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Multi-residue method for trace level determination of pharmaceuticals in environmental samples using liquid chromatography coupled to triple quadrupole mass spectrometry

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ABSTRACT

A multi-residue method for the simultaneous determination of more than 90 pharmaceuticals in water samples was developed and validated. The developed method utilizes a single liquid chromatography–tandem mass spectrometry (LC–MS/MS) run after sample enrichment using solid-phase extraction (SPE). The pharmaceuticals included in this method were chosen based on their potency (effect/concentration ratio) and potential to bioaccumulate in fish. Because the selection was based on ecotoxicological criteria and not on ease of detection, the pharmaceuticals have a wide range of physico-chemical properties and represent 27 distinct classes. No method for surface, waste water or similar matrices was previously described for 52 of the 100 target analytes. Four chromatographic columns were tested to optimize the separation prior to detection by mass spectrometry (MS). The resulting method utilizes a Hypersil Gold aQ column. Three different water matrices were tested during method validation: Milli-Q water, surface water (river water from the Umea River) and effluent from the Umea waste water treatment plant (WWTP). Four of the selected pharmaceuticals exhibited poor method efficiency in all matrices. Amiodarone, Dihydroergotamine, Perphenazine and Terbutalin were omitted from the final analytical method. In addition, five compounds were excluded from the method for surface water (Atorvastatin, Chloropromazin, Dipyridamol, Furosemid and Ranitidin) and three other pharmaceuticals (Glibenclamid, Glimepirid and Meclozine) from waste water method respectively. Absolute recoveries were above 70% for Milli-Q water, surface water, and sewage effluent for most pharmaceuticals. The limits of quantification (LOQs) ranged from 0.05 to 50 ng L^{-1} (median 5 ng L^{-1}). The use of matrix-matched standards led to the elimination of ionization enhancement or suppression. The recoveries of the method for real matrices were in the range of 23–134% for surface water (only three compounds were outside of the range of 40–130%) and in the range of 47–162% for waste water (five compounds were outside of the range of 40–130% at lower validated concentration).

1. Introduction

A wide range of pharmaceuticals have been detected in surface waters globally, raising concerns about the potential adverse environmental effects [1,2]. Pharmaceuticals are widely used and seldom fully metabolized, which results in their discharge into the aquatic environment via municipal and hospital sewage water [3,4]. It has recently been shown that pharmaceutical development and production facilities in Asia, Europe, and USA elevate surface water concentrations of antibiotics, antifungals, antidepressants, opioids and muscle relaxants considerably [5,6]. Numerous laboratory studies on aquatic organisms have illustrated that certain pharmaceuticals have negative effects on growth, development, and reproduction [1,7–9]. Consequently, there is a growing need to develop reliable analytical methods that enable rapid, sensitive, and selective determination of these emerging pollutants at trace levels in environmental samples. Multi-residue analytical methods are becoming essential tools that provide reliable information about the occurrence and fate of pharmaceuticals in the environment. Analytical methods are available for the detection of particular classes of these compounds in surface and wastewaters, including several multi-residue methods [10–18]. Current methods cover a relatively narrow range of pharmaceuticals that correspond to requests

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from regulatory bodies, e.g., priority compounds listed by the US EPA or European Water Framework Directive [11,14]. The need for relatively simple multi-residue methods for heretofore uninvestigated pharmaceutical compounds is growing.

Currently, there are more than 6000 pharmaceuticals listed in the Martindale database [19]. Therefore, prioritization approaches must be used to select those pharmaceuticals that should be included in monitoring schemes. Various strategies have been applied, including the use of sales statistics, as well as more rational strategies, such as mode-of-action-based tests [20–22]. One useful approach, suggested by Huggett [20], has been designated as the "fish plasma model." This model is based on the assumption that if two species possess the same drug target, the pharmaceuticals will activate this target at roughly the same plasma concentration. The fish plasma model generates a concentration ratio (CR) between the human therapeutic plasma concentrations (H_TPC) and a measured, or theoretically predicted, fish steady state plasma concentration ($F_{SS}PC$). If the concentration ratio is ≤ 1, then the plasma concentration in the exposed fish is equal to or higher than the plasma concentration that is known to cause a pharmacological response in humans. A lower ratio thus reflects a higher risk. A major benefit of this model is that it enables the calculation of the theoretical risks for the great majority of pharmaceuticals, because human therapeutic plasma concentrations are readily available in the literature. However, data for the measured plasma levels of pharmaceuticals in fish following exposure via water are scarce [23–26]; thus, risk calculations still largely rely on theoretically predicted $F_{SS}PCs$.

Fick et al. [27] recently calculated the surface water concentration for 500 pharmaceuticals that theoretically would result in a pharmacologically relevant fish steady state plasma concentration. This surface water concentration was described as the "critical environmental concentration" (CEC) and was derived from the theoretically predicted $F_{SS}PCs$ and the published human therapeutic plasma concentrations. By combining the predicted environmental concentrations (PECs), which are based on sales, with the CEC values for these 500 pharmaceuticals, it is possible to calculate the CRs in a specific region.

The aim of this study was to develop a sensitive, multi-residue, single chromatographic run method based on a single SPE protocol followed by LC-ESI-MS/MS for the simultaneous analysis of pharmaceuticals in surface water and sewage effluent. The selection of the pharmaceuticals included in this method was based on relevant ecotoxicological criteria and also based on a request to include pharmaceuticals from as many different classes as possible.

2. Materials and methods

2.1. Selection of pharmaceuticals

The selection of the pharmaceuticals to be included in the study was based on the CEC values for 500 pharmaceuticals [27] and the calculated PECs based on the amounts sold in Sweden in 2005 (statistics from Apoteket AB, Sweden). A full description of how the CEC values were calculated can be found in Fick et al. [27]. The PEC values were calculated according to the following equation:

$$PE,,--1..=,A\,x\,(100-R)-365\,x\,P\,x\,V\,x\,D\,x\,100. \quad (1)$$

where A is the total pharmaceutical sales ($\mu g\ year^{-1}$); R is the removal rate due to loss by adsorption to sludge particles, volatilization, hydrolysis, and biodegradation (%); P is the human population (number of individuals); V is the volume of waste water per capita per day ($L\ day^{-1}$); and D is a factor for the dilution of waste water by surface water. In order to study a worst case scenario, no removal was assumed ($R=0$). The additional parameters used were $P=9\,047\,752$, $V=200$ (default), and $D=10$ (default). The concentration ratio was calculated by dividing the CEC value by the PEC value for each pharmaceutical. The final selection of pharmaceuticals to be included was made using the criteria of low CRs and commercially available reference standards. Efforts were made to include as many therapeutic classes as possible, and several antibiotics were included to complete the selection. Fifty-two pharmaceuticals that previously lacked an analytical protocol for their determination in environmental samples are included in this selection. No LC/MS method was reported for four of the selected pharmaceuticals. An LC/MS method for pharmacokinetic or similar studies at the therapeutic concentration level in human blood and tissue was previously reported in the literature for 48 of the selected pharmaceuticals. Only 48 of the 100 compounds have a validated method for a water matrix described in the literature (based on Web of Knowledge in November 2011).

2.2. Chemicals

All of the pharmaceutical reference standards were classified as analytical grade (> 98%). Sulfuric acid (99.999%) was purchased from Sigma-Aldrich (Steinheim, Germany) and ethyl acetate (Analytical reagent, 99.8%) was purchased from Labscan Ltd. (Dublin, Ireland). $^{2}H_6$-amitriptyline, $^{2}H_{10}$-carbamazepine, $^{13}C_3^{15}N$-ciprofloxacin, $^{2}H_5$-fluoxetine, $^{13}C_6$-sulfamethoxazole, $^{13}C^{2}H_3$-tramadol and $^{13}C_3$-trimethoprim were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). $^{2}H_5$-oxazepam, $^{2}H_7$-promethazine, $^{2}H_4$-risperidone, and $^{13}C_2^{15}N$-tamoxifen were bought from Sigma-Aldrich (Steinheim, Germany). Methanol and acetonitrile were purchased in LC/MS grade quality (Lichrosolv – hypergrade, Merck, Darmstadt, Germany). The purified water was prepared by a Milli-Q Gradient ultrapure water system (Millipore, Billerica, USA), equipped with a UV radiation source. Acidification of the mobile phases was performed by addition of 1 mL of formic acid (Sigma-Aldrich, Steinheim, Germany) to 1 L of solvent.

A triple-stage quadrupole MS/MS TSQ Quantum Ultra EMR (Thermo Fisher Scientific, San Jose, CA, USA) coupled with an Accela LC pump (Thermo Fisher Scientific, San Jose, CA, USA) and a PAL HTC autosampler (CTC Analytics AG, Zwingen, Switzerland) was used as the analytical system. Xcalibur (Thermo Fisher Scientific, San Jose, CA, USA) software was used for the creation of instrument methods, the running of samples, and subsequent work with the collected chromatograms.

