeine	82 (8)	91 (4)	89 (12)	74 (4)	92 (9)	87 (8)	85 (4)	93 (6)	92 (8)
Carbamazepine	69 (3)	70 (2)	73 (6)	68 (2)	70 (2)	68 (1)	71 (3)	69 (3)	69 (4)
Lincomycin	91 (2)	90 (7)	84 (1)	82 (2)	84 (4)	81 (4)	105 (4)	95 (2)	91 (4)
Clindamycin	113 (1)	103 (3)	90 (3)	108 (1)	100 (1)	91 (4)	114 (7)	95 (2)	89 (3)
Diltiazem	113 (2)	96 (2)	89 (3)	108 (3)	92 (3)	85 (2)	104 (3)	91 (1)	90 (3)
Erythromycin-H_2O	117 (2)	114 (11)	97 (14)	107 (10)	95 (4)	97 (10)	135 (4)	115 (3)	106 (8)
Clarithromycin	115 (2)	112 (10)	85 (5)	100 (8)	91 (4)	84 (4)	113 (5)	99 (6)	92 (5)
Roxithromycin	129 (5)	123 (2)	90 (4)	113 (3)	107 (11)	88 (2)	122 (5)	97 (9)	95 (11)
Tylosin	125 (15)	125 (10)	93 (9)	133 (12)	109 (8)	88 (7)	122 (6)	104 (15)	93 (7)
Acetaminophen	51 (1)	62 (2)	75 (4)	51 (7)	53 (2)	68 (3)	62 (5)	77 (2)	81 (2)
Triclosan	73 (2)	92 (11)	73 (16)	97 (10)	84 (11)	89 (12)	87 (7)	99 (16)	80 (9)
Penicillin G	150 (10)	133 (5)	111 (3)	142 (4)	133 (6)	104 (5)	143 (4)	106 (2)	95 (6)
Oxacillin	83 (7)	86 (13)	69 (19)	71 (19)	76 (8)	64 (12)	76 (15)	69 (10)	82 (5)
Norfloxacin	165 (15)	135 (7)	123 (5)	131 (6)	125 (11)	113 (20)	131 (20)	152 (17)	168 (11)
Ciprofloxacin	157 (9)	107 (10)	107 (11)	107 (11)	105 (2)	90 (10)	135 (11)	146 (7)	154 (10)
Ofloxacin	117 (9)	118 (10)	103 (10)	93 (15)	127 (13)	112 (14)	126 (21)	151 (20)	151 (27)
Gatifloxacin	91 (4)	103 (5)	97 (18)	85 (4)	95 (6)	99 (11)	113 (6)	130 (7)	138 (7)
Gemfibrozil	55 (1)	60 (3)	57 (2)	52 (1)	56 (2)	56 (2)	53 (1)	56 (3)	55 (3)
Clofibric acid	42 (1)	44 (1)	49 (3)	40 (1)	43 (2)	46 (3)	43 (1)	45 (2)	53 (1)
Ibuprofen	66 (7)	65 (8)	51 (3)	60 (16)	62 (10)	54 (6)	43 (7)	47 (2)	50 (6)
Ketoprofen	49 (6)	56 (4)	64 (4)	53 (5)	58 (5)	58 (1)	50 (2)	46 (2)	61 (4)
Diclofenac	101 (4)	107 (3)	110 (10)	98 (3)	103 (3)	104 (3)	102 (8)	105 (3)	107 (3)
Indomethacin	53 (1)	59 (5)	57 (0)	56 (2)	57 (5)	55 (3)	59 (1)	57 (3)	55 (3)
Salicylic acid	195 (7)	203 (3)	68 (5)	201 (5)	154 (6)	60 (2)	213 (2)	74 (5)	71 (1)
Internal standard									
Simatone	74 (4)	84 (5)	84 (4)	72 (4)	76 (5)	86 (6)	87 (4)	95 (5)	92 (3)
$^{13}C_3$-Caffeine	76 (1)	86 (7)	83 (12)	75 (1)	83 (8)	87 (5)	83 (10)	89 (4)	88 (6)
Josamycin	116 (6)	124 (6)	97 (6)	122 (3)	109 (4)	98 (5)	121 (7)	114 (3)	96 (3)
2-(3-chlorophenoxy) propionic acid	23 (2)	25 (1)	28 (2)	22 (1)	24 (1)	26 (2)	23 (2)	24 (1)	35 (3)

