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Fully automated determination of 74 pharmaceuticals in environmental and waste waters by online solid phase extraction–liquid chromatography-electrospray–tandem mass spectrometry

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ABSTRACT

The present work describes the development of a fully automated method, based on on-line solid-phase extraction (SPE)–liquid chromatography-electrospray–tandem mass spectrometry (LC–MS–MS), for the determination of 74 pharmaceuticals in environmental waters (superficial water and groundwater) as well as sewage waters. On-line SPE is performed by passing 2.5 mL of the water sample through a HySphere Resin GP cartridge. For unequivocal identification and confirmation two selected reaction monitoring (SRM) transitions are monitored per compound, thus four identification points are achieved. Quantification is performed by the internal standard approach, indispensable to correct the losses during the solid phase extraction, as well as the matrix effects. The main advantages of the method developed are high sensitivity (limits of detection in the low ng L^{-1} range), selectivity due the use of tandem mass spectrometry and reliability due the use of 51 surrogates and minimum sample manipulation. As a part of the validation procedure, the method developed has been applied to the analysis of various environmental and sewage samples from a Spanish river and a sewage treatment plant.

1. Introduction

It is estimated that approximately 3000 different substances are used as pharmaceutical ingredients worldwide today. However, only a small subset of these compounds (~150) has been investigated in environmental studies. The worldwide average per capita consumption of pharmaceuticals per year is estimated to be about 15 g, but in industrialized countries the value is much higher and is estimated to be between 50 and 150 g. After administration, most pharmaceuticals are not completely metabolized. The unmetabolized parent drugs and some metabolites are subsequently excreted from the body via urine and faeces [1] reaching the Wastewater Treatment Plants (WWTPs) via wastewater. Reports have shown that many pharmaceuticals do not degrade during municipal conventional wastewater treatment [2–8] being, therefore, discharged to the receiving waters. Recent data indicate that, as much as, 80% of the total load of pharmaceuticals entering a WWTP may be discharged into surface water [9,10]. Disposal of unused or unwanted medications to the toilet or household waste is another route of their entry to the environment.

The concentrations of individual compounds in surface waters are typically in the range of several tens to hundreds of ng L^{-1}, although concentrations at the μg L^{-1} level are also reported for some compounds and specific sites [11]. Generally, these concentrations are lower than typical maximum concentrations (in the tens of μg L^{-1}) reported for some industrial contaminants (e.g. surfactants, plasticizers), but due to their continuous introduction into the environment and bioactive properties, pharmaceuticals may pose a risk to the aquatic organisms and ultimate to humans. One of main concerns is contamination of groundwater through surface water filtration and landfill leakage [1].

Generally, very little is known about the long-term effect and behaviour of pharmaceutical residues in the aquatic environment [12], and in groundwater in particular [13]. In addition, environmental risk assessment is often carried out for individual pharmaceutical compound (active ingredients), while pharmaceutical compounds are typically detected in mixtures with other anthropogenic contaminants [11]. Studies have shown that combinations of pharmaceutical compounds exert a much stronger toxic effect that could be expected from the weak toxic effects related to

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exposure to each compound individually [14,15], i.e., the combination of drugs that share a common mechanism of action exhibits synergistic effects [16].

Therefore, monitoring of wide-range pharmaceuticals in surface and ground waters is as a prerequisite for proper risk assessment. Nowadays, a large number of analytical methodologies, mainly using liquid chromatography–tandem mass spectrometry (LC–MS/MS), are already available for pharmaceutical determination in both environmental and wastewaters [17–19] being antibiotics the most widely studied group [20–22]. Majority of the methods employ rather tedious and time-consuming sample preparation based on off-line solid-phase extraction (SPE). However, the growing number of samples to be analyzed in laboratories carrying out monitoring studies requires employment of high-throughput and fully automated analytical techniques. Because of these reasons, great effort is going into the development of fast, cost-effective and "greener" alternative methods for environmental analysis. Over the past several years, there has been an increase in the use of automated instruments that integrate extraction, purification and detection step (i.e. on-line solid phase extraction systems such as Symbiosis™ and Prospekt-2 systems manufactured by Spark Holland). On line SPE followed by LC–MS/MS that has been used to analyze trace emerging contaminants in water, such as drugs of abuse, pesticides, and hormones [23–27]. With respect to the analysis of pharmaceuticals in aqueous environmental samples several papers were published recently [24,27–29]. For example [28], used on-line SPE in the analysis of six pharmaceutical indicators in water, while [29] reported on the application on line SPE for the analysis of macrolide antibiotics.

In this work, a reliable, fully automated method for the determination of 74 pharmaceuticals in environmental waters (groundwater (GW) and superficial water (SW)) and wastewater (WWTP effluent (WWE) and WWTP influent (WWI) has been developed, validated and applied to real samples. Target compounds, which are listed in Table 1, belong to different medicinal classes and were selected based on their high human consumption, ecotoxicological relevance and ubiquity in the aquatic environment, according to the information found in the scientific literature [30–42].

The objective of this work is to develop an analytical method for simultaneous analysis of a large number of target compounds belonging to different therapeutical classes, that will have clear advantages and improvements over existing methods in terms of (i) minimum sample manipulation; (ii) maximum sensitivity; (ii) reliability, and (iv) selectivity and thus to fulfil the stringent criteria set by the EU regulations (EU Commission Decision 2002/657/EC) [43].

The developed method was successfully applied to the analysis of pharmaceutical residues in WWTP as well as river and drinking water samples.

2. Material and methods

2.1. Chemicals

All pharmaceutical standards were of high purity grade (>90%) and are listed in the Supplementary data 1.

Both individual stock standard and isotopically labelled internal standard solutions were prepared on a weight basis in methanol (MeOH), except fluoroquinolones which were dissolved in a water–methanol (H_2O/MeOH) mixture (1:1) containing 0.2% (v/v) hydrochloric acid, as they are slightly soluble in pure MeOH [44]. After preparation, standards were stored at −20 °C. Special precautions have to be taken into account for tetracycline antibiotics, which have to be stored in the dark in order to avoid their exposure to the light, since it has been demonstrated that they are liable to photodegradation [45]. Fresh stock solutions of antibiotics were prepared monthly due to their limited stability while stock solutions for the rest of substances was renewed every three months. On the other hand, compounds with number (see Table 1) 26, 5, 10, 12 and 8, were obtained as solutions in acetonitrile (ACN), while 67 and 65 were dissolved in MeOH, at a concentration of 1 mg mL^{-1}.

A mixture of all pharmaceuticals was prepared by appropriate dilution of individual stock solutions in MeOH/H_2O (25:75, v/v). Working standard solutions, also prepared in MeOH/H_2O (25:75, v/v) mixture, were renewed before each analytical run. Working solutions were prepared in amber glass vials while standard mixtures were prepared in volumetric flasks wrapped with aluminium foil, in order to prevent the exposure of tetracycline antibiotics to light. A separate mixture of isotopically labelled internal standards, used for internal standard calibration, was prepared in MeOH and further dilutions also in MeOH/H_2O (25:75, v/v) mixture.

HPLC grade MeOH, ACN, water, hydrochloric acid 37% and formic acid 98% were supplied by Merck (Darmstadt, Germany). Ethylenediaminetetraacetic acid disodium salt dehydrate (thereon Na_2EDTA) was 99% from Sigma–Aldrich (Steinham, Germany). Nitrogen for drying 99.995% of purity was from Air Liquide (Madrid, Spain).

2.2. Sample pre-treatment

The method was optimized using groundwater, river water, WWTP influent and effluent. Amber glass bottles pre-rinsed with ultra-pure water were used for sample collection. Water samples were filtered through 1 μm fiberglass filters from Whatman (Fairfield, Connecticut, USA) followed by 0.45 μm nylon membrane filters from Teknokroma (Barcelona, Spain). Na_2EDTA 0.1% (m/v) was added to all samples in order to form complexes with inorganic elements. As it is indicated in [19], this addition improves in a great extent the extraction efficiency of tetracycline, macrolide and fluoroquinolone antibiotics. This could be explained by the fact that these compounds can potentially bind residual metals present in the sample matrix and glassware, resulting in low extraction recoveries [46–50]. The amount of Na_2EDTA added was the same for all types of water analyzed and was considered to be sufficient to enabled formation of complexes with inorganic compounds in all types of matrices, even in waters with high mineral content. Finally, 200 μL of a 0.05 ng μL^{-1} standard mixture containing 37 surrogates for the analysis in positive ion (PI) mode, and 14 surrogates for the analysis in negative ion (NI) mode (see Table 1), were added in every 100 mL of sample for surrogate control and internal standard calibration.

2.3. On-line trace enrichment

Preconcentration of the samples and its chromatographic separation was performed using an automated on-line SPE–LC device Symbiosis™ Pico from Spark Holland (Emmen, The Netherlands). The base of the Symbiosis™ Pico system is a high-end HPLC system with a high performance injector that handles sample volumes from 10 μL up to 10 mL fully automated. This equipment also counts with the Alias™ autosampler that includes positive headspace pressure, extensive wash routines for minimal carry over and 2 injection modes, offline and online SPE. Offline mode was only used in the optimization procedure to assess the recovery by comparing the peak areas obtained in the on-line analyses of spiked waters samples with those obtained from the injection of standard mixtures of the analytes in MeOH/H_2O (25:75, v/v) at equivalent concentrations.

