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Fast liquid chromatography–quadrupole-linear ion trap mass spectrometry for the analysis of pharmaceuticals and hormones in water resources

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ABSTRACT

This paper presents the development of a fast multi-residue method for the determination of 49 pharmaceuticals and 6 metabolites from different therapeutic classes in water resources by means of Ultra-performance™ liquid chromatography (UPLC) coupled to tandem mass spectrometry. The use of the UPLC technology enabled all the 55 compounds to be separated chromatographically in less than 9 min (6.3 min positive mode and 2.7 min negative mode) and with a total analysis time of 18 min when considering column conditioning. Improved resolution, sensitivity and a reduction of matrix effects were obtained under these conditions. Unequivocal identification and quantification of the target compounds was also performed by using the dual acquisition modes of the hybrid triple quadrupole-linear ion trap (QqLIT) system. Triple quadrupole mode by means of selected reaction monitoring (SRM) was used for quantification, whilst a second SRM transition together with information-dependent analysis (IDA) experiments was used for confirmation. Additionally, one general, single solid-phase extraction (SPE) method was developed by using Oasis HLB cartridges. Quality parameters of the method in wastewaters were established obtaining a fast, robust, reproducible and cost-effective method for all the target pharmaceuticals. Finally, the optimized SPE-UPLC/QqLIT method was used for the analysis of the target compounds in wastewaters from Spain. Thirty-one out of fifty-five compounds were identified in the samples collected.

1. Introduction

Pharmaceuticals have already been shown to be present in the aquatic environment as a class of so-called "emerging" contaminants. These compounds can enter the water system by means of direct disposal and excretion and, depending on the efficiency of wastewater treatments, they may be present in surface waters at low concentration levels. Despite these low concentrations, the ubiquity of pharmaceuticals in the aquatic environment together, with their persistent biological activities explains the concern over this specific group of water contaminants. Although first reports dealing with the presence of pharmaceutical residues in the environment were performed by using gas chromatography (GC) [1–4], nowadays liquid chromatography coupled to mass spectrometry (MS; MS/MS) is the preferred technique for multi-analyte determinations since it reduces the analysis time and avoids derivatization procedures. Several reviews on the application of this technique can be found in the literature [5–11].

Ultra-performance™ liquid chromatography (UPLC) using narrow diameter particles (<2 μm) enables efficiency gains and increased sensitivity and peak capacity per unit time. However, despite the important benefits related to UPLC (coupled to MS), few multi-residue methods using this technique can be found in the literature for the determination of pharmaceuticals in the water environment [12–16]. For instance, Batt et al. [12] developed a UPLC method coupled to a triple quadrupole mass spectrometer for the determination of 48 drugs and 6 metabolites in wastewater and surface waters. However, total analysis time was 48 min since four chromatographic conditions were used to determine all the compounds. Conley et al. [13] and Kasprzyk-Hordern et al. [14] also developed UPLC methods coupled to triple quadrupole mass spectrometers for the analysis of pharmaceuticals in rivers in the USA. The former described the determination of 13 pharmaceuticals and 1 metabolite in less than 4 min, whilst the latter achieved a separation of the 26 compounds selected in 16 min. Langford and Thomas [16] also used a similar mass analyzer; however, chromatographic separation of the 40 pharmaceuticals analyzed took more than 50 min. A different MS analyzer was selected by Petrovic et al. [15] who developed an UPLC method coupled to a Q-TOF mass spectrometer for the determination of 29 pharmaceuticals in 14 min.

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Finally, some works can be found in the literature using QqLIT hybrid quadrupole instruments coupled to conventional liquid chromatography (LC) systems for the analysis of pharmaceuticals in waters [17–19]. For instance Martínez Bueno et al. [17] developed a method for the determination of 38 pharmaceuticals and 10 metabolites by LC–MS/MS using this hybrid analyzer under SRM acquisition mode with a total analysis time of 90 min. More recently, Gros et al. [19] proposed a (LC–MS/MS-QqLIT) method for the determination of 73 pharmaceuticals by using both SRM and IDA acquisition modes with a total analysis time of 87 min.

In the present work, a UPLC–MS/MS multi-residue method for the determination of 49 pharmaceuticals and 6 metabolites in water resources has been developed. The aim of this work was to explore the possibilities of combining improved chromatographic resolution, increased peak capacity and rapid elution of a UPLC separation with the dual quantitation and confirmation power of the hybrid mass analyzer QqLIT. Both SRM and information-dependent analysis (IDA) acquisition modes were used. To the best of our knowledge, this is the first time that both techniques (UPLC and QqLIT) have been combined for the analysis of pharmaceuticals in the environment, achieving low limits of quantification and a reduction of matrix effects. In addition, the established method was applied to the estimation of pharmaceutical residues occurrence in six wastewater treatment plants (WWTPs). Some of the compounds investigated have been found for the first time in water resources.

2. Experimental

2.1. Chemicals and materials

Alprazolam, atenolol, bromazepam, carbamazepine, chlordiazepoxide, chlorpromazine, diazepam, diltiazem, fluoxetine, furosemide, hydrochlorotiazide, loratadine, lorazepam, metoprolol, oxazepam, paroxetine, phenytoin, propanolol, sertraline, venlafaxine and zolpidem were obtained from Cerilliant (Austin, TX, USA). Demethylsertraline, desmethylvenlafaxine, irbesartan, Ldopa, losartan, terbutaline, valsartan and warfarin were purchased from Toronto Research Chemicals (North York, Canada), whilst all the other standards were obtained from Aldrich Chemical (St. Louis, MO, USA). Isotopically labeled compounds alzaprolam-d_5, chlorpromazine-d_3, diazepam-d_5, lorazepam-d_4, oxazepam-d_6, paroxetine-d_6 and zolpidem-d_6 were obtained from LGC Promochem (Austin, TX, USA). Carbamazepine-d_2, furosemide-d_5 and losartan-d_3 were from Toronto Research Chemicals (North York, Canada) whereas atenolol-d_7, was purchased from Aldrich Chemical (St. Louis, MO, USA). The characteristics of the studied compounds such as use, psychochemical properties and CAS number are given in Table 1.