3.3 *Solvent reduction and filtration*

Solvent reduction is another step that may lead to increased loss of analytes during sample preparation. Recoveries from solvent reduction using the optimised method indicated that losses of analytes were negligible for most analytes. Only ranitidine (69%) and norfloxacin

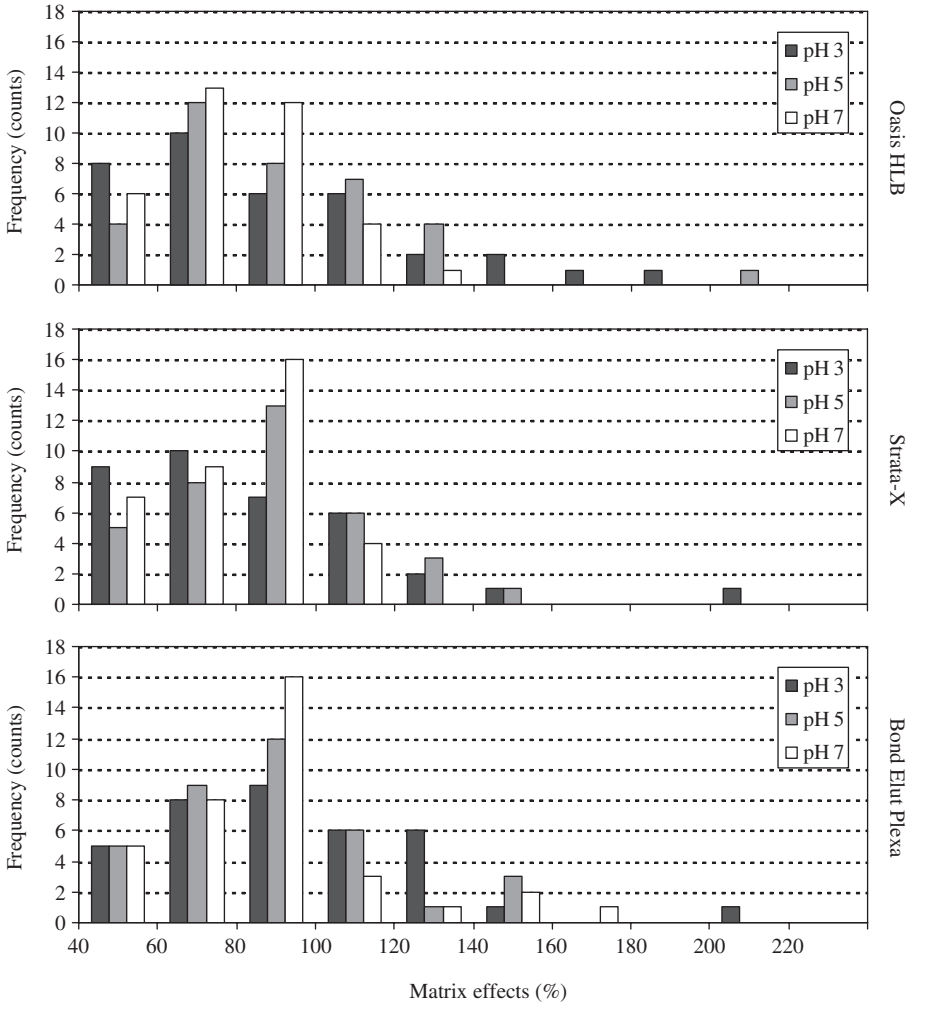

Figure 1. Frequency distributions of matrix effects from spiked groundwater.

(79%) had loss >20%, probably due to sorption of analytes onto the glass vial or degradation during solvent reduction.