A meticulous experiment design was carried out to optimize SPE (see Table 2A and B). Three different disposable trace enrichment

Table 1
Target compounds organized in their therapeutical groups and their assigned surrogates.

Therapeutic groups	Compounds	Number	CAS Number	Corresponding surrogate
Analgesics/anti-inflammatories (12)	Ketoprofen	1	22071-15-4	*Ketoprofen-13C-d3*
	Naproxen	2	22204-53-1	*Naproxen-d3*
	Ibuprofen	3	15687-27-1	*Ibuprofen-d3*
	Indomethacin	4	53-86-1	*Indomethazine-d4*
	Diclofenac	5	15307-86-5	*Diclofenac-d4*
	Mefenamic acid	6	61-68-7	*Mefenamic acid-d3*
	Acetaminophen	7	103-90-2	*Acetaminophen-d4*
	Salicylic acid	8	69-72-7	*Salicylic acid-α-13C*
	Propyphenazone	9	479-92-5	*Antipyrine-d3*
	Phenylbutazone	10	50-33-9	
	Phenazone	11	60-80-0	
	Codeine	12	76-57-3	*Codeine-d3*
Lipid regulators and cholesterol lowering stain drugs (7)	Clofibric acid	13	882-09-7	*Clofibric acid-d4*
	Bezafibrate	14	41859-67-0	*Bezafibrate-d4*
	Fenofibrate	15	49562-28-9	*Fenofibrate-d6*
	Gemfibrozil	16	25812-30-0	*Gemfibrozil-d6*
	Mevastatin	17	73573-88-3	*Carbamazepine-d10*
	Pravastatin	18	81093-37-0	*Pravastatin-d3*
	Atorvastatin	19	134523-00-5	*Atorvastatin-d5*
Psychiatric drugs (5)	Paroxetine	20	61869-08-7	*Paroxetine-d4*
	Fluoxetine	21	54910-89-3	*Fluoxetine-d5*
	Diazepam	22	439-14-5	*Diazepam-d5*
	Lorazepam	23	846-49-1	
	Carbamazepine	24	298-46-4	*Carbamazepine-d10*
Histamine H2 receptor antagonists (4)	Loratadine	25	79794-75-5	*Loratadine-d4*
	Famotidine	26	76824-35-6	*Famotidine-13C3*
	Ranitidine	27	66357-35-5	*Ranitidine-d6*
	Cimetidine	28	51481-61-9	*Cimetidine-d3*
Tetracycline antibiotics (4)	Tetracycline	29	60-54-8	*Demeclocycline*
	Doxycycline	30	564-25-0	
	Oxytetracycline	31	79-57-2	
	Chlortetracycline	32	57-62-5	
Macrolide antibiotics (4)	Erythromycin	33	114-07-8	*Erythromycin-13C-d3*
	Azithromycin	34	83905-01-5	*Azithromycin-d3*
	Tilmicosin	35	10850-54-0	
	Roxithromycin	36	80214-83-1	*Clarithromycin-N-methyl-d3*
	Clarithromycin	37	81103-11-9	
	Josamycin	38	16846-24-5	
	Tylosin A	39	1401-69-0	
	Spiramycin	40	8025-81-8	*Spiramycin-d3*
Sulfonamide antibiotics (3)	Sulfamethoxazole	41	723-46-6	*Sulfamethoxazole-d4*
	Sulfadiazine	42	68-35-9	*Sulfadiazine-d4*
	Sulfamethazine	43	57-68-1	*Sulfamethazine-d4*
Fluoroquinolones antibiotics (7)	Danofloxacin	44	112398-08-0	*Ofloxacin-d8*
	Enoxacin	45	74011-58-8	
	Ofloxacin	46	82419-36-1	
	Ciprofloxacin	47	85721-33-1	*Ciprofloxacin-d8*
	Enrofloxacin	48	93106-60-6	*Enrofloxacin-d5*
	Norfloxacin	49	70458-96-7	*Norfloxacin-d5*
	Flumequine	50	42835-25-6	*Flumequine-13C3*
Other antibiotics (4)	Trimethoprim	51	738-70-5	*Carbamazepine-d10*
	Nifuroxazide	52	965-52-6	
	Chloramphenicol	53	56-75-7	*Ibuprofen-d3*
	Metronidazole	54	443-48-1	*Metronidazole-hydroxy-d2*
β-Blockers (9)	Atenolol	55	29122-68-7	*Atenolol-d7*
	Betaxolol	56	63659-18-7	
	Carazolol	57	57775-29-8	
	Pindolol	58	13523-86-9	
	Nadolol	59	42200-33-9	
	Timolol	60	26839-75-8	*Timolol-d5*
	Sotalol	61	3930-20-9	*Sotalol-d6*
	Metoprolol	62	37350-58-6	*Metoprolol-d7*
	Propranolol	63	525-66-6	*Propranolol-d7*
β-Agonists (2)	Salbutamol	64	18559-94-9	*Albuterol-d3*
	Clenbuterol	65	37148-27-9	*Clenbuterol-d9*
Barbiturates (3)	Butalbital	66	77-26-9	*Phenobarbital-d5*
	Pentobarbital	67	76-74-4	
	Phenobarbital	68	50-06-6	
Antihypertensives (3)	Enalapril	69	75847-73-3	*Enalapril-d5*
	Hydrochlorothiazide	70	58-93-5	*Hydrochlorothiazide-d2*
	Lisinopril	71	83915-83-7	*Atenolol-d7*
Dirutics (1)	Furosemide	72	54-31-9	*Furosemide-d5*
Antidiabetic (1)	Glibenclamide	73	10238-21-8	*Glyburide-d3*
To trat cancer (1)	Tamoxifen	74	10540-29-1	*Tamoxifen-(N,N-dimethyl-13C2)*

Table 2
Experiments tested during the online SPE optimization procedure.

Type of water	Type of cartridge	Sample extraction volume (mL)	Wash volume after extraction (mL)
(A) Online SPE experiments in HPLC grade water			
HPLC grade water	HySphere Resin GP	1	1
			2
		2.5	1
			2
		5	1
			2
	PRLP-s	1	1
			2
		2.5	1
			2
		5	1
			2
	Oasis HLB	1	1
			2
		2.5	1
			2
		5	1
			2
(B) Online SPE experiments in real aqueous samples			
GW	HySpere Resin GP	1	1
			2
		2.5	1
			2
		5	1
			2
SW		1	1
			2
		2.5	1
			2
		5	1
			2
WWE		1	1
			2
		2.5	1
			2
		5	1
			2
WWI		1	1
			2
		2.5	1
			2
		5	1
			2

cartridges were evaluated for their efficiency in the on-line SPE of the target pharmaceuticals from water: Oasis HLB (macroporous copolymer of divinylbenzene and *N*-vinylpyrrolidone, 30-μm particle size) from Waters Corporation (Milford, Massachusetts, USA), PLRP-s (cross-linked styrenedivinylbenzene polymer, 15–25-μm particle size) from Spark Holland (Emmen, The Netherlands), and HySphere Resin GP (polydivinyl-benzene, 5–15-μm particle size) also from Spark Holland (Emmen, The Netherlands). In order to evaluate which of these three cartridges yielded higher recoveries of target compounds, HPLC grade water was spiked with 100 ng L^{-1} of each target compound. The experiment is summarized in Table 2A. After cartridge conditioning with 2 mL of MeOH and 2 mL of water (flow rate 5 mL min^{-1}), three different sample loading volumes (1, 2.5 and 5 mL) were tested. The flow through the cartridge was in all cases 1 mL min^{-1}. Afterwards and prior to the elution, cartridges were rinsed with HPLC grade water at a flow rate of 5 mL min^{-1} to complete the transfer of the sample and remove interferences such as inorganic salts. Two wash volumes (1 and 2 mL) were tested in order to optimize it. Upon completion of each SPE protocol, the trapped analytes are eluted from the cartridge to the LC column. Two elution modes can be chosen in Symbisis™ Pico device: a "focusing" approach where a pre-selected quantity of solvent or mixture of solvents can be chosen; or a so called "standard" approach, where the full chromatographic gradient passes through the SPE cartridge before being led to the LC column. Due to the elevated number of target compounds and their different chemical properties, the last option is the more appropriate one because of the wide range of polarity given by the mixture of the mobile phases during the gradient. So, the chance of a successful elution is higher. The full eluate is conducted to the LC column where the chromatographic separation and the subsequent detection by the mass spectrometer are carried out. In meanwhile, during the elution, a new cartridge is put in place and pre-concentration of the next sample is simultaneously performed. This kind of configuration allows short cycle times, which in our approach are 30 and 37 min (the duration of the chromatographic run time) for NI and PI mode, respectively.

Once selected the cartridge which yielded the best SPE recoveries, the same extraction and wash volumes trials were carried out on real matrices (GW, SW, WWE and WWI) previously spiked with a standard mixture of target analytes at environmentally realistic concentrations: 20 and 100 ng L^{-1} for GW and SW, respectively, and 50 and 500 ng L^{-1} for WWE and WWI, respectively (see Table 2B). SPE recoveries as well as the method detection limits (MDLs) achieved in each case where the parameters observed to choose the more suitable extraction and wash volumes.

According to the results obtained by preliminary trials, HySphere Resin GP cartridge, 2.5 mL of sample extraction volume and 1 mL of cartridge wash after extraction, were selected for further experiments and analysis of water samples.