2.3. Mass spectrometry

Heated electrospray (HESI) in positive or negative ion mode was used for ionization. The fused-silica capillary (standard set up) was replaced with a metal capillary. The key parameters were set as follows: ionization voltage 3.5 kV; sheath gas 50, and auxiliary gas 35 arbitrary units; vaporizer temperature 200 °C; capillary temperature 325 °C; and collision gas (argon) flow 1.5 mL min^{-1}. Both the first and third quadrupoles were operated at a resolution of 0.7 FMWH. The above-mentioned ionization conditions were set as tuning conditions for the single reaction monitoring SRM of individual compounds. The tuning was performed with an infusion of 1 $\mu g\ mL^{-1}$ solution of each analyte into the stream of the mobile phase (250 $\mu L\ min^{-1}$ of water/MeOH/ACN 50/30/20 all solvents acidified by 0.1% of formic acid). The tube lens voltage and collision energy of the three most abundant transitions were optimized.

2.4. Liquid chromatography

Due to the wide range of physico-chemical properties of the studied pharmaceuticals, four different reversed chromatography stationary phases were used for the LC/MS/MS method development.

Fully endcapped C18 phase Hypersil GOLD aQ (50 mm × 2.1 mm ID × 5 μm particles, Thermo Fisher Scientific, San Jose, CA, USA) was tested as an alternative to the conventional C18 phase Hypersil GOLD (50 mm × 2.1 mm ID × 3 μm particles, Thermo Fisher Scientific, San Jose, CA, USA). Less hydrophobic C-phenyl phase Hypersil GOLD Phenyl (50 mm × 2.1 mm ID × 3 μm particles, Thermo Fisher Scientific, San Jose, CA, USA) and porous graphite column Hypercarb (50 mm × 2.1 mm ID × 5 μm particles, Thermo Fisher Scientific, San Jose, CA, USA) were tested with the expectation of different separation properties compared with the C18. All columns were preceded by a guard column (2 mm × 2.1 mm i.d., 3 or 5 μm particles) of the same packing material from the same manufacturer. The separation of the pharmaceuticals was performed under the same or very similar conditions. Generally, a gradient of MeOH and ACN in water (all solvents were acidified by 0.1% formic acid) was used for the elution of analytes. The elution conditions were programmed as follows: 200 μL min^{-1} 5% methanol in water for 1 min, isocratically followed by a gradient change to 20/20/60 water/ACN/MeOH at a flow of 250 μL min^{-1} in 8 min and a final gradient change to ACN/MeOH 40/60 at a flow of 300 μL min^{-1} in 11 min. These parameters were held for 1 min and then changed to the starting conditions and held for 4 min to equilibrate the column for the next run. The only difference among the columns was in the initial mobile phase composition. Pure water was used for the Hypersil Gold aQ and Hypercarb columns due to their ability to work under this condition. All of the experiments were performed at 22 °C ambient temperature.

A 20 μL injection loop was used for injection of the standards and samples. The injected volume ranged from 5 to 20 μL depending on the content of MeOH or ACN in the sample and the expected concentration of target analytes.

2.5. Pre-treatment of water samples and solid phase extraction

All samples (100 mL of Milli-Q, surface water, and sewage effluent from the Umea WWTP) were filtered through a 0.45 μm membrane filter (MF, Millipore, Sundbyberg, Sweden) and acidified to pH 3 using sulfuric acid. Five nanograms of each surrogate standard were added to each sample. The solid phase extraction of samples was carried out with a Visiprep SPE manifold (Supelco, Bellefonte, PA, USA). Oasis HLB (200 mg) cartridges were sequentially conditioned with 5 mL MeOH and 5 mL pure water. Samples were applied to the SPE cartridges at a flow rate of 5 mL min^{-1}. Water with 5% methanol was used to wash the SPE column before elution with 5 mL methanol and 3 mL ethyl acetate. The eluate was collected in 10 mL vials, evaporated to 20 μL under a gentle air stream, and dissolved in 5% acetonitrile in water with 0.1% formic acid to a final volume of 1.0 mL. The most diverse pre-treatment protocol among the multi-residue methods was selected and validated [28,29] due to the problems associated with the simultaneous optimization of the extraction efficiencies and matrix effects of 97 different analytes.

2.6. Quality assurance/quality control

Stock solutions of each of the pharmaceuticals were prepared in methanol and stored at −18 °C. Calibration standards were prepared in the mobile phase. Any pharmaceutical that lacked a labeled internal standard was matched with a suitable surrogate standard based on the physico-chemical properties, retention time, negative or positive ionization.

Possible memory effects were evaluated by a blank injection of Milli-Q water following the injection of the standard samples at varying concentrations.

A seven-point calibration curve was constructed with a broad concentration range (0.005–500 ng mL^{-1} with the same concentration of IS). The calibration curve was used for evaluating the linearity. The instrumental limits of quantification (LOQs) were estimated from the calibration curve. The LOQs for the different matrices were calculated based on the instrument LOQs and relative response ratios in real samples. The recoveries were determined by spiking the standard solution of the matrices at the following concentration levels: Milli-Q water at 100 ng L^{-1}; surface water at 100 ng L^{-1} and 500 ngL^{-1}; and sewage effluent at 1000 ng L^{-1} and 2500 ng L^{-1}. To distinguish between ionization suppression or enhancement and recovery efficiency on SPE, the matrix-matched standards were prepared as follows: The same aliquots of surface or waste water were extracted, and the native compounds/internal standards were added at a level of 500/5 ng for surface water and 2500/5 ng for waste water to 1 mL of mobile phase reconstituted extract.

3. Results and discussion

3.1. HESI –MS/MS

The quantification and qualification of the SRM transitions for each target compound and internal standard are listed in Table 1. The time window for each SRM is defined by the retention time (RT) and by a window factor, a number that multiplies the default time window (1 min). This procedure is possible only in the EZ

Table 1
MS transitions used in this method.

Analyte	Group	Mod	Precursor	Product	CE (V)	Tube lens (V)	Type	Ratio quan/qual	tR (min)
Alfuzosin	Urological	+	390.1	156.2	26	111	Quan	1.4490	6.85
		+	390.1	235.1	26	111	Qual		
Alprazolam	Psycholeptics	+	309.0	205.1	39	103	Quan	1.3020	8.18
		+	309.0	281.0	25	103	Qual		
Amiodarone	Antiarrhythmics	+	645.9	100.2	30	134	Quan	1.3670	12.00
		+	645.9	58.2	46	134	Qual		
Amitryptyline*	IS	+	284.1	191.1	27	99	Quan		
		+	284.1	233.2	15	99	Qual		
Amytriptyline	Antidepressant	+	278.1	233.2	15	99	Quan	1.6143	8.80
		+	278.1	117.2	23	99	Qual		
Atenolol	Hypertension drug	+	267.0	145.1	26	98	Quan	1.1501	4.49
		+	267.0	190.1	19	98	Qual		
Atorvastatin	Statin	+	559.2	250.0	43	120	Quan	1.7372	9.04
		+	559.2	440.4	20	120	Qual		
Atracurium	Muscle relaxant	+	358.1	151.2	29	108	Quan	4.2569	6.50
		+	358.1	206.1	18	108	Qual		
Azelastine	Anti-histamine	+	382.1	112.2	25	110	Quan	14.8620	8.63

Table 1 (*continued*)