For some dirty samples, precipitation may occur after solvent reduction. Sometimes, filtration using a syringe filter is needed to avoid clogging of the LC column. Loss of analyte during filtration was tested by comparing the response of analytes in standards with that in standards filtered with 0.45 μm PTFE and nylon filters. Filtration recoveries using polytetrafluoroethylene (PTFE) filters were better than 80% for most of analytes, except doxycycline (64%) and ofloxacin (68%). However, filtration using a nylon filter caused serious loss for more analytes. Recoveries were less than 30% for tetracyclines and around 50% for fluoroquinolones. Sorption to the filter is the main reason for the loss of analytes during filtration. As no precipitation was observed in our samples, no filtration was used to avoid further loss of analytes.

3.4 *LC–MS/MS analysis*

For chromatographic separation, several columns, mobile phases (H_2O, methanol and acetonitrile) and additives (formic acid, ammonium acetate and ammonia formate) were tested. The best chromatography for the analytes was obtained by using a Supelco Discovery HS C18 column with 0.1% (v/v) formic acid and acetonitrile as a binary mobile phase. Although analytes like sulphonamides, NSAIDs and triclosan are more sensitive when using ammonium acetate as additives, formic acid was used instead to simplify the LC methodology. Chromatograms of extracted stream water samples spiked at 100 ng L^{-1} with mixed standard solution are presented in Figure 2. Tetracyclines exhibited multiple epimers, which have been observed in previous research [27]. As those minor epimers could not be well identified and their abundances were negligible, only the major epimer was used for quantification. Erythromycin-H_2O, which is the main degradation product of erythromycin at acidic condition [3], eluted as two peaks. A previous NMR spectrum has shown that erythromycin consists of two slowly interconverting isomers [28]. The minor peak observed here is likely an isomer. Two peaks were also found for tylosin and roxithromycin and could represent the (Z, Z)-isomer of tylosin [29], this isomerisation has also been observed at a low pH condition for roxithromycin [30]. These isomers have the same molecular weight but differ in structure. Thus, isomers can be separated by the LC column and form double peaks with the same MRM. For erythromycin-H2O, clarithromycin and roxithromycin, the sum of isomers was used for quantification.

The protonated molecular ion ($[M+H]^+$) or the deprotonated molecular ion ($[M-H]^-$) was chosen as a precursor ion for all analytes, with the exception of erythromycin for which the protonated molecular ion of erythromycin-H_2O was used. Most transitions agreed well with current literature, with the exception of ketoprofen and indomethacin. Other research has analysed these compounds in ESI negative mode [31], while in this work we found they were more sensitive in ESI positive mode. Their most abundant product ions corresponded to $[M+H-CH_2O_2]^+$ (m/z 209), and $[M+H-C_{12}H_{12}NO_3]^+$ (m/z 139), respectively. Two precursor ions were used for triclosan, which can be attributed to the Cl isotopes in the triclosan molecular ($C_{12}H_7Cl_3O_2$).

3.5 *Quantification and method validation*

Target analytes were identified based on two MRM transitions and a comparison of retention time with reference standard. After the presence of target analytes was confirmed, the most abundant ion was used for quantification to ensure better sensitivity. Due to the poor fragmentation, clindamycin, salicylic acid, diclofenac and gemfibrozil were analysed using one transition. Although the probability of false positive detection might increase, using one MRM transition for compounds with only one dominate breakdown ion is also reported in the literatures [15,32]. To compensate for the matrix effect, the standard addition method was used for quantification. Although standard addition method is time-consuming and laborious, it aids in overcoming the variation of matrix effects found among different environmental matrices and ensures precise results [20]. Standards were added after extraction to compensate for the matrix effects but not the recoveries of sample preparation, which have been evaluated during the method development. Although adding standards before extraction can help correct the loss of analyte during the sample preparation, the sample and the standard added sample need to be prepared separately, which will increase the amount of time and expense for