2.4. LC-ESI-(QqLIT) MS/MS analysis

For chromatographic separation, an analytical column was used: a reversed-phase Purospher Star RP-18 endcapped column (125 mm × 2.0 mm, particle size 5 μm) from Merck (Dramstadt, Germany) [19]. For MS/MS analyses, Symbiosis™ Pico was connected in series with a 4000QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer equipped with a Turbo Ion Spray source from Applied Biosystems-Sciex (Foster City, California, USA), where mass spectrometry detection is carried out. 4000QTrap is controlled by means of the Analyst 1.4.2 Software from Applied Biosystems-Sciex (Foster City, California, USA) and a companion software appendix for controlling the Symbiosis™ Pico from Spark Holland (Emmen, The Netherlands).

The chromatographic conditions were adapted from an analytical method previously developed and described elsewhere [19]. For PI mode, this involves a flow rate of 0.3 mL min^{-1}, and ACN/0.1% (v/v) formic acid as mobile phases. The proportion of the organic solvent was programmed to increase from 5 to 95% in the first 20 min and then to 100% in the following 2 min; afterward the column was readjusted to the initial conditions. These conditions were held for 10 min to allow re-equilibration of the column before the next injection. The total time of chromatographic analysis (and cartridge elution) is 37 min. In this mode 57 pharmaceuticals are analyzed. For NI mode, this involves a flow rate of 0.2 mL min^{-1}, and ACN:MeOH (1:1, v/v)/H_2O as mobile phases. The proportion of the organic solvent was programmed to increase from 20 to 80% in the first 15 min and then to 90% in the following 2 min; afterward the column was readjusted to the initial conditions by programming the amount of organic solvent to 20% in 3 min. These conditions were held for 10 min to allow re-equilibration of the column before the next injection. The total time of chromatographic analysis (and the cartridge elution) is 30 min. In this mode 17 compounds are analyzed. In both modes, the injection volume was 20 μL.

For quantitative analysis, the ESI-MS/MS method was modified and adapted from [19]. For most of compounds two SRM transitions between the precursor ion and two most abundant fragment ions were monitored (full list of SRMs and instrumental conditions are given in Supplementary data 2). Only one transition was monitored for the isotopically labelled standards since they are added in a concentration elevated enough (100 ng L^{-1}) to be reliably quantified in its more intense transition. In order to obtain additional confirmation, especially for compounds showing poor fragmentation, an Information Dependent Acquisition (IDA) experiment was performed, with SRM as the survey scan and an Enhanced Product Ion Scan (EPI), at three different collision energies, as dependent scan. The obtain spectra were compared with library data based on EPI spectra at the three collision energies used. This allows broad accomplishment of the requirements set by the EU regulations (EU Commission Decision 2002/657/EC) [43] related to identification and confirmation of pharmaceuticals in LC–tandem MS analysis.

Improvements of the existing MS/MS method included: (i) a total of 51 isotopically labelled compounds (37 in PI and 14 in NI mode) were added before the SPE, (ii) an additional compound, the antibiotic flumequine, was included; (iii) a second transition has been tuned for the hydrochlorothiazide, lisinopril, acetaminophen, pravastatin and norfloxacin. For all these ones, the selection of parent ions and optimum ionization mode were performed by infusing 100 μg L^{-1} individual standard solutions in full-scan mode at different values of declustering potential (DP). In all cases, $[M-H]^-$ for NI and $[M+H]^+$ for PI mode were selected. Subsequent identification of the two most abundant fragment ions (one for surrogate standards) and selection of the optimum collision energies (CEs) and collision cell exit potentials (CXP) for each one was carried out in the product ion scan mode, also infusing standard solutions of each individual substance.

In order to obtain enough points per peak to fulfil the European Directive and, at the same time, to get the highest sensitivity possible, the dwell time values were adjusted to 12 in PI (providing a total scan time of 2.15 s) and 31 ms for NI (with a total scan time of 2.12 s), with pauses between ranges of 2 (PI) and 5 ms (NI).

3. Results and discussion

3.1. Solid phase extraction

Three parameters were optimized for the performance of the method in environmental waters (groundwater and superficial water) and sewage water (influent and effluent to a waste water treatment plant (WWTP)): the type of cartridge, the sample extraction volume and the wash volume after extraction. SPE recoveries and method detection limits (MDLs) were the criteria used to make the more appropriate choice for every parameter.

Type of cartridge optimization: Table 2A shown the experimental set up. Extraction recovery of each compound was compared among all the experiments realized for every type of cartridge. For hydrophilic compounds, such as salbutamol, famotidine, sotalol, ranitidine, cimetidine, HySphere Resin GP cartridges are clearly the best performing cartridge. As the hydrophobia increases, the differences among the performing of the three cartridges decrease. For the most hydrophobic compounds (betaxolol, paroxetine, propyphenazone), Oasis HLB cartridges are the ones with better performing, nevertheless, differences with the other two cartridges compared are not significant, especially with HySphere Resin GP. In general, in PI as well as NI mode, the best recoveries (near 100%) were obtained for HySphere Resin GP, for a higher number of compounds. In Fig. 3 extraction recoveries of some representative compounds are shown.

Sample volume optimization: In comparison with conventional methods, where hundreds or even thousands of mL of sample were needed [7,19,51–53], in the present method, much smaller sample size (units of mL) was needed since the whole eluate goes into the analytical column. Three extraction volumes have been tested (1 mL, 2.5 mL and 5 mL). In general, volumes that gave best SPE recovery were 1 and 2.5 mL for PI mode, and 2.5 mL for NI mode. In Fig. 4A extraction yield of some representative compounds is shown.

The next step included experiments with real samples in order to check the influence of the matrix on the required sample volume, and consequently on SPE recoveries and MDLs. Less complex matrices, such as GW and SW showed the same tendency seen in experiments with HPLC grade water (as the hydrophobicity of compounds increases the required volume decreased). For the most hydrophobic compounds, 1 mL was the one that gave the best results. For samples with a complex matrix (WWE and WWI), preference of smaller volumes (1 and 2.5 mL) was even more pronounced. That can be due to signal suppression in the ESI because of the matrix (see Section 2). The bigger volume of sample is extracted, the higher amount of matrix is trapped in the cartridge that subsequently gets to the ESI source. In Fig. 4B and C, SPE recoveries comparing extraction volumes were represented for GW and WWE waters, respectively. In general, 1 and 2.5 mL were the volumes that provided the best recoveries in environmental samples (GW and SW) as well as in sewage waters (WWE and WWI) with no big differences between them, so finally, 2.5 mL was selected as the

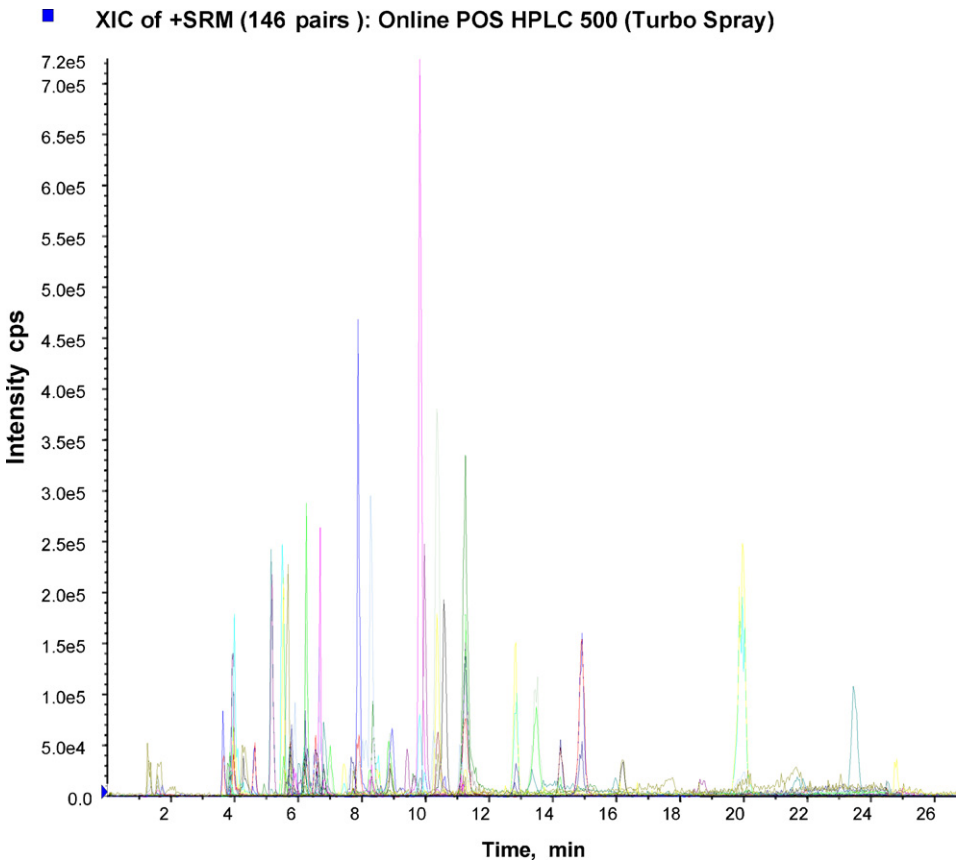

Fig. 1. Chromatogram in positive ESI mode of a HPLC grade water sample spiked with a mixture of standards at 500 ng L^{-1} after being underwent to the online-SPE extraction through GP, 2.5 mL of samples 1 mL of wash.