Analyte	Group	Mod	Precursor	Product	CE (V)	Tube lens (V)	Type	Ratio quan/qual	tR (min)
		+	382.1	58.4	50	110	Qual		
Azithromycine	ATB	+	749.4	158.2	37	148	Quan	3.7330	7.90
		+	749.4	591.6	28	148	Qual		
Beclomethasone	Antiinflammatory corticoide	−	453.2	297.2	27	92	Quan	1.3709	8.17
		−	453.2	377.3	19	92	Qual		
Bezafibrate	Cholesterol statin	−	360.0	154.1	30	101	Quan	3.4903	8.57
		−	360.0	274.1	21	101	Qual		
Biperiden	Anti-Parkinson	+	312.1	294.3	15	103	Quan	9.8316	8.78
		+	312.1	98.3	23	103	Qual		
Bisoprolol	Hypertension drug	+	326.1	116.2	17	105	Quan	6.0582	7.28
		+	326.1	74.4	26	105	Qual		
Bromocriptine	Anti-Parkinson	+	654.1	301.0	35	124	Quan	1.8565	8.66
		+	654.1	346.0	26	124	Qual		
Budesonide	Antiinflammatory corticoide	+	431.2	413.4	7	116	Quan	1.0374	8.90
		+	431.2	323.1	12	116	Qual		
Buprenorphine	Analgesic	+	468.2	468.2	25	126	Quan	118.0852	7.90
		+	468.2	55.4	54	126	Qual		
Bupropion	Antidepressant	+	240.0	131.2	25	77	Quan	1.6599	7.21
		+	240.0	184.1	12	77	Qual		
Carbamazepin	Psycholeptics	+	237.0	193.2	35	118	Quan	3.3815	7.57
		+	237.0	194.2	19	118	Qual		
Carbamazepin*	IS	+	247.0	204.0	19	118	Quan		
Chlorpromazine	Psycholeptics	+	319.0	214	35	76	Quan	9.8304	9.23
		+	319.0	86.3	19	76	Qual		
Chlorprothixene	Psycholeptics	+	316.0	231	29	103	Quan	1.0738	9.41
		+	316.0	271	18	103	Qual		
Cilazapril	Hypertension drug	+	418.1	114.2	34	92	Quan	27.7288	8.50
		+	418.1	211.1	19	92	Qual		
Ciprofloxacin	ATB	+	332.0	288.2	16	117	Quan	4.8062	6.40
		+	332.0	231.1	35	117	Qual		
Ciprofloxacin*	IS	+	336.0	318	20	106	Quan		
Citalopram	Antidepressant	+	325.1	109.2	27	104	Quan	1.6882	7.90
		+	325.1	262.1	18	104	Qual		
Clarithromycine	ATB	+	748.4	158.1	27	156	Quan	1.5751	9.30
		+	748.4	590.5	17	156	Qual		
Clemastine	Antidepressant	+	344.0	180	31	73	Quan	4.4051	9.65
		+	344.0	215.1	18	73	Qual		
Clindamycine	ATB	+	425.1	126.2	31	110	Quan	8.3053	7.69
		+	425.1	377.3	18	110	Qual		
Clomipramine	Antidepressant	+	315.0	242.1	26	77	Quan	10.0845	9.33
		+	315.0	86.3	17	77	Qual		
Clonazepam	Psycholeptics	−	313.9	278	17	94	Quan	2.0840	7.84
		−	313.9	286.1	18	94	Qual		
Clotrimazol	Antimycotic	+	277.0	165.1	26	81	Quan	2.2601	8.90
		+	277.0	241.1	26	81	Qual		
Codeine	Analgesic	+	300.1	215.1	23	102	Quan	1.0014	4.86
		+	300.1	165.2	41	102	Qual		
Cyproheptadine	Anti-histamine	+	288.1	191.1	28	100	Quan	1.1406	8.55
		+	288.1	96.2	24	100	Qual		
Desloratidin	Anti-histamine	+	311.0	259.1	20	81	Quan	3.6887	7.40
		+	311.0	294	16	81	Qual		
Diclofenac	NSAID	−	294.0	250	15	96	Quan	1.7302	9.22
		−	296.0	252	16	84	Qual		
Dicycloverine	Drug for gastrointestinal disorders	+	310.1	109.2	19	103	Quan	1.0246	10.10
		+	310.1	237.2	26	103	Qual		
Dihydroergotamine	Analgesic	+	584.2	253.1	31	134	Quan	2.9101	8.10
		+	584.2	270.1	28	134	Qual		
Diltiazem	Hypertension drug	+	415.1	150.1	37	95	Quan	3.3427	8.15
		+	415.1	178.1	23	95	Qual		
Diphenhydramine	Anti-histamine	+	256.1	165.1	37	73	Quan	2.6942	7.82
		+	256.1	167.1	13	73	Qual		
Dipyridamole	Antithrombotic agent	+	505.3	385.3	40	127	Quan	1.3469	8.29
		+	505.3	429.5	39	127	Qual		
Duloxetine	Antidepressant	+	298.1	123.5	50	74	Quan	56.8644	8.70
		+	298.1	44.3	12	74	Qual		
Eprosartan	Hypertension drug	+	425.1	207.1	23	117	Quan	1.9458	7.42
		+	425.1	107.1	47	117	Qual		
Etonogestrel	Hormonal contraceptive	+	325.1	91.2	50	110	Quan	1.4819	9.62
		+	325.1	257.2	18	110	Qual		
Ezetimibe	Cholesterol statin	−	408.1	119.3	59	132	Quan	35.5912	8.77
		−	408.1	271.2	19	132	Qual		
Fentanyl	Analgesic	+	337.1	105.2	35	106	Quan	1.8395	7.60
		+	337.1	188.2	22	106	Qual		
Fenofibrate	Cholesterol statin	+	361.0	139.0	27	99	Quan	1.6734	10.23
		+	361.0	233.1	16	99	Qual		
Fexofenadine	Anti-histamine	+	502.2	171.1	35	101	Quan	2.1410	8.60

Table 1 (*continued*)

Analyte	Group	Mod	Precursor	Product	CE (V)	Tube lens (V)	Type	Ratio quan/qual	tR (min)
		+	502.2	466.5	26	101	Qual		
Finasteride	Urological	+	373.2	305.2	29	110	Quan	15.6825	8.98
		+	373.2	95.4	36	110	Qual		
Flecainide	Antiarrhythmics	+	415.1	301.1	31	114	Quan	3.1111	7.94
		+	415.1	398.2	22	114	Qual		
Fluconazole	Antimycotic	+	307.1	238.1	16	91	Quan	1.0401	6.06
		+	307.1	220.1	17	91	Qual		
Fluoxetine	Antidepressant	+	310.1	44.3	13	83.6	Quan	one SRM	9.00
Fluoxetine*	IS	+	315.1	44.0	13	83.6	Quan		
Flupentixol	Psycholeptics	+	435.1	265.0	34	116	Quan	1.1536	10.10
		+	435.1	305.1	28	116	Qual		
Fluphenazine	Psycholeptics	+	438.1	143.2	28	93	Quan	1.1992	9.90
		+	438.1	171.2	22	93	Qual		
Flutamide	Antiandrogen	−	275.0	202.0	25	90	Quan	1.2387	8.72
		−	275.0	205.0	23	90	Qual		
Furosemide	Diuretic	−	328.9	205.0	23	81	Quan	1.1965	7.28
		−	328.9	285.0	18	81	Qual		
Glibenclamide	Anti diabetic	−	492.1	369.2	11	137	Quan	4.1732	9.57
		+	494.1	170.0	31	105	Qual		
Glimepiride	Anti diabetic	−	489.2	352.2	11	121	Quan	3.1344	9.27
		+	491.2	225.0	36	122	Quan		
Haloperidol	Psycholeptics	+	376.0	123.1	36	88	Quan	1.1849	7.81
		+	376.0	165.1	22	88	Qual		
Hydroxyzine	Psycholeptics	+	375.1	166.1	35	97	Quan	2.0692	8.70
		+	375.1	201.1	18	97	Qual		
Irbesartan	Hypertension drug	+	429.2	180.1	38	110	Quan	5.3791	8.39
		+	429.2	207.1	22	110	Qual		
Ketoconazole	Antimycotic	+	531.1	254.9	34	134	Quan	1.1539	9.27
		+	531.1	244.0	33	134	Qual		
Levonorgestrel	Hormonal contraceptive	+	313.0	109.2	29	103	Quan	1.2621	9.51
		+	313.0	245.2	17	103	Qual		
Loperamide	Antipropulsive	+	477.2	210.2	45	121	Quan	2.2773	9.23
		+	477.2	266.2	24	121	Qual		
Maprotiline	Antidepressant	+	278.1	219.2	24	99.3	Quan	1.6236	8.80
		+	278.1	250.2	17	99.3	Qual		
Meclozine	Anti-histamine	+	391.1	200.1	16	100	Quan	376.4917	10.03
		+	391.1	166.1	36	100	Qual		
Medroxyprogesterone	Hormonal contraceptive	+	345.1	123.3	24	107	Quan	3.6353	9.68
		+	345.1	97.2	34	107	Qual		
Megestrol	Cancer treatment	+	385.1	267.2	18	98	Quan	1.2117	9.91
		+	385.1	325.2	15	98	Qual		
Memantine	Psycholeptics	+	180.1	107.2	24	77	Quan	2.9151	7.87
		+	180.1	163.2	16	77	Qual		
Metoprolol	Hypertension drug	+	268.1	191.1	16	98	Quan	1.1511	6.44
		+	268.1	159.1	20	98	Qual		
Mianserin	Antidepressant	+	265.0	118.2	30	98	Quan	7.5547	7.90
		+	265.0	208.1	20	98	Qual		
Miconazole	Antimycotic	+	414.9	159.0	29	114	Quan	1.5514	10.70
		+	416.9	161.0	31	114	Qual		
Mirtazapine	Antidepressant	+	266.1	194.1	40	98.2	Quan	2.9146	6.39
		+	266.1	195.1	25	98.2	Qual		
Morphine	Analgesic	+	286.1	201.1	24	110	Quan	1.1019	3.63
		+	286.1	165.1	35	110	Qual		
Naloxone	Opoid overdose drug	+	328.0	212.0	36	119	Quan	5.3991	4.88
		+	328.0	310.2	18	119	Qual		
Nefazodone	Antidepressant	+	470.1	246.2	32	120	Quan	3.5340	9.06
		+	470.1	274.2	27	120	Qual		
Norfloxacin	ATB	+	320.0	233.1	23	114	Quan	4.6996	6.00
		+	320.0	302.1	20	114	Qual		
Ofloxacin	ATB	+	362.1	261.1	25	138	Quan	1.1384	6.04
		+	362.1	318.2	17	138	Qual		
Orphenadrine	Anti-histamine	+	270.1	165.1	44	70	Quan	1.9599	8.35
		+	270.1	181.1	13	70	Qual		
Oxazepam	Psycholeptics	+	287.0	241.1	22	84	Quan	1.4517	7.98
		+	287.0	269.1	15	84	Qual		
Oxazepam*	IS	+	292.0	246.1	22	84	Quan		
Paroxetine	Antidepressant	+	330.0	192.1	20	105	Quan	2.5801	8.60
		+	330.0	70.4	30	105	Qual		
Perphenazine	Psycholeptics	+	404.1	143.2	27	113	Quan	1.5155	9.60
		+	404.1	171.2	21	113	Qual		
Pizotifen	Analgesic	+	296.0	199.1	26	101	Quan	11.8679	8.60
		+	296.0	96.3	21	101	Qual		
Progesterone	Hormon therapy	+	315.0	109.2	26	103	Quan	1.0684	8.90
		+	315.0	97.2	24	103	Qual		
Promethazine	Anti-histamine	+	285.1	86.3	16	65	Qual	2.1383	8.40
		+	285.1	198.0	26	65	Quan		