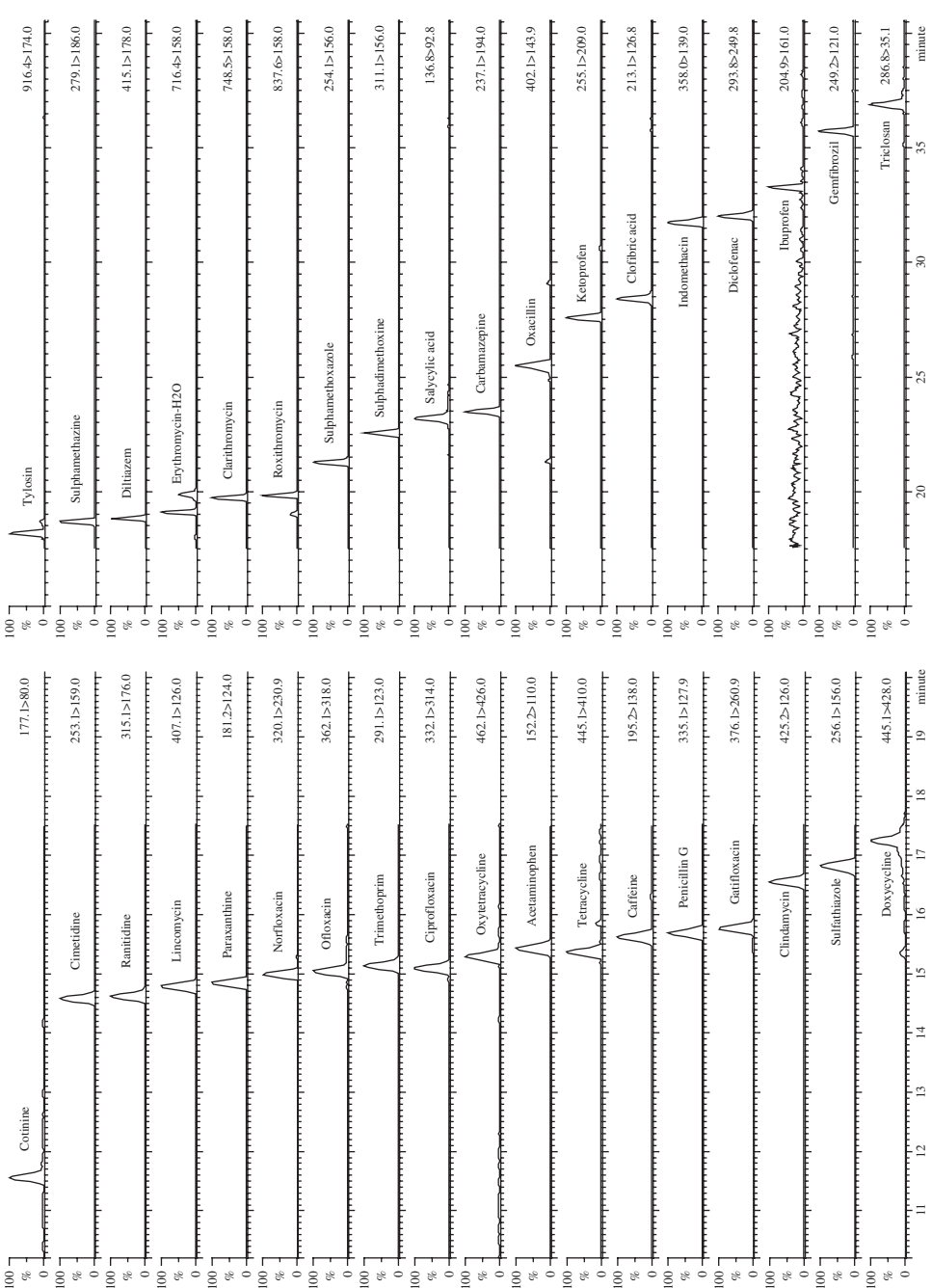

Figure 2. MRM Chromatograms of extracted stream water sample spiked at 100 ng L^{-1}.

the analysis. Internal standards, which were not found in surface waters samples, were also added in order to compensate for the systematical variation.

Method validation data are presented in Table 5. Linearity and dynamic range of the method were determined by analysing stream water samples spiked at concentrations with low values of 10, 50 or 100 ng L^{-1}, analyte dependent, and a high value of 2000 ng L^{-1}. These cover ranges typically reported in surface waters. The dynamic range for all analytes ranged up to 2000 ng L^{-1} and the coefficients of determination (r^2) calculated for the

Table 5. Linearity, dynamic range, recoveries, variability, instrumental and MQLs in surface water samples.