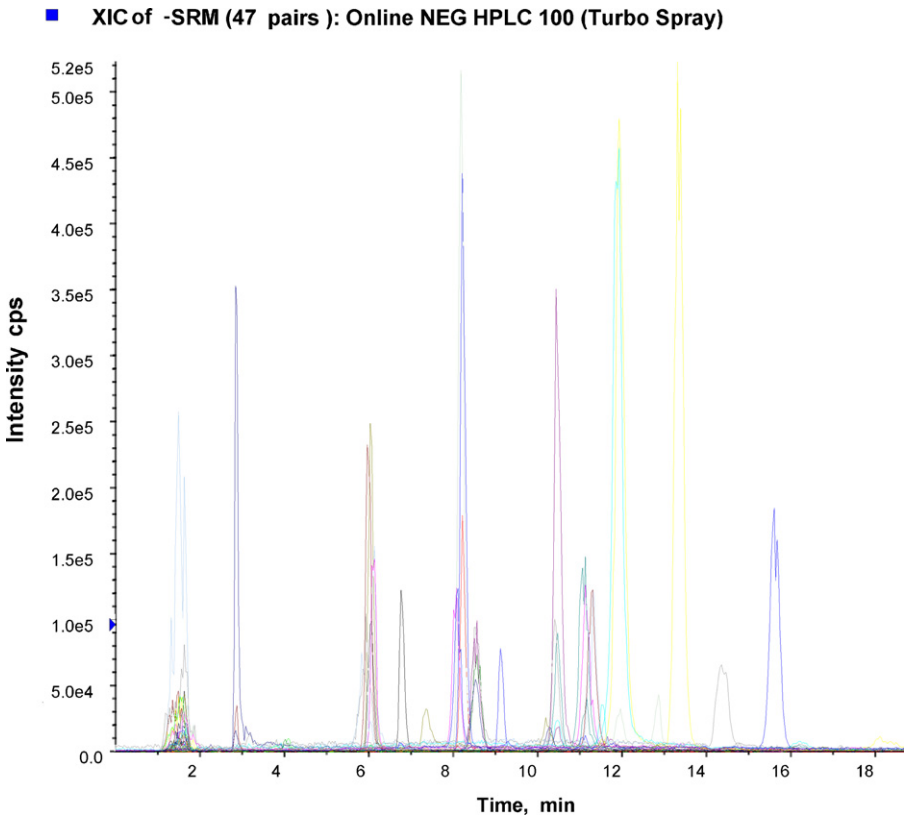

Fig. 2. Chromatogram in negative ESI mode of a HPLC grade water sample spiked with a mixture of standards at 100 ng L^{-1} after being underwent to the online-SPE extraction through GP, 2.5 mL of samples 1 mL of wash.

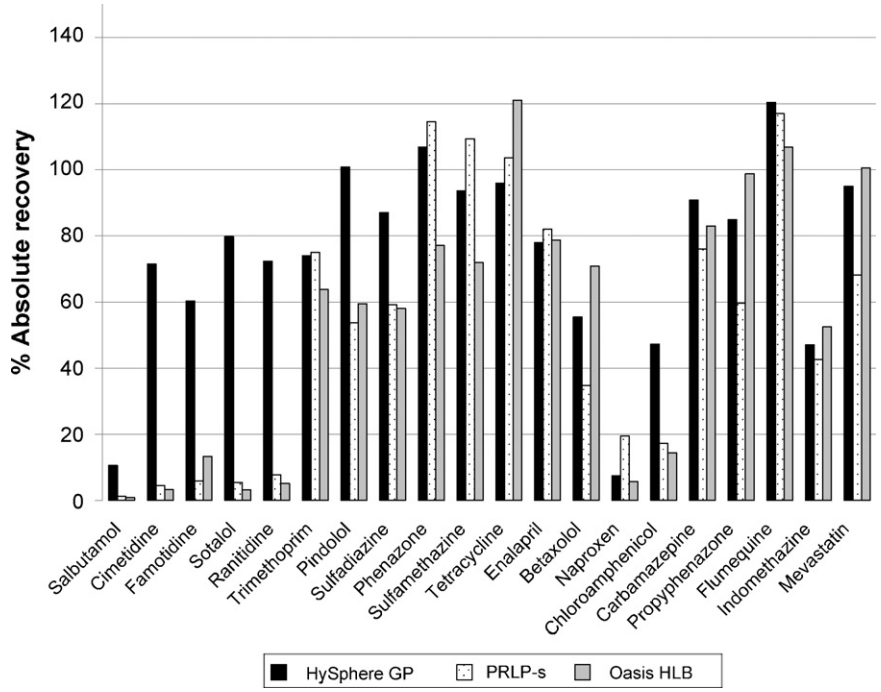

Fig. 3. Cartridge election (HPLC grade water, extraction volume 2.5 mL, wash volume 1 mL).

sample extraction volume, in PI mode as well as in NI one, for all type of samples, since it yielded better MDLs.

Wash cartridge step optimization: Two cartridge wash volumes of water were tested (1 mL and 2 mL). In spiked HPLC grade water samples (experiment in Table 2A), polar compounds gave better SPE recoveries with 1 mL (Fig. 5A). This is consistent with the fact that the solvent used for washing is water, and part of the polar compounds will run with it. For them the less washing volume used the best. For the rest of compounds this parameter is not so influential. In real samples (Table 2B), the same tendency was observed (see Fig. 5B and C). In general, washing with 1 mL of water resulted in best recovery for a higher number of compounds and was chosen for further analyses.

3.2. ESI-(QqLIT) MS/MS detection

Optimization of MS/MS parameters: In the present method, a total of 51 isotopically labelled surrogates (37 in PI and 17 in NI mode) were included which controlled all the steps the samples underwent, in comparison with only 10 internal standards added to the sample after the SPE, just before the LC–MS/MS analysis in [19] where only the matrix effect can be corrected. For a small number of compounds, the corresponding isotopically labelled compound was not commercially available or their price was extraordinarily elevated. An additional compound, the antibiotic forbidden for selling, flumequine is now included in the method. A second transition has been tuned for the hydrochlorothiazide, lisinopril, acetaminophen, pravastatin and norfloxacin improving the reliability of the method compared with [19] where only one transition was registered for those compounds.

Thus, the resulting method includes 125 substances (74 compounds and 51 surrogates), 94 of them (57 compounds and 37 surrogates) monitored in the PI mode and 31 (17 pharmaceuticals and 14 surrogates) in NI mode (Figs. 1 and 2). Transitions between the precursor ion and the two most abundant product ions for each target analyte were recorded for all compounds with the only exception of ibuprofen, phenobarbital, flumequine, ofloxacin, carbamazepine and fenofibrate, for which only one product ion could be obtained. In total, 146 transitions in positive ionization mode (corresponding to 57 compounds and 37 surrogates) and 47 transitions in negative ionization mode (17 compounds and 14 surrogates) were recorded in one single retention time window (Figs. 1 and 2). It should be remarked the fact of that elevated number of transitions were recorded in one single retention time window, without losing sensitivity, due to the setting of appropriate values for the dwell time and pause between mass ranges. Adjusting the dwell time to an appropriate value is a key parameter to monitor large number of transitions in the same time segment and still obtain enough points per chromatographic peak (>15), which is very important for a precise quantification. Dwell time in NI (31 ms) was higher than in PI (12 ms) because the number of transitions was lower, so the detector can devote more time in monitoring every transition in each cycle. Nevertheless, the ionization in PI is better than in NI mode, so the sensitivity for both modes is similar.

3.3. Method performance

Extraction recoveries for target compounds were determined for all different matrices by spiking samples ($n = 3$) at two levels of concentrations 20 ng L^{-1} and 100 ng L^{-1} for HPLC grade water, GW and SW and 50 ng L^{-1} and 500 ng L^{-1} for both WWI and WWE. Those levels were chosen as typical low and high concentrations for most of compounds in those types of waters. For each type of water samples, recoveries were determined by comparing the concentrations obtained after the whole procedure, calculated by internal standard calibration, with the initial spiking levels. As real samples (ground, surface and wastewaters) already contained target compounds, non-spiked samples were analysed in order to determine their concentrations, which were afterwards subtracted to the spiked samples. Due to huge quantity of data, and in order to be easily observed, validation parameters are presented in figures (see Figs. 6 and 7). Complete numerical data is given in Supplementary data 3. Two types of SPE recoveries are provided. Absolute recoveries, determined by comparing the peak areas obtained for spiked water samples in the on-line Symbiosis™ Pico mode of work-