Table 1 (*continued*)

Analyte	Group	Mod	Precursor	Product	CE (V)	Tube lens (V)	Type	Ratio quan/qual	tR (min)
Promethazine*	IS	+	292.1	89.3	16	65	Quan		
Ranitidine	Drug for peptic ulcer	+	315.0	176.1	17	82	Qual	4.1880	4.62
		+	315.0	125.1	26	82	Quan		
Repaglinide	Anti diabetic	+	453.2	162.2	19	118	Quan	2.5285	8.76
		+	453.2	230.2	25	118	Qual		
Risperidone	Psycholeptics	+	411.1	110.2	44	94	Quan	21.1823	7.20
		+	411.1	191.1	27	94.0	Qual		
Risperidone*	IS	+	415.1	195.1	27	95	Quan		
Rosuvastatin	Statin	+	482.1	258.1	32	118.0	Quan	2.1341	8.21
		+	482.1	272.2	33	118.0	Qual		
Roxithromycine	ATB	+	837.4	158.1	33	148.0	Quan	1.6586	9.41
		+	837.4	679.6	20	148.0	Qual		
Sertraline	Antidepressant	+	306.0	159.0	27	100	Quan	1.0710	9.55
		+	306.0	275	12	100	Qual		
Sotalol	Hypertension drug	+	273.0	213.1	17	86.3	Quan	1.6099	4.38
		+	273.0	255	11	86.3	Qual		
Sulfamethoxazol	ATB	+	254.0	156	15	92	Quan	1.4587	5.92
		+	254.0	108.2	22	92	Qual		
Sulfamethoxazol*	IS	+	260.0	162.1	15	97	Quan		
		+	260.0	114.2	23	97	Qual		
Tamoxifen	Anticancer	+	372.2	129.1	26	113	Quan	12.0177	10.90
		+	372.2	72.4	22	113	Qual		
Tamoxifen*	IS	+	375.2	75.2	22	110	Quan		
Telmisartan	Hypertension drug	+	515.2	276.1	43	114.6	Quan	6.3890	9.13
		+	515.2	305.1	41	114.6	Qual		
Terbutaline	Adrenergic	+	226.1	107.2	29	94	Quan	3.2882	4.37
		+	226.1	152.1	15	94	Qual		
Tramadol	Analgesic	+	264.1	246.2	10	82	Quan	54.2862	6.37
		+	264.1	58.4	16	82	Qual		
Tramadol*	IS	+	268.1	58.4	16		Quan		
Trihexyphenidyl	Anti-Parkinson	+	302.2	70.3	39	102	Quan	18.7338	8.70
		+	302.2	98.3	20	102	Qual		
Trimethoprim	ATB	+	291.0	123.2	25	106	Quan	1.7931	5.61
		+	291.0	230.1	23	106	Qual		
Trimethoprim*	IS	+	294.1	233.2	22	101	Quan		
		+	294.1	126.2	24	101	Qual		
Venlafaxine	Antidepressant	+	278.1	121.2	29	99.3	Quan	1.6165	7.40
		+	278.1	260.2	10	99.3	Qual		
Verapamil	Hypertension drug	+	455.2	165.1	28	118	Quan	3.5710	8.26
		+	455.2	303.3	23	118	Qual		
Zolpidem	Psycholeptics	+	308.1	235.2	32	103	Quan	2.4769	6.89
		+	308.1	263.1	24	103	Qual		
Zuclopenthixol	Psycholeptics	+	401.0	231	35	112	Quan	1.2926	9.80
		+	401.0	271.1	25	112	Qual		

mode of the MS/MS method. The algorithm of the measurement is as follows: The system is switched to the negative mode and all of the transitions in this mode are measured. The mode is then changed to the positive mode, and all of the SRM transitions in this mode are measured. The EZ method contains some simplifications: only a resolution of 0.7 or 0.2 FWHM is allowed, and there is a fixed cycle time that is divided equally among all of the SRMs at a defined time. However, it is the only method for easily handling hundreds of SRMs.

As shown in Table 1, most of the compounds showed at least two MS/MS transitions (with the exception of Fluoxetin, which had only one SRM found by tuning) from a protonized (positive mode) or deprotonized (negative mode) molecule. Unfortunately, the intensities of the second (qualifying) MS/MS transition varied from the intensity obtained for the quantifying SRM to values of less than 1% of the quantifying SRM. The histogram of the intensity ratios is shown in Fig. 1. The separation to four fractions was done in accordance with the requirements of the Commission Decision 2002/657/EC on the performance of analytical methods and the interpretation of the results (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32002D0657:EN:HTML). There are different tolerances for mass ratios in different groups; 20%, 25%, 30%, and 50%, tolerances of the qualification/quantification ratio are allowed for intensities of qualifying mass $>50\%$, 20–50%, 10–20%, and $<10\%$, respectively. It is obvious from the histogram that most (78 of 100) qualifying ions show intensities higher than 20% of the quantifying mass transition.

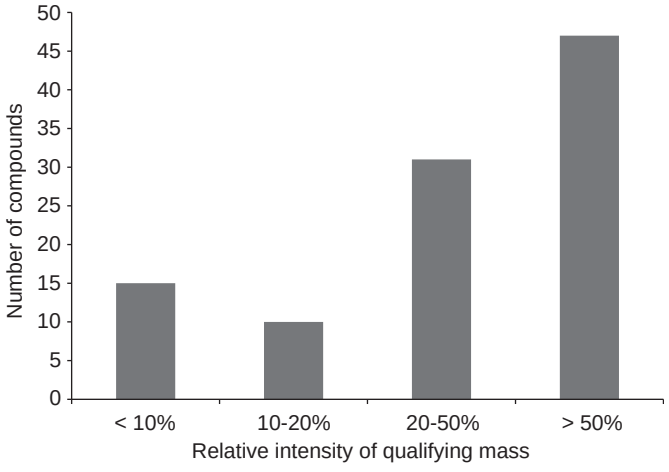

Fig. 1. Histogram of quantification/qualification transition ratios.

3.2. Liquid chromatography

Because the selection of the pharmaceuticals in this study was based solely on the potential environmental effects, the pharmaceuticals consisted of a heterogeneous set with a wide range of physico-chemical properties. It was difficult to find conditions providing acceptable chromatographic behavior for the suitable quantification of this selection in a single run. The four above-mentioned columns were used to achieve, as much as possible, quantifiable compounds in a single run. The tRs for all of the pharmaceuticals in the four separation systems are given in Supplementary Table S1.