Compound	Linearity (r^2)	Dynamic range (ng L^{-1})	Recovery ± SD (%)[a]	Within-day variation (RSD%)	Between-day variation (RSD%)	IQL (pg)	MQL (ng L^{-1})
Cotinine	0.9915	10–2000	89 ± 7	4	17	40	9.8
Cimetidine	0.9924	50–2000	78 ± 13	2	15	112	44
Ranitidine	0.9913	50–2000	54 ± 3	5	8	50	31
Trimethoprim	0.9988	10–2000	85 ± 8	7	12	27	9.4
Doxycycline	0.9925	50–2000	79 ± 14	13	18	96	27
Tetracycline	0.9912	50–2000	79 ± 8	20	22	68	18
Oxytetracycline	0.9909	50–2000	109 ± 11	21	17	73	16
Sulphamethoxazole	0.9993	10–2000	88 ± 4	8	7	16	4.4
Sulphathiazole	0.9985	10–2000	103 ± 6	3	19	18	4.6
Sulphamethazine	0.9989	10–2000	74 ± 3	2	5	21	7.2
Sulphadimethoxine	0.9990	10–2000	82 ± 5	9	9	10	3.1
Paraxanthine	0.9932	10–2000	78 ± 8	10	16	14	4.7
Caffeine	0.9943	10–2000	85 ± 9	13	9	40	10
Carbamazepine	0.9996	10–2000	115 ± 4	5	9	9	2.2
Lincomycin	0.9988	10–2000	102 ± 11	3	13	10	2.4
Clindamycin	0.9984	10–2000	87 ± 2	4	11	2	0.5
Diltiazem	0.9937	10–2000	82 ± 3	7	5	4	1.1
Erythromycin-H_2O	0.9987	10–2000	99 ± 8	7	12	45	9.6
Clarithromycin	0.9983	10–2000	79 ± 10	6	9	13	3.6
Roxithromycin	0.9984	50–2000	90 ± 13	19	16	96	20
Tylosin	0.9908	50–2000	70 ± 5	18	24	60	16
Acetaminophen	0.9915	50–2000	97 ± 17	10	18	72	29
Triclosan	0.9922	50–2000	79 ± 6	14	17	132	40
Penicillin G	0.9934	50–2000	65 ± 6	20	19	72	18
Oxacillin	0.9976	10–2000	77 ± 8	16	14	18	6.2
Norfloxacin	0.9951	50–2000	51 ± 8	13	16	87	27
Ciprofloxacin	0.9970	50–2000	55 ± 8	10	17	54	19
Ofloxacin	0.9952	100–2000	64 ± 2	12	18	181	98
Gatifloxacin	0.9942	50–2000	54 ± 2	8	12	75	23
Gemfibrozil	0.9987	10–2000	91 ± 16	18	22	20	4.7
Clofibric acid	0.9991	10–2000	89 ± 2	6	12	23	8.8
Ibuprofen	0.9979	50–2000	98 ± 3	15	17	94	45
Ketoprofen	0.9990	100–2000	75 ± 8	8	22	48	61
Diclofenac	0.9934	10–2000	113 ± 3	5	12	50	8.4
Indomethacin	0.9912	50–2000	95 ± 2	9	22	42	15
Salicylic acid	0.9943	10–2000	60 ± 12	6	9	15	3.3

Note: [a]Recoveries and SDs ($n = 3$) determined by analysing spiked stream water samples (200 ng L^{-1}).

concentration ranges were greater than 0.99, indicating good linearity of the method. Recoveries of target analytes from spiked stream water ranged from 51% to 115% with 29 out of the 36 targeted compounds exceeding 70%. The recoveries obtained from spiked stream water were similar to those found from spiked groundwater, indicating that the difference of matrices here has minor impact on the performance of this SPE method. This result agrees with previous observations by Zhang and Zhou [13], who found that the recovery of some pharmaceuticals was slightly increased with increasing salinity and the presence of colloids but not affected by surfactant. The method variation reflected by the within- and between-day variations were generally small with a RSD% ranging from 2 to 24. IQL is expressed as pico gram per injection and varied between 2 and 181 pg. MQL ranged from 0.5 to 98 ng L^{-1} with a standard deviation less than 20% for the majority of analytes. These values are comparable to previous published methods [11,16,19,32]. With quantification limits at nanogram per litre level, the method is sufficient for the detection of trace level pharmaceutical residuals typically found in surface waters, and with slight alteration by either increasing the initial volume of sample water used for the extraction and/or decreasing the volume of reconstructed samples, more sensitivity can be achieved as needed, although care should be taken as matrix effects may also change.