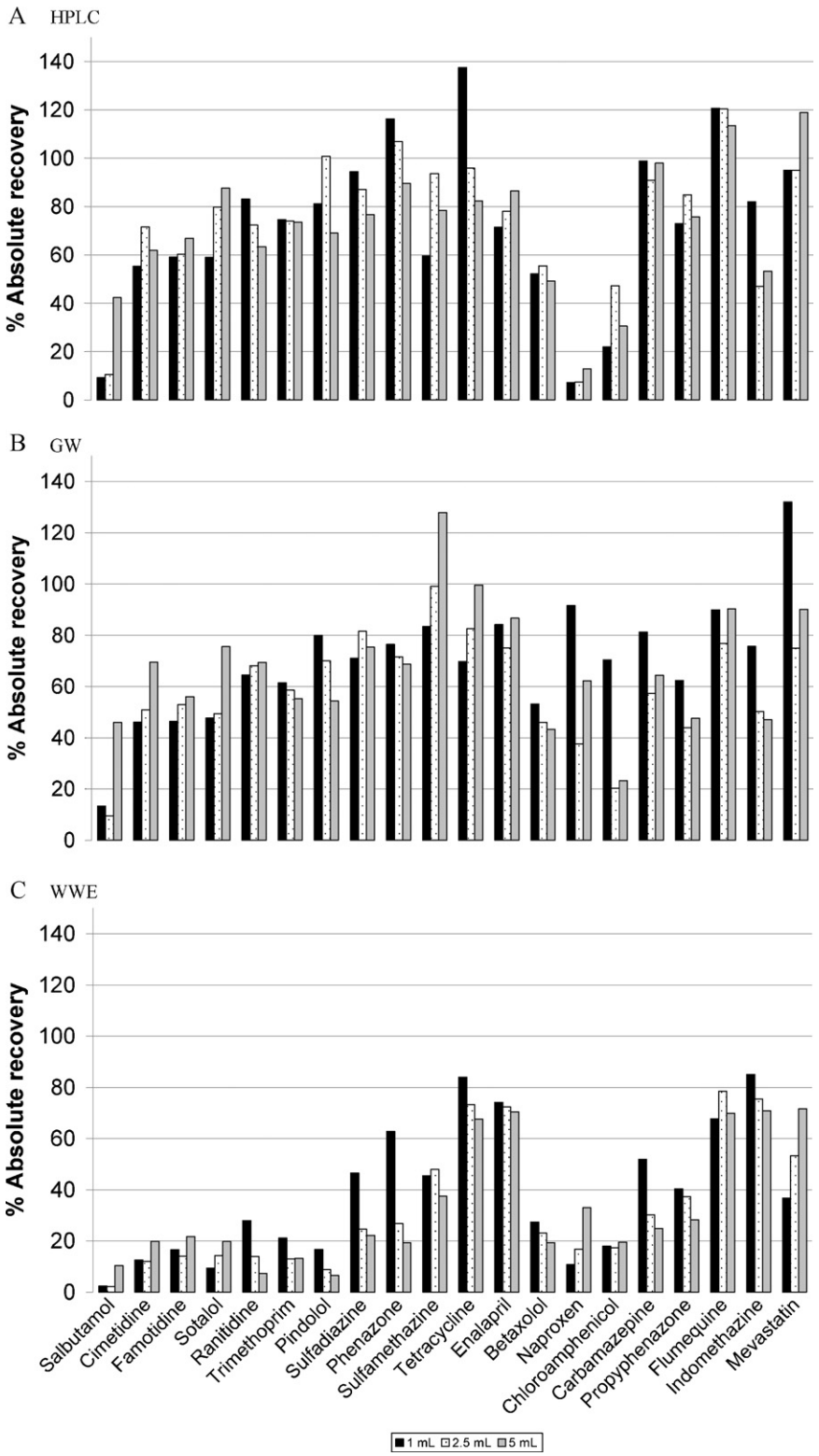

Fig. 4. Extraction volume election (GP cartridge, wash volume 1 mL).

ing, with those achieved from the injection of standards mixtures of the analytes in MeOH/H_2O (25:75, v/v) through off-line mode at equivalent concentrations. Relative recoveries were calculated afterwards by comparing absolute recoveries for every compound and its respective surrogate.

Absolute recoveries achieved were in the range of 50–150% for the 70%, 73%, 61%, 42% and 36% of target compounds in HPLC grade water, GW, SW, WWE and WWI, respectively. See Supplementary data 3A. Thus, it was clear that as the matrix was more complex, the extraction performance and/or the mass spectrometry detection got worse. For polar compounds, as salbutamol, atenolol, cimetidine, famotidine low absolute SPE recovery is obtained (10.1%, 46.0%, 14.4% and 29.2%, absolute recovery in HPLC grade water, respectively). The poor affinity for the cartridge and/or the removal

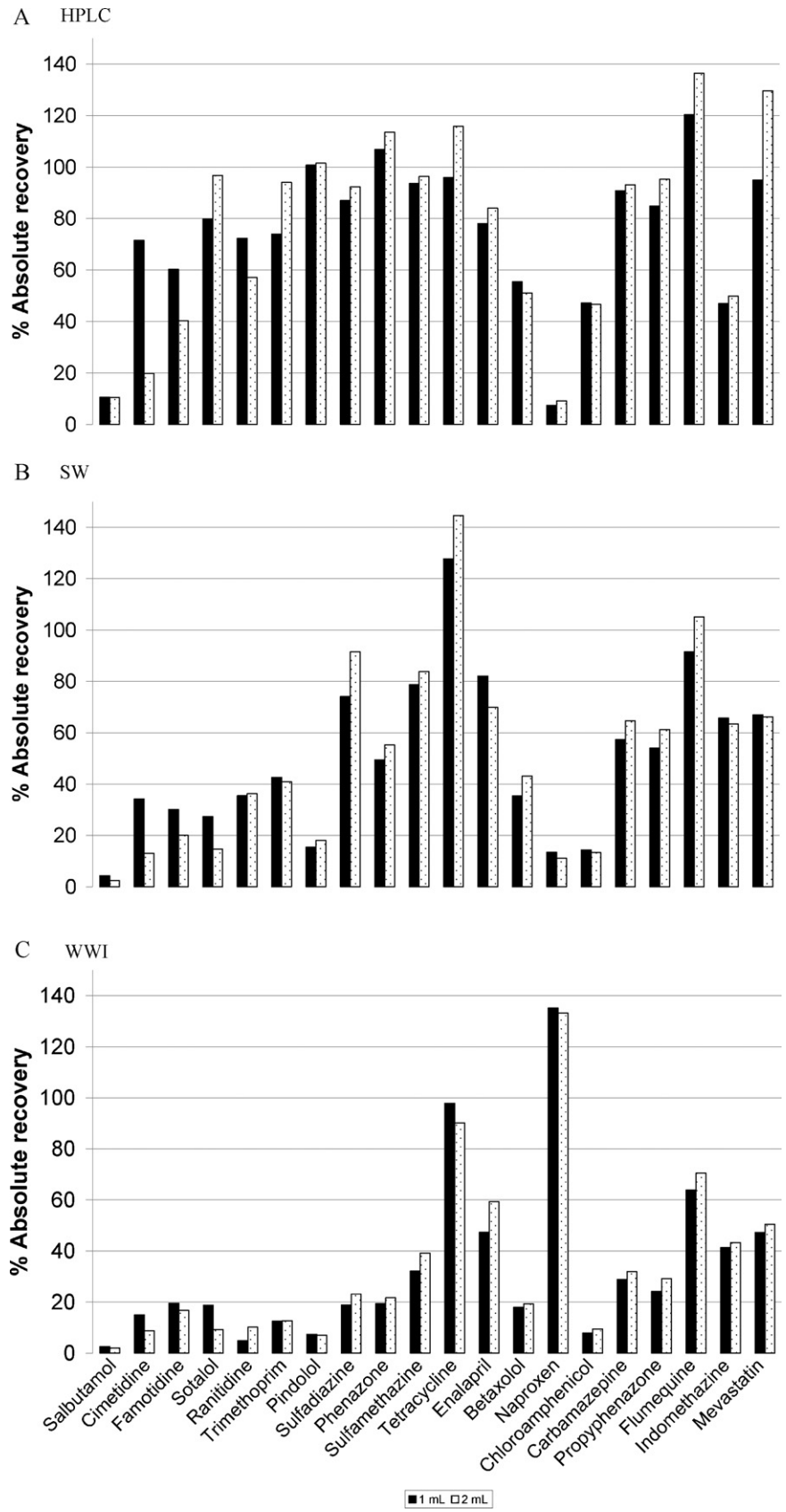

Fig. 5. Cartridge wash volume election (GP cartridge, extraction volume 2.5 mL).