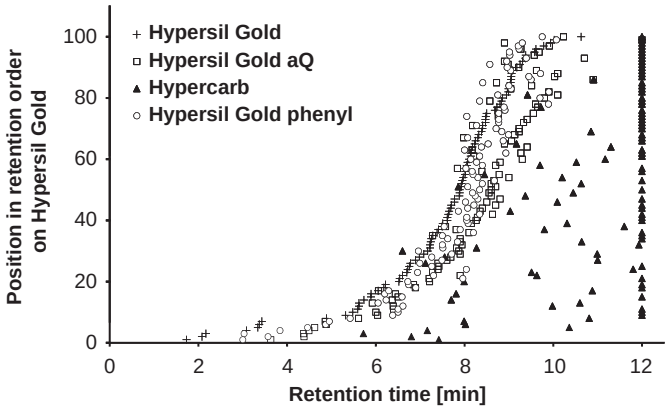

Fig. 2. Retention time shift compared to Hypersil Gold RT for all studied compounds.

The differences among the columns were visualized on a graph, with the *x*-axis representing the retention time and the *y*-axis representing the position in retention order in the Hypersil Gold column. Fig. 2 shows the shift of the tR for the individual compounds compared to Hypersil Gold. The porous graphite column is not a good choice for multi-residue analysis due to the irreversible retention of 58 of the 100 tested pharmaceuticals. Even prolonging the time at 100% organic phase to 5 min did not lead to the elution of these compounds. The best peak shape was obtained in Hypercarb column at least for the early eluting compounds (see Fig. 3). The peak shape of the first few eluted compounds on the Hypersil Gold column is not acceptable. In addition, the peaks are eluted too close to the dead volume. Both of the remaining columns show acceptable peak shapes for early eluting compounds but only one compound is eluted earlier then in 4 min in aQ column (four in Gold Phenyl). Target compounds are less affected with ionic compounds eluted close to dead volume in Hypersil Gold aQ column. At the given chromatographic conditions, the Hypersil Gold Phenyl column has a steeper elution profile of pharmaceutical mixtures than the Hypersil Gold aQ column. The method characteristics for all four columns are given in Table 2. The Hypersil Gold Phenyl has the shortest elution window of target compounds, which results in higher target analytes overlapping. Median number of SRM in cycle is comparable for both columns (51 for aQ and 53 for Phenyl) but maximal values are quite different (76 for aQ and 100 for Phenyl). Insufficient separation can be most likely resolved with a slower gradient, but this will prolong analysis time. The Hypersil Gold aQ was chosen for the single run analyses based on the best separation of the analytes, on good peak shapes at chromatogram

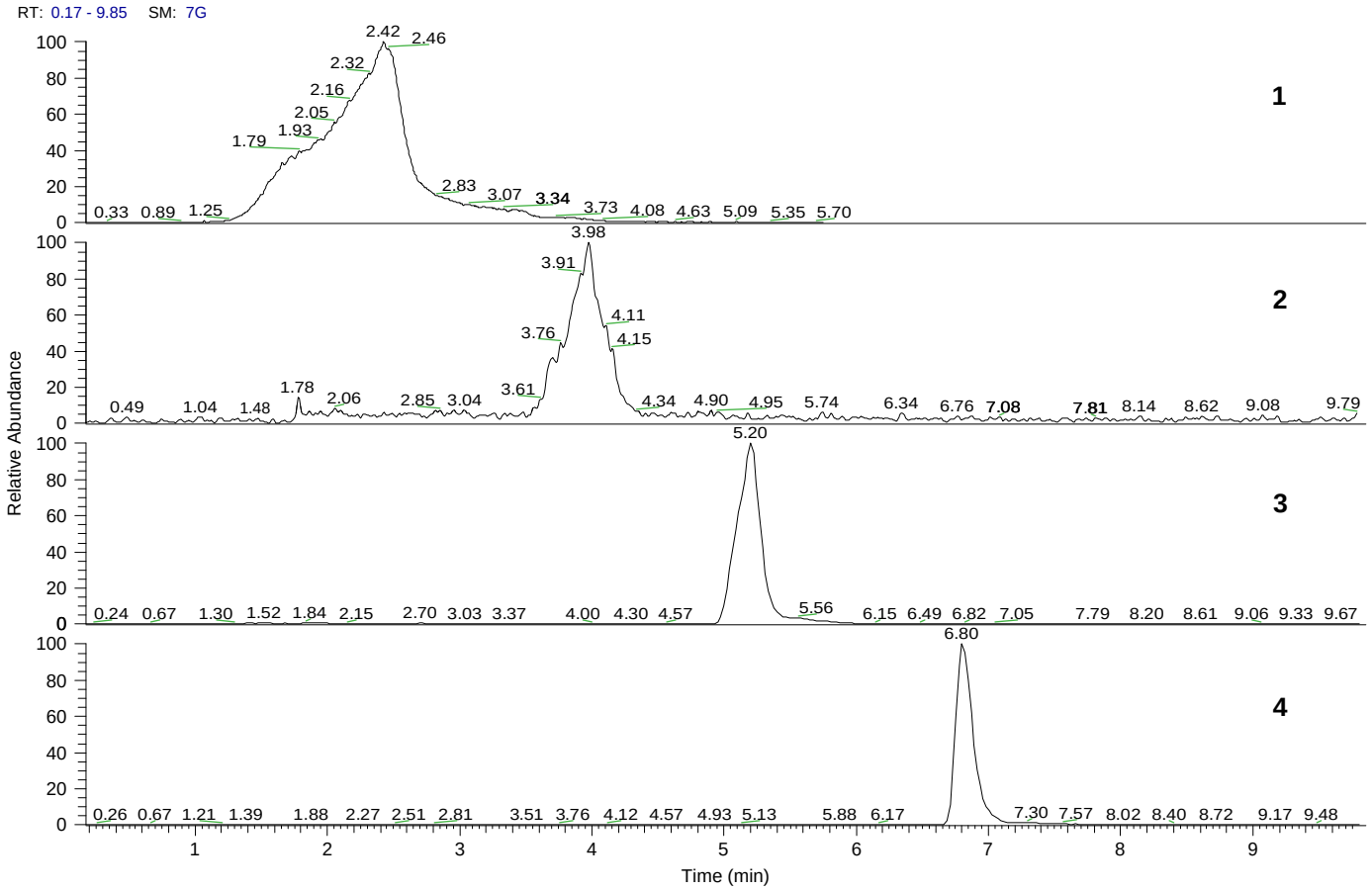

Fig. 3. Chromatogram of Sotalol on four columns under conditions as described in Section 2.4. (1) Hypersil Gold (50 × 2, 1 mm, 3 μm particles), (2) Hypersil Gold Phenyl (50 × 2, 1 mm, 3 μm particles), (3) Hypersil Gold aQ (50 × 2, 1 mm, 5 μm particles), (4) HyperCarb (50 × 2, 1)

Table 2
Comparison of method parameters for four analytical columns under the constant parameter settings of peak width, cycle time, points per peak.

Method parameters	Hypercarb	Gold Phenyl	Gold aQ	Hypersil Gold
Total No. of analytes and IS	114	114	114	114
Cycle time (s)	1	1	1	1
SRM per analyte	2	2	2	2
Retention time of first compound (min)	5.72	3.00	3.63	1.73
Retention time of final compound (min)	12.00	10.07	12.00	10.74
Ret. Window (min)	6.28	7.07	8.37	9.00
Median No. of SRM in cycle	134	53	51	46
Maximal No. of SRM in cycle	138	100	76	84
Median time per SRM (ms)	7.0	19.0	20.0	22.0
Minimal time per SRM (ms)	7.0	10.0	13.2	11.9

beginning and on compatibility of the column with 100% water in mobile phase, This column shows somewhat slow equilibration in 100% water. When the column is equilibrated for approximately 4 min, some of the early eluted compounds have different retention times compared to the same run following 20 min equilibration in water. However, the repeatability of the retention time with a 4 min equilibration is excellent; it is only necessary to run the first sample in the sequence twice to get the same tR in the second and following runs.

3.3. Method performance

3.3.1. Linearity

The quantification of the target compounds was based on the internal standard calibration. The isotope-labeled pharmaceuticals that were selected as the surrogates for each analyte are shown in Table 3, together with the other method parameters,

Table 3
IS used, LOQ and linearity.