3.6 *Analysis of environmental samples*

After validation, the optimised method was used to detect the targeted analytes in environmental samples. Surface water samples were collected after a rainfall event in December 2007 from the Maumee Bay Lake Erie coastal area of Oregon, Ohio, including water from a stream draining agriculture lands previously amended with treated sewage sludge produced at a local wastewater treatment plant and lake water from the shore of Maumee Bay to the west and east of the stream outfall. In the stream and lake water samples, 17 pharmaceuticals were detected with a concentration from 0.5 to 212 ng L^{-1} (Table 6). Greater than 100 ng L^{-1} concentrations were found for caffeine, sulphamethoxazole, trimethoprim and salicylic acid. The concentrations found here were comparable to those previously reported in other areas. For instance, caffeine and clindamycin are of the same magnitude as those detected in a stream receiving effluent discharge in the US [33]. The concentrations of carbamazepine, erythromycin-H_2O and clarithromycin are within the range reported in Italian rivers [34]. Detection of pharmaceuticals in the stream suggests that possible anthropogenic input of pharmaceuticals from areas within this watershed. For example, land application of sewage sludge and septic systems may be potential sources, and an ongoing study is assessing this hypothesis. Most pharmaceuticals detected in the stream were also detected in the lake with lower concentrations. However, some compounds showed a higher concentration in the lake than in the stream, indicating that sources other than the stream examined in this study contribute to the occurrence of pharmaceuticals in the lake at that location.

4. Conclusion

In this work, an analytical method was developed for the determination of several widely used pharmaceuticals from different therapeutic classes using SPE and LC–MS/MS. Matrix and recovery data is presented for three SPE sorbent materials, at three differing extraction pH's. Compared with previously published methods encompassing many of the

Table 6. Detection of pharmaceuticals in surface water samples (ng L^{-1}).

Compounds	LW1	LW2	SW1	SW2
Cotinine	n.d.	n.d.	55	16
Trimethoprim	26	21	212	38
Sulphamethoxazole	211	201	131	35
Sulphamethazine	17	8.5	38	29
Paraxanthine	31	18	5.7	27
Caffeine	76	60	144	109
Carbamazepine	20	15	4	2.7
Lincomycin	<MQL	<MQL	n.d.	<MQL
Clindamycin	1.7	0.5	1.6	1.2
Diltiazem	3.7	<MQL	<MQL	n.d.
Erythromyin-H_2O	12	n.d.	n.d.	n.d.
Clarithromycin	9.6	4	n.d.	<MQL
Ofloxacin	n.d.	<MQL	n.d.	n.d.
Gemfibrozil	5.4	16	n.d.	n.d.
Diclofenac	11	11	48	82
Indomethacin	n.d.	<MQL	<MQL	n.d.
Salicylic acid	70	90	121	103

Notes: LW1: east outfall lake water, LW2: west outfall lake water; SW1: upstream water; SW2: downstream water; n.d.: below the method detection limit; <MQL: below the method quantification limit.

same analytes the presented method simplifies analytical procedure and enables simultaneous determination of 36 pharmaceutical compounds using a single extraction step and one LC method while retaining comparable sensitivity. This method eliminates the need for use of multiple SPE extraction steps, laborious derivatisation associated with GC, or several LC gradients. Utilising the standard added method allowed for compensation for matrix effects present. The presented data allows researchers to use the method as a whole or subset a list of analytes based on sorbent choice, pH, matrix and recovery data.

The proposed method was subsequently used to measure pharmaceuticals from several environmental matrices. Seventeen compounds were detected with concentrations up to the hundreds nanogram per litre in the stream and lake water samples. The method is a useful tool for the analysis of pharmaceuticals in the aquatic environment and will be used to study the transportation and fate of pharmaceuticals following the land application of treated sewage sludge in an on going project in the Maumee River Watershed.

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