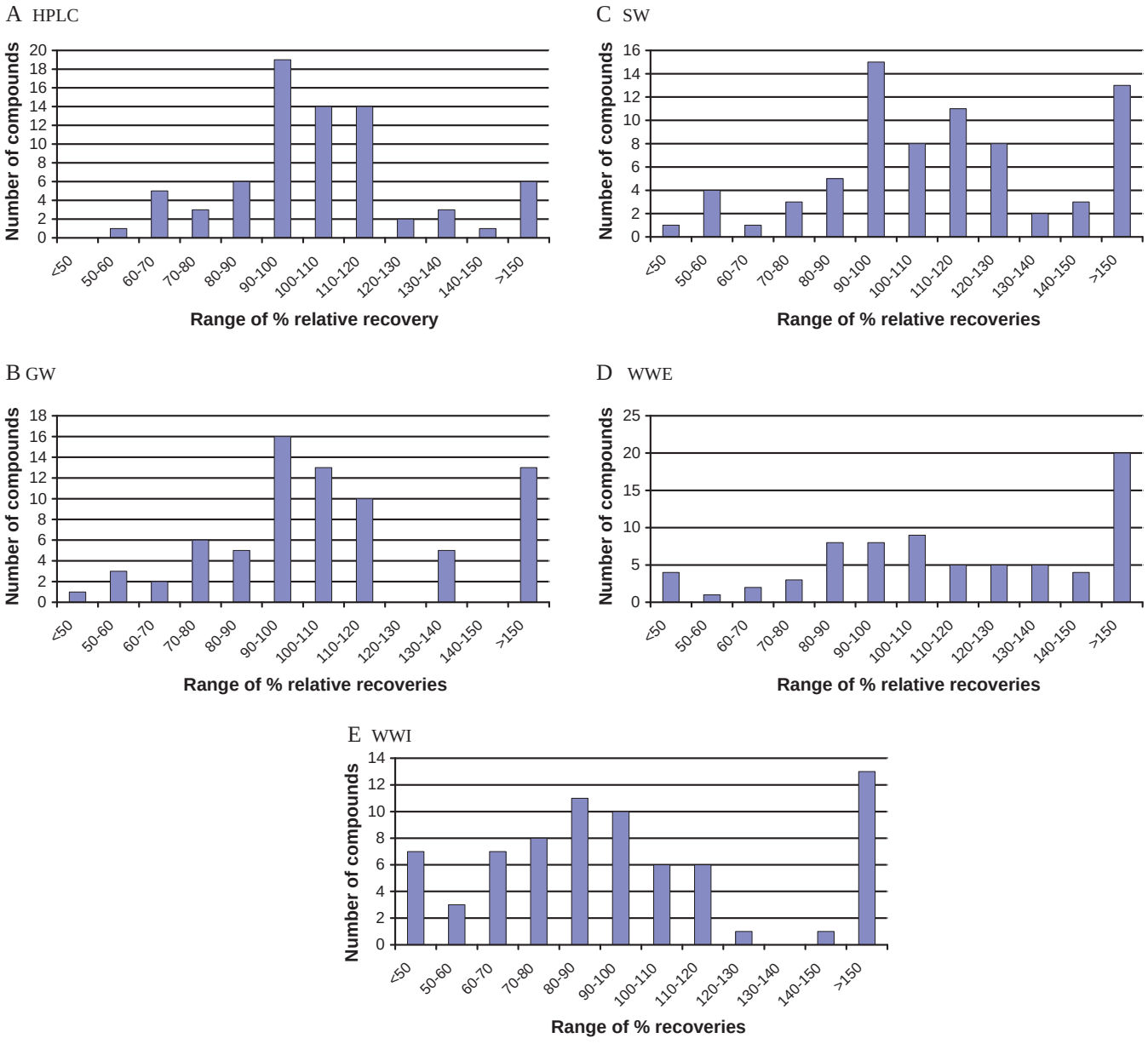

Fig. 6. Relative SPE recoveries organized in ranges for HPLC grade water, GW, SW, WWE and WWI.

from it during the cartridge wash step was the reason of those low absolute recoveries, as no matrix was involved. For chloramphenicol, the absolute SPE recoveries were 87.1%, 82.5%, 78.9%, 28.9% and 16.3% for HPLC grade water, GW, SW, WWE and WWI, respectively. In this case, a clear influence of matrix on the extraction and MS/MS detection occurred. Anyhow, when the SPE recoveries were corrected by the ones for the corresponding surrogates, the percentages of compounds with relative SPE recovery around 100% increased significantly. In this manner, the 92%, 81%, 81%, 68% and 72% of compounds showed a relative SPE recovery between 50 and 150%. Thereby, 111.2%, 117.4%, 97.6% and 122.5% were the relative SPE recoveries, for the same polar compounds named before, respectively. And, 98.0%, 99.2%, 76.1%, 74.4 and 89.8% were the relative SPE recoveries for the chloramphenicol in HPLC grade water, GW, SW, WWE and WWI, respectively. Consequently, poor percentages of absolutely recovery were not considered an obstacle for their reliable determination in water, as their sensitivity was fairly good for being corrected by the corresponding surrogate. The overall method precision, calculated as the relative standard deviation (RSD) was satisfactory, with RSD values ranging from 1 to 30% for most of the compounds in all matrices.

Regarding sensitivity, Method Detection Limits (MDLs) and Method Quantification Limits (MQLs) were determined, for environmental and wastewater samples, as the minimum detectable amount of analyte with a signal-to-noise of 3 and 10, respectively. Spiked GW, SW, WWE and WWI samples ($n=3$) at the two level of concentrations indicated before were used for their calculation. As it can be seen in the Fig. 7 and Supplementary data 3B, MDLs achieved ranged from 0.01 to 5 ng L^{-1} for most of compounds in HPLC grade water, GW and SW, and from 0.01 to 20 ng L^{-1} for the majority of them in wastewaters.

To ensure correct quantification, precision of the chromatographic method, determined as relative standard deviation (RSD), was determined from repeated injections ($n=5$) of a 100 ng L^{-1} spiked HPLC grade water sample during the same day (repeatability) and on different days (reproducibility). RSD achieved were lower than 20 and 30% for most of compounds for intra- and inter-day analysis, respectively.

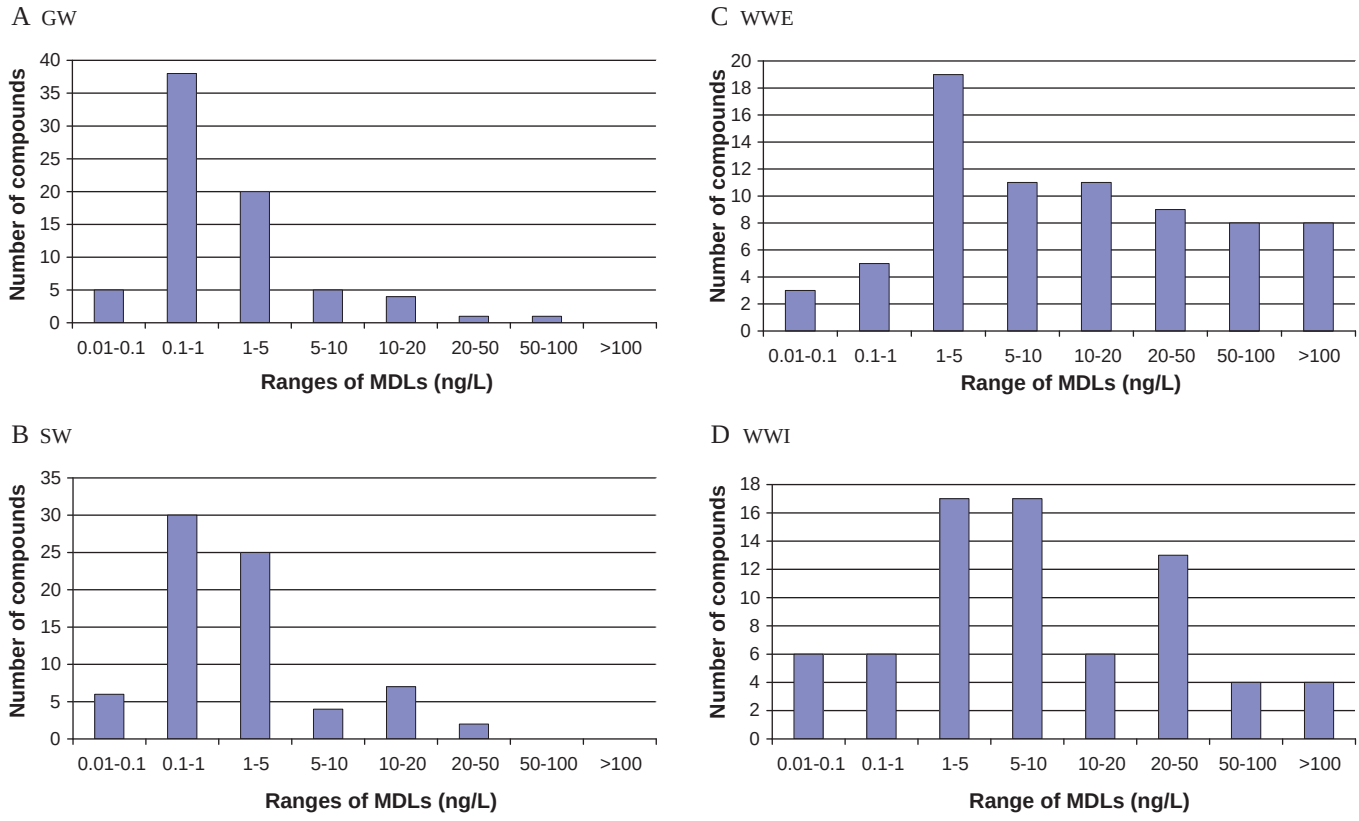

Fig. 7. MDLs organized in ranges for GW, SW, WWE and WWI.

Regarding quantitative performance in terms of dynamic range, linear response covered, giving good fits ($r^2 > 0.99$), four and even five orders of magnitude for the majority of compounds. Calibration curves were generated in HPLC grade water and linear regression analysis was used over the concentration range of 0.01–10,000 ng L^{-1}. Only, glibenclamide, phenyl-butazone, propyphenazone and diclofenac showed a narrower linear response from their MQLs to 500 ng L^{-1}. Thanks to that wide range of linearity, no sample dilution is needed for highly concentrated samples before performing the analysis in order to get a concentration inside the lineal range. For quantification purposes, the internal standard calibration approach was used, performing thirteen-point calibration standards daily, and the possible fluctuation in signal intensity was checked by injecting a standard solution at two concentration levels after each 8–10 injections.