Analyte	Surrogate standard used	LOQ (ng L^{-1})[a]				R^2 from LOQ to 5000 (ng L^{-1})[a]
		Instrumental	Milli-Q water[b]	Surface water[b]	Waste water[b]	
Alfuzosin	Tramadol	0.1	0.09	0.11	0.15	0.998
Alprazolam	Tramadol	10	9.9	13	8.9	0.993
Amiodarone	Tramadol	50	119	185	61	0.981
Amytriptyline	Amitryptiline	5	5.9	4.9	5.1	1.000
Atenolol	Tramadol	5	8.6	8.8	5.2	1.000
Atorvastatin	Amitryptiline	50	245	196	59	0.996
Atracurium	Tramadol	0.5	0.46	0.53	0.49	1.000
Azelastine	Tramadol	5	4.5	5.9	5.9	1.000
Azithromycine	Carbamazepin	5	6.9	6.1	4.6	1.000
Beclomethasone	Oxazepam	50	47	44	47	0.999
Bezafibrate	Oxazepam	1	1.3	0.96	1.6	1.000
Biperiden	Amitryptiline	0.1	0.11	0.09	0.09	1.000
Bisoprolol	Tramadol	0.1	0.10	0.11	0.12	1.000
Bromocriptin	Tramadol	5	6.0	6.8	6.1	0.982
Budesonide	Fluoxetine	5	5.8	4.2	5.1	0.998
Buprenorphin	Tramadol	10	10	12	12	1.000
Bupropion	Tramadol	0.1	0.09	0.12	0.11	1.000
Carbamazepin	Carbamazepin	1	0.93	1.2	0.94	1.000
Chlorpromazine	Amitryptiline	10	12	11	11	1.000
Chlorprothixen	Amitryptiline	5	8.7	14	6.1	0.999
Cilazapril	Tramadol	1	0.79	1.20	0.98	0.996
Ciprofloxacin	Ciprofloxacin	10	12	8.1	11	0.997
Citaprolam	Tramadol	5	4.2	6.2	6.3	1.000
Clarithromycin	Amitryptiline	1	1.3	1.4	0.62	1.000
Clemastine	Oxazepam	0.5	0.50	0.46	0.56	0.997
Clindamycine	Tramadol	1	1.0	1.9	0.98	1.000
Clomipramine	Amitryptiline	0.5	0.54	0.50	0.51	1.000
Clonazepame	Tramadol	5	4.2	4.9	6.1	0.959
Clotrimazol	Amitryptiline	1	1.2	0.97	1.2	1.000
Codeine	Tramadol	0.5	0.61	0.52	0.46	0.997
Cyproheptadine	Tramadol	5	4.3	5.3	5.8	0.996
Desloratidine	Risperidone	0.5	0.49	0.46	0.43	0.999
Diclofenac	Tramadol	10	15	11	12	0.999
Dicycloverin	Oxazepam	5	4.0	4.7	4.2	0.999
Dihydroergotamine	Tramadol	50	88	168	78	0.576
Diltiazem	Tramadol	0.5	0.47	0.61	0.53	1.000
Diphenhydramine	Tramadol	0.05	0.04	0.06	0.07	1.000
Dipyridamol	Tramadol	50	61	56	50	0.997
Duloxetin	Tramadol	1	1.0	1.5	1.1	1.000
Eprosartan	Carbamazepin	5	6.2	5.5	4.9	0.999
Etonogestrel	Amitryptiline	0.5	0.65	0.43	0.60	1.000
Ezetimibe	Amitryptiline	50	88	44	46	1.000
Fentanyl	Fluoxetin	50	56	51	54	0.998
Fenofibrate	Carbamazepin	0.5	1.3	1.0	0.4	0.999
Fexofenadine	Amitryptiline	5	7.0	5.9	4.5	1.000
Finasteride	Oxazepam	10	11	7.5	8.5	1.000
Flecainide	Tramadol	0.1	0.08	0.09	0.12	1.000
Fluconazole	Trimetoprim	0.5	0.43	0.46	0.36	0.999
Fluoxetine	Fluoxetine	5	5.3	5.6	4.7	1.000
Flupentixol	Oxazepam	5	5.5	5.9	3.2	1.000
Fluphenazine	Oxazepam	10	12	19	7.2	1.000

Table 3 (*continued*)

Analyte	Surrogate standard used	LOQ (ng L^{-1})[a]			R^2 from LOQ to 5000 (ng L^{-1})[a]
		Instrumental	Milli-Q water[b]	Surface water[b]	Waste water[b]	
Flutamide	Amitryptiline	5	5.6	4.6	5.1	0.998
Furosemid	Carbamazepin	50	46	37	53	1.000
Glibenclamide	Oxazepam	5	6.5	5.4	3.2	1.000
Glimepiride	Oxazepam	5	6.1	4.7	3.3	1.000
Haloperidol	Tramadol	0.1	0.09	0.12	0.17	0.999
Hydroxyzine	Amitryptiline	0.5	0.54	0.49	0.48	1.000
Irbesartan	Amitryptiline	0.5	0.61	0.57	0.49	0.999
Ketoconazole	Amitryptiline	50	80	80	50	1.000
Levonorgestrel	Oxazepam	50	71	48	52	1.000
Loperamide	Amitryptiline	0.5	0.53	0.51	0.50	1.000
Maprotiline	Amitryptiline	5	5.9	4.8	4.7	1.000
Meclozine	Oxazepam	5	4.3	4.0	3.7	0.998
Medroxyprogesterone	Oxazepam	50	74	52	55	1.000
Megestrol	Oxazepam	50	57	53	55	1.000
Memantin	Tramadol	0.5	0.40	0.49	0.51	1.000
Metoprolol	Tramadol	5	4.6	4.6	4.0	1.000
Mianserin	Tramadol	1	0.88	1.2	1.4	1.000
Miconazole	Tramadol	5	6.6	7.3	7.1	0.998
Mirtazapine	Tramadol	10	8.9	8.4	9.6	1.000
Morphine	Trimetoprim	10	17	20	24	0.998
Naloxon	Tramadol	1	1.3	0.89	0.97	1.000
Nefazodon	Amitryptiline	0.5	0.70	0.57	0.52	1.000
Norfloxacin	Ciprofloxacin	10	11	9.3	9.2	0.998
Ofloxacin	Ciprofloxacin	10	12	7.9	14	0.997
Orphenadrine	Amitryptiline	0.1	0.12	0.09	0.07	1.000
Oxazepam	Oxazepam	5	5.3	5.3	3.8	1.000
Paroxetin	Amitryptiline	10	14	10	11	1.000
Perphenazine	Oxazepam	10	15	65	12	1.000
Pizotifen	Amitryptiline	0.5	0.66	0.60	0.54	1.000
Progesterone	Oxazepam	10	13	12	11	1.000
Promethazine	Amitryptiline	10	14	18	10	1.000
Ranitidine	Amitryptiline	0.5	0.97	1.5	0.69	1.000
Repaglinide	Amitryptiline	0.5	0.56	0.52	0.48	1.000
Risperidone	Risperidone	0.1	0.10	0.11	0.09	1.000
Rosuvastatin	Tramadol	10	10	18	10	1.000
Roxithromycin	Amitryptiline	50	76	73	47	0.999
Sertraline	Amitryptiline	10	11	9.0	11	0.964
Sotalol	Tramadol	0.5	0.63	0.51	0.54	1.000
Sulfamethoxazole	Sulfamethoxazol	5	5.3	4.9	5.1	1.000
Tamoxifen	Tamoxifen	5	5.2	4.8	5.0	1.000
Telmisartan	Amitryptiline	50	67	61	72	0.982
Terbutaline	Tramadol	0.5	1.9	1.6	0.71	1.000
Tramadol	Tramadol	0.5	0.50	0.51	0.43	1.000
Trihexyphenidyl	Amitryptiline	0.1	0.11	0.09	0.10	0.999
Trimethoprim	Trimetoprim	0.1	0.10	0.11	0.10	1.000
Venlafaxine	Tramadol	0.5	0.40	0.48	0.43	1.000
Verapamil	Tramadol	10	8.6	12	8.5	1.000
Zolpidem	Tramadol	0.5	0.43	0.60	0.55	1.000
Zuclopenthixol	Oxazepam	5	7.6	9.5	7.2	0.998
Median		5	5.3	4.9	4.6	
Min		0.05	0.04	0.06	0.07	
Max		50	245	196	78	

[a] Based on Instrumental LOQ and SPE enrichment 100 times (100 mL of samples and 1 mL final volume).
[b] Median of LOQs calculated in validation data set (calculated to matrix matched standard and recovery of native compounds).

including the linear range, R^2, coefficient of concentration versus ratio peak area analyte to peak area IS, and LOQs for the different matrices. The developed LC–MS/MS chromatographic procedure exhibits excellent linearity ($R^2 > 0.980$) except for three compounds – Clonazepam, Dihydroergotamine and Sertraline. Dihydroergotamine exhibited poor precision and recovery efficiency, and in the end, we decided to omit this compound from the other experiments. However, both the precision and accuracy of the method for the Clonazepam and Sertraline were acceptable.