Influence of matrix effect in the quantitative LC–MS/MS analysis is a widely observed and studied phenomena [19,25,54]. The ESI source is highly susceptible to other components present in the matrix, which may result in a signal suppression or enhancement leading to erroneous results. Natural organic matter, salts, ion-pairing agents, non-target contaminants have shown to be responsible for ion suppression. The more complex is the matrix the stronger matrix effect will be present. Therefore, any analytical method where MS is used as detection technique should include a matrix effect study, especially if it deals with complex samples, as in the present case, wastewaters. If relevant ion suppression (or signal enhancement) occurs, appropriate quantitative approaches should be applied for its correction and/or minimization in order to get an accurate quantification. The most common approaches consist of the use of suitable calibration, such as external calibration using matrix-matched samples, standard addition or internal standard calibration with structurally similar unlabelled pharmaceuticals or isotopically labelled standards, as well as dilution of sample extracts [55–58]. In order to evaluate the degree of ion suppression or enhancement in each target compound, matrix effects in all types of validated samples (GW, SW, WWE and WWI) were evaluated by comparing the peak areas from the analysis of spiked real samples (after subtracting the peak areas corresponding to the native analytes present in the sample), with peak areas from spiked HPLC grade water. In the absence of matrix effects, analyte peak areas should be similar in both HPLC grade water and real samples. Nevertheless, when matrix effects occurs the signal intensity for the analytes decreases (ion suppression) or increases (enhancement). Matrix effect was quantified comparing the areas of compounds in spiked matrix samples with the areas obtained in spiked solvent. The effect was expressed by percentage of signal suppression (positive value) or enhancement (negative values). It is clearly observed an increase in the effect as the matrix becomes more and more complex. However, the impact of the matrix is different for every compound. Two extreme examples were bezafibrate, for which rather low effect (−1.07%, 5.72%, 39.06% and 34.51% of matrix effect in GW, SW, WWE and WWI, respectively) is observed, in comparison to phenobarbital for which a much stronger effect was evidenced with 10.90%, 24.54%, 57.48% and 84.85% ion suppression for the same samples. It should be noticed that ion suppression/enhancement is different for every sample analysed even among the same type samples. Therefore, it is of high significance to use any of the aforementioned approaches to correct ion suppression in order to avoid inaccurate quantification and underestimate levels of compounds when analyzing real samples. In our study, the approach used was internal standard calibration. In general, a corresponding isotopically labelled internal standard was selected for each compound (51 surrogates for 74 target analytes). Thus, all the therapeutic groups and within them every family of compounds count with at least an internal standard. The assignation of an appropriate internal standard for

Table 3
Average concentrations and relative standard deviation (expressed in brackets) for target pharmaceuticals in drinking water, superficial water (2 points) and effluent wastewater in the Llobregat River basin (NE Spain).

Therapeutic groups	Compounds	Concentration (ng L^{-1})[a]			
		Point #1 – Llobregat River upstream to the spill point	**Point #2** – Llobregat River downstream to the spill point	**Point #3** – drinking water	**Point #4** – WWE tertiary treatment
Analgesics and antiinflammatories	**Ketoprofen**	*n.d.*	*3.18 (1.56)*	*n.d.*	*57.73 (0.55)*
	Naproxen	**81.05 (0.27)**	**67.38 (0.27)**	**n.d.**	**72.17 (0.33)**
	Ibuprofen	**186.68 (0.33)**	**134.75 (0.32)**	**3.71 (0.15)**	**43.57 (0.60)**
	Indomethacin	**16.27 (0.18)**	**37.75 (0.29)**	**n.d.**	**93.88 (0.52)**
	Diclofenac	**89.53 (0.25)**	**176.78 (0.31)**	**n.d.**	**421.50 (0.26)**
	Mefenamic acid	*n.d.*	*6.76 (0.30)*	*12.82 (2.24)*	*17.38 (0.63)*
	Acetaminophen	**307.00 (0.59)**	**146.67 (0.91)**	**n.d.**	**77.83 (1.77)**
	Salicylic acid	**208.17 (0.07)**	**333.17 (0.61)**	**201.20 (0.23)**	**674.33 (0.26)**
	Propyphenazone	*3.25 (1.11)*	*11.10 (0.68)*	*n.d.*	*22.55 (0.88)*
	Phenylbutazone	n.d.	n.d.	n.d.	n.d.
	Phenazone	*5.90 (1.57)*	*40.27 (0.58)*	*n.d.*	*56.30 (0.28)*
	Codeine	**45.85 (0.8)**	**109.68 (0.41)**	**n.d.**	**350.12 (0.45)**
Lipid regulators	**Clofibric acid**	8.40 (0.36)	24.25 (1.10)	n.d.	22.43 (0.16)
	Bezafibrate	**15.89 (0.34)**	**67.32 (0.47)**	**0.11 (2.24)**	**217.50 (0.50)**
	Fenofibrate	23.85 (0.36)	82.08 (0.49)	n.d.	293.67 (0.64)
	Gemfibrozil	**1.90 (0.39)**	**2.14 (0.57)**	**n.d.**	**8.58 (0.54)**
	Mevastatin	n.d.	n.d.	n.d.	n.d.
	Pravastatin	n.d.	n.d.	n.d.	n.d.
	Atorvastatin	*2.99 (1.19)*	*2.39 (1.10)*	*27.60 (2.24)*	*2.71 (1.32)*
Psiquiatric drugs	**Paroxetine**	*n.d.*	*n.d.*	*n.d.*	*7.30 (0.91)*
	Fluoxetine	*n.d.*	*<LOQ*	*2.74 (2.24)*	*15.87 (0.25)*
	Diazepam	*n.d.*	*6.52 (0.64)*	*n.d.*	*18.92 (0.23)*
	Lorazepam	**22.58 (0.14)**	**41.27 (0.23)**	**n.d.**	**114.92 (0.26)**
	Carbamazepine	**31.28 (0.29)**	**58.43 (0.30)**	**n.d.**	**156.83 (0.24)**
Histamine H2 receptor antagonists	**Loratadine**	*3.68 (1.68)*	*2.51 (0.80)*	*10.48 (1.14)*	*6.99 (1.08)*
	Famotidine	n.d.	n.d.	n.d.	n.d.
	Ranitidine	**33.87 (0.40)**	**61.23 (0.70)**	**n.d.**	**197.67 (1.20)**
	Cimetidine	*17.33 (2.45)*	*n.d.*	*n.d.*	*32.05 (1.17)*
Tetracycline antibiotics	**Tetracycline**	*n.d.*	*29.00 (0.91)*	*n.d.*	*171.47 (1.06)*
	Doxycycline	n.d.	n.d.	n.d.	n.d.
	Oxytetracycline	*n.d.*	*n.d.*	*n.d.*	*42.12 (0.86)*
	Chlorotetracycline	n.d.	n.d.	n.d.	n.d.
Macrolide antibiotics	**Erythromycin**	**50.38 (0.55)**	**174.73 (0.42)**	**n.d.**	**677.00 (0.28)**
	Azithromycin	**14.73 (0.34)**	**71.67 (0.70)**	**17.00 (0.58)**	**1031.67 (0.53)**
	Tilmicosin	n.d.	n.d.	n.d.	n.d.
	Roxythromycin	*n.d.*	*n.d.*	*n.d.*	*3.90 (0.43)*
	Clarithromycin	**42.60 (0.27)**	**88.83 (0.35)**	**3.67 (0.22)**	**237.83 (0.23)**
	Josamycin	*1.82 (0.60)*	*0.81 (1.56)*	*1.41 (0.77)*	*3.03 (0.58)*
	Tylosin	*n.d.*	*n.d.*	*n.d.*	*7.17 (0.57)*
	Spiramycin	**39.90 (0.44)**	**68.32 (0.44)**	**20.54 (0.92)**	**141.58 (0.33)**
Sulfonamide antibiotics	**Sulfamethoxazol**	**39.70 (0.23)**	**78.38 (0.37)**	**n.d.**	**140.48 (0.46)**
	Sulfadiazine	*n.d.*	*13.40 (1.17)*	*n.d.*	*20.38 (0.56)*
	Sulfamethazine	*1.68 (2.45)*	*112.27 (1.55)*	*4.08 (2.24)*	*373.84 (1.92)*
Fluoroquinolones	**Danofloxacin**	n.d.	n.d.	n.d.	n.d.
	Enoxacin	*4.83 (1.56)*	*4.65 (1.57)*	*16.04 (0.96)*	*8.27 (1.13)*
	Ofloxacin	**23.28 (0.24)**	**75.017 (0.48)**	**15.30 (0.73)**	**276.67 (0.46)**
	Ciprofloxacin	**8.32 (0.79)**	**28.02 (0.90)**	**13.28 (0.68)**	**151.25 (1.43)**
	Enrofloxacin	**5.82 (0.56)**	**40.12 (0.84)**	**18.93 (0.79)**	**255.67 (0.35)**
	Norfloxacin	**15.83 (0.31)**	**15.17 (0.86)**	**32.88 (0.94)**	**63.72 (1.02)**
	Flumequine	n.d.	n.d.	n.d.	n.d.
Other antibiotics	**Trimethoprim**	**16.43 (0.24)**	**33.53 (0.34)**	**0.51 (2.24)**	**65.92 (0.37)**
	Nifuroxazide	n.d.	n.d.	n.d.	n.d.
	Chloroamphenicol	n.d.	n.d.	n.d.	n.d.
	Metronidazole	*n.d.*	*44.88 (0.92)*	*n.d.*	*211.83 (0.74)*
Beta blockers	**Atenolol**	**38.40 (0.43)**	**63.17 (0.48)**	**n.d.**	**117.82 (0.56)**
	Betaxolol	n.d.	n.d.	n.d.	n.d.
	Carazolol	n.d.	n.d.	n.d.	n.d.
	Pindolol	n.d.	n.d.	n.d.	n.d.
	Nadolol	n.d.	n.d.	n.d.	n.d.
	Timolol	n.d.	n.d.	n.d.	n.d.
	Sotalol	**15.28 (0.30)**	**44.32 (0.94)**	**n.d.**	**91.98 (0.30)**
	Metoprolol	**54.47 (0.15)**	**327.40 (1.91)**	**38.48 (0.30)**	**96.8 (0.25)**
	Propranolol	*n.d.*	*14.94 (0.39)*	*n.d.*	*51.60 (0.30)*
Beta agonists	**Salbutamol**	*n.d.*	*4.87 (1.70)*	*n.d.*	*27.05 (0.30)*
	Clenbuterol	n.d.	n.d.	n.d.	n.d.
Barbiturates	**Butalbital**	n.d.	n.d.	n.d.	n.d.
	Pentobarbital	n.d.	n.d.	n.d.	n.d.
	Phenobarbital	n.d.	n.d.	n.d.	n.d.