3.3.2. Limits of quantification

Quantification in the method is based on the ratio of the target analyte peak area to the internal standard peak area. For highly selective detection method such as MS/MS, the S/N ratio is an auxiliary parameter for the LOQ estimation. Stability of the above mentioned analyte/IS ratio is the determining factor for the LOQ estimation. Relative response factor was used in calculation (ratio of peak area native/IS normalized to concentration of both native compounds and IS in calibration standard(s) used) as it is defined in EPA methods. The second point in the linear range of the calibration curve (i.e., the concentration range where the relative response factor is constant) was set as the instrumental LOQ with an auxiliary criterion of $S/N > 10$ in the real sample. The recalculated LOQs for 100 mL sample aliquots with a final sample volume of 1 mL ranged from 0.05 to 50 ng L^{-1} (median 5 ng L^{-1}) (Table 3). Sixty-eight of the 100 compounds showed LOQs $\leq$ 5 ng L^{-1}, 18 had an LOQ of 10 ng L^{-1}, and 14 analytes had an LOQ of 50 ng L^{-1}. In the real

samples, the LOQs are calculated the same way as concentration of analytes but peak area corresponding to instrument LOQ is taken for the calculation instead of peak area of native compound. It means that all compounds in each sample have individual LOQs. Medians of LOQs obtained in validation data set (triplicates of no fortified matrix waters) are given in Table 3. These data were calculated using matrix matched standards and recovery. The highest LOQ values, i.e., the worst sensitivities, were found for Milli-Q and surface water due to the low recoveries of some compounds which were omitted from the method (see later). The variation in the LOQ was expected due to the wide selection of pharmaceuticals and was satisfactory for the main target to produce a simple single-run method. In addition, the LOQ value is a confusing parameter due to its relevancy. An LOQ of 50 ng L^{-1} is acceptable for antibiotic determination in waste water, whereas 5 ng L^{-1} is inadequate for hormones in surface water.

3.3.3. Recovery and matrix effect

The absolute recoveries in Milli-Q water, surface water, and sewage effluent are given in Table 4. The recoveries ranged from 5.2% for Perphenazine in surface water to 246% for Furosemide in effluent. There are greater differences among the matrices than between concentration levels of the same matrix. Atorvastatin and Terbutalin exhibited absolute recovery lower than 40% in Milli-Q water (20 and 27%). In the case of Atorvastatin, it can be addressed to ion suppression effect of coeluting pharmaceuticals. The ratio Atorvastatin to IS measured for individual compound is about 40% higher than the same ration measured in mixture of all pharmaceuticals. This effect is eliminated in the presence of waste water matrix. However, Atorvastatin was removed from the method for surface water due to both low recovery and high variability of results. Terbutalin as the second eluted compound cannot be affected the same way. Its recoveries are low in all matrices even using matrix matched standard and this compound was removed from the method.

The group of compounds with much lower recoveries in surface water than in other two matrices was found (Amiodarone, Dihydroergotamin, Dipyridamol, Chlorpromazine, Fluphenazine, Flupetixol, Perphenazine, Ranitidine). The data were recalculated to relative recoveries using the recovery of the native analogues of internal standards (IS) as a calculation factor. A summary of the recalculated data is given in Supplementary Table S2. Some of the lowest recoveries were enhanced but do not represent a real improvement. To distinguish between ion suppression or enhancement and other changes in the recovery, matrix-matched standards were used. The recoveries that were obtained with matrix-matched standards are given in Supplementary Table S3. The comparison of both data sets for all matrices is shown in Fig. 4. Box graphs show that variation in the recoveries is significantly lower for the matrix matched standard calculated data with some outlaying extremes. The recoveries of above mentioned compounds did not improve in surface water but it was satisfactory in waste water. It can be concluded that ion suppression in waste water is eliminated with using matrix matched standards. Low recoveries in surface water are obviously caused by different effects (ionization suppression from other pharmaceuticals as in the case of Atorvastatin was not confirmed by single compounds/mixture measurements). Amiodarone and Perphenazine showed high uncertainty of determination so they were removed from the final method for both matrices. Dihydroergotamin was omitted from the method too due to both low recovery and nonlinear response. Chlorpromazine, Dipyridamol

Table 4
Recoveries and recovery uncertainties of pharmaceuticals from different matrices

Analyte	Milli-Q water		Surface water		Surface water		Effluent		Effluent	
	100 ng L^{-1} Recovery	$n=10$ RSD (%)	100 ng L^{-1} Recovery	$n=10$ RSD (%)	500 ng L^{-1} Recovery	$n=10$ RSD (%)	1000 ng L^{-1} Recovery	$n=10$ RSD (%)	2500 ng L^{-1} Recovery	$n=10$ RSD (%)
Alfuzosin	111	10	92	4.2	103	3.1	70	11	81	9.7
Alprazolam	101	12	82	6.7	101	4.5	68	5.5	67	8.8
Amiodarone	42	36	22	27	11	23	38	37	40	59
Amytriptyline	85	2.4	98	9.7	100	4.9	102	3.8	91	7.1
Atenolol	58	15	56	8.5	65	10	60	13	35	13
Atorvastatin	20	62	26	48	34	12	39	25	51	17
Atracurium	108	11	91	3.8	83	5.6	90	5.8	79	7.2
Azelastine	112	16	71	4.6	79	1.0	87	15	103	13
Azithromycine	72	19	63	26	60	11	105	7.9	103	6.5
Beclomethasone	106	13	119	25	105	8.6	151	31	132	8.3
Bezafibrate	80	5.0	117	17	105	15	58	23	55	17
Biperiden	91	6.0	110	5.7	104	4.5	104	4.1	97	4.1
Bisoprolol	99	18	97	7.4	107	6.5	126	3.4	164	8.7
Bromocriptin	84	11	47	29	38	12	59	28	80	33
Budesonide	86	14	132	25	104	14	90	8.4	101	15
Buprenorphin	99	14	82	8.8	88	4.6	57	16	70	15
Bupropion	115	11	99	4.6	105	6.3	89	9.6	107	9.2
Carbamazepin	107	10	88	5.4	110	3.2	112	6.9	109	7.7
Chlorpromazine	58	16	21	49	24	56	85	9.0	73	8.7
Chlorprothixen	85	18	63	6.3	62	11	75	12	74	12
Cilazapril	127	15	102	6.5	138	4.5	120	2.7	158	9.2
Ciprofloxacin	87	8.9	68	14	36	35	81	20	88	21
Citaprolam	119	12	84	4.9	100	2.3	60	17	70	11
Clarithromycin	74	38	73	26	89	11	112	11	67	18
Clemastine	100	23	60	21	56	11	85	33	66	11
Clindamycine	98	19	58	18	111	5.3	128	4.7	139	9.6
Clomipramine	93	12	95	5.4	92	5.0	89	9.2	86	9.2
Clonazepame	119	16	96	11	90	5.5	73	11	91	10
Clotrimazol	84	3.4	106	3.5	99	5.5	82	9.5	82	7.3
Codeine	82	15	104	10	96	5.1	92	7.8	89	9.7
Cyproheptadine	115	14	86	7.5	98	8.2	80	14	124	13
Desloratidine	102	6.8	81	8.3	79	6.2	108	5.2	95	3.2
Diclofenac	67	19	104	13	93	20	78	16	82	27
Dicycloverin	125	16	81	5.8	76	1.2	125	27	99	6.5

Table 4 (*continued*)

Analyte	Milli-Q water		Surface water		Surface water		Effluent		Effluent	
	100 ng L^{-1} Recovery	$n=10$ RSD (%)	100 ng L^{-1} Recovery	$n=10$ RSD (%)	500 ng L^{-1} Recovery	$n=10$ RSD (%)	1000 ng L^{-1} Recovery	$n=10$ RSD (%)	2500 ng L^{-1} Recovery	$n=10$ RSD (%)
Dihydroergotamine	57	21	18	35	13	11	54	26	69	18
Diltiazem	107	7.2	88	3.4	99	3.5	84	18	117	9.9
Diphenhydramine	114	21	87	6.2	95	6.0	74	12	95	9.9
Dipyridamol	82	14	45	47	20	55	150	17	126	14
Duloxetin	101	22	62	15	90	5.2	96	15	132	17
Eprosartan	80	7.2	81	6.4	92	8.2	113	17	98	9.3
Etonogestrel	77	20	111	11	88	8.8	88	15	83	8.3
Ezetimibe	57	12	113	26	40	20	50	27	35	28
Fentanyl	90	5.0	74	7.2	75	10	82	12	81	9.3
Fenofibrate	40	40	52	16	24	37	62	27	41	38
Fexofenadine	71	7.9	100	3.8	106	6.2	113	8.4	99	7.9
Finasteride	95	13	90	9.4	75	4.3	90	37	64	9.1
Flecainide	121	12	98	5.8	95	4.1	85	9.7	112	8.2
Fluconazole	115	10	128	5.4	122	4.4	157	6.5	107	5.4
Fluoxetine	94	4.7	107	8.1	85	22	110	5.3	109	9.5
Flupentixol	90	12	26	22	18	12	77	37	41	26
Fluphenazine	85	10	16	20	18	11	72	36	43	24
Flutamide	90	6.9	100	13	110	5.5	93	8.5	81	9.1
Furosemid	108	12	64	83	69	49	246	28	252	24
Glibenclamide	77	14	70	19	52	28	62	50	41	36
Glimepiride	82	18	65	14	54	19	62	48	41	27
Haloperidol	106	7.3	80	4.7	85	2.9	54	24	106	17
Hydroxyzine	93	4.4	100	3.9	102	5.6	115	4.5	97	3.4
Irbesartan	82	5.4	103	5.5	124	6.0	109	4.0	96	6.1
Ketoconazole	63	11	60	24	53	8.1	105	8.1	88	13
Levonorgestrel	70	15	121	25	106	6.7	107	29	104	6.5
Loperamide	95	12	94	6.3	77	12	71	18	65	16
Maprotiline	85	2.5	103	4.2	100	4.7	102	3.9	91	7.3
Meclozine	117	12	43	19	32	19	62	44	34	38
Medroxyprogesterone	68	14	102	9.8	104	7.3	102	30	98	16
Megestrol	87	17	103	9.6	103	6.4	85	32	88	12
Memantin	124	13	106	6.9	95	3.4	116	7.7	122	9.2
Metoprolol	108	7.6	104	6.3	99	8.2	151	5.8	127	35
Mianserin	114	11	73	3.6	82	2.2	71	12	119	11
Miconazole	75	23	46	14	34	19	45	28	76	34
Mirtazapine	112	11	86	4.0	42	12	104	2.7	100	6.6
Morphine	60	13	37	7.9	40	13	42	14	54	12
Naloxon	80	20	117	7.9	107	7.3	101	4.1	76	11
Nefazodon	71	13	68	6.0	72	8.7	67	18	78	16
Norfloxacin	92	8.4	68	18	50	32	104	21	92	27
Ofloxacin	81	4.9	57	11	45	33	47	20	31	20
Orphenadrine	82	15	113	9.0	102	6.4	105	8.0	83	4.1
Oxazepam	94	3.3	96	7.0	102	3.9	126	17	82	9.2
Paroxetin	71	14	80	8.1	81	5.1	95	7.7	78	7.3
Perphenazine	69	14	5.2	46	6.1	44	77	35	65	24
Pizotifen	76	14	92	4.2	98	4.9	96	4.0	88	5.5
Progesterone	80	12	93	6.9	98	6.4	82	31	90	26
Promethazine	72	10	41	27	63	11	88	4.2	74	8.0
Ranitidine	52	20	16	42	16	7.2	61	9.7	55	32
Repaglinide	89	5.5	113	3.5	117	7.0	112	2.8	96	4.1
Risperidone	98	2.2	95	4.1	100	3.7	103	2.9	89	3.0
Rosuvastatin	100	12	103	11	170	4.0	102	14	101	9.8
Roxithromycin	66	32	75	25	81	10	98	8.6	72	15
Sertraline	92	15	101	6.2	89	12	84	11	83	14
Sotalol	80	10	88	6.2	81	11	106	6.9	104	13
Sulfamethoxazole	95	2.9	97	8.4	101	5.6	103	5.9	90	7.5
Tamoxifen	96	3.6	109	4.0	121	5.6	133	6.3	111	13
Telmisartan	74	12	63	11	75	14	66	19	92	16
Terbutaline	27	20	29	12	38	16	35	7.9	19	13
Tramadol	100	10	100	16	102	4.3	116	9.7	86	13
Trihexyphenidyl	94	6.5	120	4.5	102	4.8	100	6.9	93	4.3
Trimethoprim	96	6.4	100	3.6	103	2.7	106	2.9	93	4.9
Venlafaxine	124	17	96	4.6	102	7.7	102	5.5	100	7.6
Verapamil	116	10	101	5.7	99	4.2	110	13	122	12
Zolpidem	115	11	103	4.0	109	3.6	91	4.8	116	10
Zuclopenthixol	66	15	30	19	45	15	69	19	76	12
Average	89	14	81	14	80	11	91	15	88	14
Median	90	12	88	8.4	90	7.1	90	12	88	11
Max. value	127	62	132	83	170	56	246	50	252	59
Min. value	20	2.2	5.0	3.4	6.0	1.0	35	2.7	19	3.0

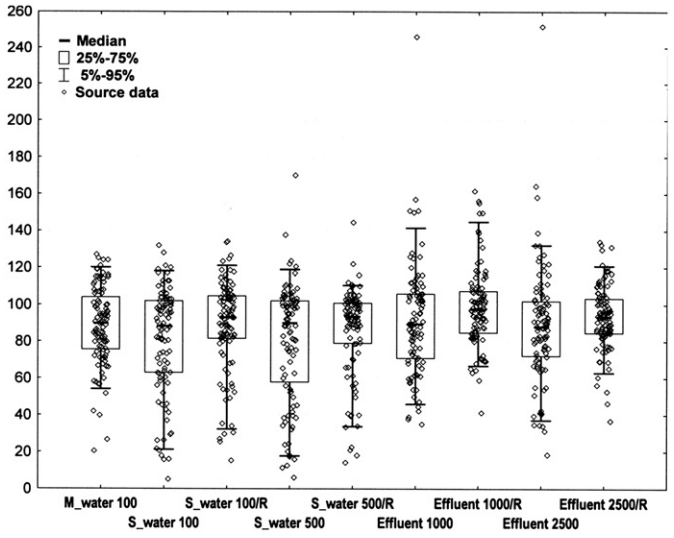

Fig. 4. The comparison of absolute recoveries and recoveries recalculated to matrix matched standard. Recalculated data are indicated with *R* in the name. The line in the box represents the median of the data set; the box is the 50th percentile; the range between bottom and upper line segments represents 90th percentile; and individual points are projected as empty rhombi.

and Ranitidine were removed from the surface water method for the same reasons as Amiodarone and Perphenazine but the results for waste water are acceptable. Low recovery for Atorvastatin and Ranitidine in surface water (40% and 50%, respectively) was also found by Gross [10]. Ionization suppression was not identified as the reason of low recoveries for above mentioned pharmaceuticals group. The sorption on glass surface during sample handling can be excluded too because of good recoveries in Milli-Q water. Humic acid mediated degradation (sorption) of some pharmaceuticals was described recently [30,31]. Low extraction efficiency in Swedish river water could be related to sorption or binding the pharmaceuticals to humic acids.

Excessively high recoveries of Furosemide in waste water were caused by ionization enhancement in this matrix; recoveries using the matrix-matched standard were 94.1% and 96% instead of 246% and 252%. Furosemide in surface water and Glibenclamide, Meclozine, and Glimepiride in waste water showed high variability in the results despite the fact that they had good recoveries (RSD > 30% at both concentration levels). Those pharmaceuticals have not been included to the method for corresponding matrices too. Finally, 91 compounds in surface water and 93 compounds in waste water showed acceptable performance. Cut off criterions were set as follows: 40–140% recovery at least at one of the concentration levels analyzed and RSD lower than 30%. Fifty-two of 100 studied pharmaceuticals were lacking analytical protocol for environmental matrices (water or soil), 49 of them remains in surface water method and 47 in waste water method.

The method was successfully applied for the screening of pharmaceuticals presence in effluent from small WWTPs in Sweden. The results of this screening are given in Supplementary Table S4. Forty-seven to 66 target compounds were found above LOQ (45–64 compounds when omitted pharmaceuticals are not included) in six WWTPs effluent analyzed.

4. Conclusions

An efficient LC/MS/MS method based on one injection, one pre-treatment protocol and a 15 min retention time was developed. The method measures multiple ecotoxicologically relevant pharmaceuticals. Due to the selection approach based on the potential of the pharmaceuticals to bioconcentrate in fish, the included compounds represent 27 different classes with a wide variety of physico-chemical properties. Forty-nine pharmaceuticals in surface water (47 pharmaceuticals in waste water respectively) previously lacking an analytical protocol for their determination in environmental samples are included in this method. The use of internal standard addition combined with matrix-matched standards resolves most of the challenges with ionization suppression or enhancement. Both of the above mentioned approaches must be included in the quantification method, i.e. preparation of matrix matched standard for each series of samples and each analyzed matrix.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.08.032.

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