Table 3 (*Continued*)

Therapeutic groups	Compounds	Concentration ($ng\,L^{-1}$)[a]			
		Point #1 – Llobregat River upstream to the spill point	**Point #2** – Llobregat River downstream to the spill point	**Point #3** – drinking water	**Point #4** – WWE tertiary treatment
Antihypertensives	**Enalapril**	n.d.	n.d.	n.d.	n.d.
	Hydrochlorothiazide	**3.26 (0.16)**	**7.96 (0.32)**	**1.26 (0.05)**	**33.53 (0.28)**
	Lisinopril	n.d.	n.d.	n.d.	n.d.
Diuretic	**Furosemide**	**50.98 (0.50)**	**173.72 (0.63)**	**n.d.**	**1120.33 (0.88)**
Antidiabetics	**Glibenclamide**	*0.46 (1.55)*	*1.72 (0.87)*	*3.51 (1.00)*	*13.13 (0.14)*
To treat cancer	**Tamoxifen**	n.d.	n.d.	n.d.	n.d.

[a] values below the limit of detection and below the limit of quantification were considered 0 to calculate the mean value and the RSD.

substances without a specific one, was based on the similarity of their chemical structures and/or their retention times. In Table 1 and Supplementary data 2, internal standards used for each substance, which in this method work as surrogates, are indicated. In this way, the limitation in the number of internal standards presented in [19] was clearly overcome.

3.4. Monitoring results

To demonstrate the applicability of the developed method, two river waters from the Llobregat River (NE Spain), one WWE and the effluent of a drinking water treatment plant (DWTPE) were analyzed River samples correspond to Llobregat River (NE Spain) in two strategic sites up- (point #1) and downstream (point #2) to the point of discharge of treated waters form one WWTP respectively. Point #2 coincides also with the entrance to the DWTP, which was located a few kilometres downstream to the point #2. Point #3 corresponds to the effluent of DWTP (drinking water) and point #4 the WWE after the tertiary treatment which was recirculated towards the discharge point (Fig. 8). Samples from all four points were collected twice a week during three consecutive weeks (six samples per point) during November 2009. The object of this sampling was monitoring the feasibility in the reuse of WWE after a tertiary treatment. Despite the point #3 did not correspond to a specific type of water validated for this method, it was considered similar to a groundwater because of their poor matrix and the low levels of pharmaceuticals expected.

Average concentration for the six samples per point is summarized in Table 3. Levels detected were in the range of hundreds of $pg\,L^{-1}$ to low tens of $ng\,L^{-1}$ for drinking water, and up to low hundreds of $ng\,L^{-1}$ for surface water. Levels in wastewater effluent samples were from units to hundreds of $ng\,L^{-1}$ depending on the compound or even thousands of $\mu g\,L^{-1}$ in some cases such as the antibiotic azithromycin and the diuretic furosemide. Data from the most frequently detected and at higher concentration compounds is presented in bold. Antibiotics, analgesics and anti-inflammatories were the most ubiquitous compounds. The azithromycin and diclofenac must be remarked among them, respectively. As expected, higher concentration were shown at point #4 (WWE after the tertiary treatment). For the diuretic furosemide, this concentration was especially elevated ($1120\,ng\,L^{-1}$), but after the spill into the river, the concentration decreased in a great extent ($173\,ng\,L^{-1}$). Anyhow, that concentration was still higher regarding to the one in the river upstream in the point #1 ($51.0\,ng\,L^{-1}$). This tendency was observed for most of compounds. So it can be said that, after the discharge of effluent

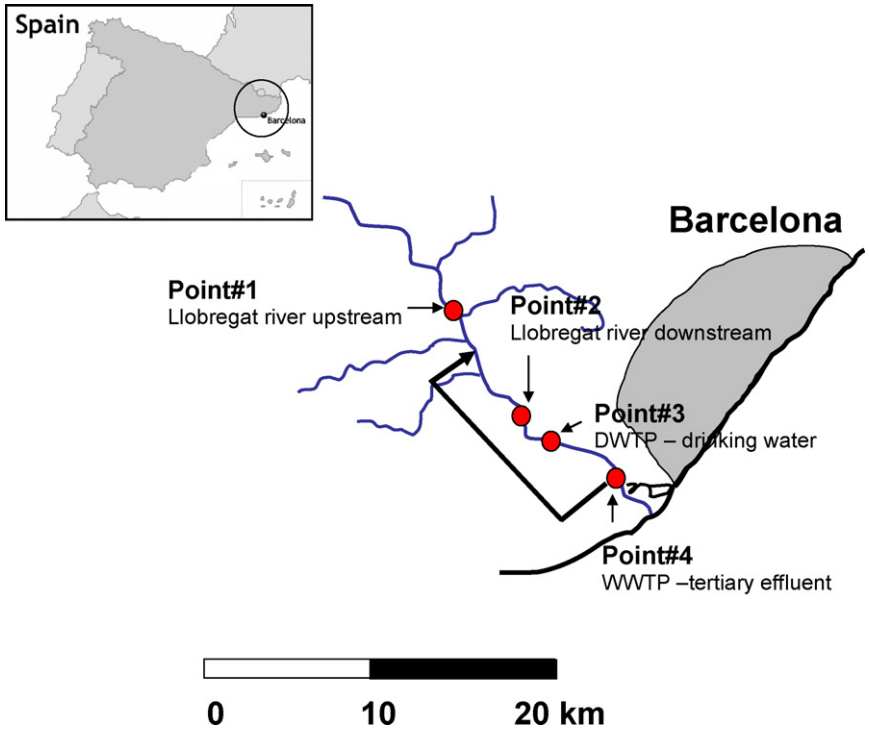

Fig. 8. Sampling location.

the dilution effect is quite effective, but anyway, the perturbation can be observed. However, the decrease in the levels of concentration after the discharge into the river is not the same for all the compounds, even taking into consideration the quantity already present in the river upstream. Thus, in addition to the dilution which is physical phenomenon which should affect all compounds in the same extent, other process like adsorption to sediments or suspended solids, biodegradation or even photodegradation must be taken into account. Anyhow, levels of pharmaceutical at the entrance of the DWTP (point #2) were low and after the treatment at DWTP (point #3) drinking water contained undetectable or very low concentrations for most pharmaceuticals, with the exception of salicylic acid that was detected at 200 ng L^{-1}.

Compounds occasionally detected or detected at low levels even at point #4 were presented in italics. For some of them, quantifications were only possible at point #4. But after the discharge into the river the levels decreased under the limits of quantification or even detection. The macrolides tylosin and roxythromycin, and the cycline oxytetracycline were some examples of that. The presence of compounds, whose quantification at point #2 was still got, could be attributed exclusively to the discharge from the WWTP. In those cases, purification in the DWTP treatment was responsible for reducing their levels down the limit of quantification and/or detection, (e.g. 20–30 ng L^{-1}). 23 compounds were not detected in any sample at any point of sampling.

4. Conclusions

The fully-automated multi-residue analytical method developed, based on on-line SPE–LC–MS/MS allowed the analysis of 74 multiple-class pharmaceuticals in two environmental types of water as well as waste water (influent and effluent to a WWTP). Since the SPE is carried out fully automated, on-line and simultaneously to the chromatographic separation and mass spectrometry detection, a minimum sample manipulation is involved, and therefore a clear decrease in the error introduction is achieved. In fact, filtration is the only sample pre-treatment required. In this way, the method increases in reliability in comparison with conventional off-line methods. To this feature also contributes the fact that most of compounds count with a specific isotopically labelled compound as surrogate (quasi isotopic dilution approach). The method yielded detection limits in the low ng L^{-1} range for both environmental and wastewaters, what is essential for proper monitoring of the target compounds in those type of samples. Moreover, regarding to selectivity, the method fulfil the stringent criteria set by the EU regulations (EU Commission Decision 2002/657/EC) [43]. Other advantages of this method is its high throughput (total analysis time is 30 min in NI mode and 37 min in PI mode) and the wide linear range for most of compounds, which avoids the necessity of diluting the samples for determining compounds present at higher concentrations. It must also be remarked the small size of sample needed, 2.5 mL per ionization mode (total of 5 mL), what relieves the storage problems so usual in analytical laboratories. Application of the method to the analysis of drinking, surface and effluent wastewaters showed a widespread occurrence of pharmaceuticals in such matrices, with general levels, when detected, in the range of units and tens of ng L^{-1} for drinking and river water, respectively, and tens and hundreds of ng L^{-1} in wastewaters.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.09.046.

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