Both stock standards and isotopically labeled compounds were prepared in methanol at a concentration of 1 mg/mL and 0.1 mg/mL respectively, except for Ldopa which was dissolved in a water:methanol mixture (1:1) since it showed to be slightly soluble in methanol. Standard solutions were stored at −20 °C and fresh ones were prepared every 2 months to avoid degradation. Individual working solutions at a concentration of 15 mg/L were prepared for tuning and stability studies. A mixed working solution of these compounds at 0.2 mg/L in methanol was used for the preparation of calibrators. Fresh mixed solutions were prepared weekly.

Analytical grade ammonium formate and formic acid were obtained from Sigma Chemical (MO, USA). LC–MS grade acetonitrile, methanol and water were obtained from Merck (NJ, USA). Methanol Purge and Trap grade was obtained from Riedel-de-Haën (Germany).

2.2. Sample collection

Samples were collected in glass bottles, stored in the dark at below 4 °C and extracted by solid-phase extraction (SPE) within 48 h. Twenty-four-hour composite water samples were taken from influents and effluents of six wastewater treatment plants (WWTPs) in Catalonia (NE, Spain) in September 2008. These plants received different amounts of wastewater with flow rates varying from 80 to 1000 m^3/h and treated population sizes ranging from 9500 to 320,000 inhabitants. All the wastewater samples exhibited pH values ranging from 7.5 to 8.2, conductivities higher than 3500 μS/cm and total organic content (TOC) values ranging from 150 to 200 mgC/L for influents and from 15 to 30 mgC/L for effluents. Four of the selected WWTPs (# 2, 3, 4 and 5) operate in a similar way, which consisted of a conventional secondary treatment using activated sludge, whilst for WWTPs 1 and 6, the secondary treatment consisted of on aerated lagoon.

2.3. Solid-phase extraction

All the samples collected were filtered through glass microfiber GF/A filters (Whatman, UK) and filters were cleaned by using 5 mL of MeOH prior to solid-phase extraction (SPE). Solid-phase extraction was carried out on a Zymark Rapid Trace SPE Workstation from Zymark (Zymark, Hopkinton, MA). The extraction method was optimized by comparing hydrophilic–lipophilic balance sorbent Oasis HLB (6 mL, 200 mg), the mixed cation exchange reversed-phase Oasis MCX (6 mL, 200 mg) mixed weak cation exchange reversed-phase Oasis WCX (6 mL, 200 mg) and mixed anion exchange reversed-phase Oasis MAX (6 mL, 200 mg), operating under different conditions depending on the cartridge selected. For Oasis HLB, cartridges were washed with 10 mL of methanol and 10 mL of Milli-Q water, rinsed with 8 mL of water, dried with nitrogen gas for 10 min and eluted twice using 3 mL of pure methanol. For Oasis MCX, samples were acidified at pH 3 with formic acid and loaded after washing cartridges with 10 mL of methanol and 10 mL of Milli-Q water. Rinsing was performed with 8 mL of a 2% of formic acid aqueous solution and drying with nitrogen gas for 10 min. Finally, elution using 3 mL of pure methanol and 3 mL of methanol with 5% of ammonia solution was performed. For Oasis MAX and WCX a common procedure was selected. Samples were loaded at pH 9 (ammonia solution) after washing cartridges with 10 mL of methanol and 10 mL of Milli-Q water. Next, cartridges were rinsed with 8 mL of a 5% of ammonia aqueous solution, dried with nitrogen gas for 10 min and eluted using 3 mL of pure methanol and 3 mL of methanol with 2% of formic acid. In all the cases, 100 mL of wastewater was loaded into the system at a flow rate of 10 mL/min. Prior to extraction, isotopically labeled surrogates (chlorpromazine-d_3, lorazepam-d_4 and paroxetine-d_6) were added to water samples, resulting in a concentration of 10 ng/L for each compound.

Extracts were evaporated to dryness at 35 °C under a gentle stream of nitrogen in a TurboVap LV evaporator from Zymark (Zymark, Hopkinton, MA) and dried samples were reconstituted in 200 μL of a 10% methanol aqueous solution. Finally, a mixed standard solution containing atenolol-d_7, alprazolam-d_5, carbamazepine-d_2, diazepam-d_5, furosemide-d_5, losartan-d_3, oxazepam-d_5 and zolpidem-d_6 was added as internal standard at a concentration of 200 ng/L for each compound. Extracts were analyzed immediately after extraction; when no immediate analysis could be performed, extracts were frozen for not more than 7 days.

2.4. Ultra-performance™ liquid chromatography–tandem mass spectrometry analysis

Chromatographic separations were carried out with a Waters Acquity Ultra-performance™ liquid chromatograph system,

Table 1
Physicochemical properties of pharmaceuticals studied.

Therapeutics	Compound	CAS#	Mw	log K_{ow}	pK_a	Excr. unch.[a]	Metabolites
Psychiatric	Alprazolam	28981-97-7	308	2.12	2.39	<1%	
	Bromazepam	1812-30-2	316	2.10	2.9–11		
	Carbamazepine	298-46-4	236	2.46	13.9	1–3%	10,11-Epoxide carb.
	Chlordiazepoxide	58-25-3	299	2.44	4.8		
	Chlorpromazine	69-09-0	318	5.20	9.3		
	Diazepam	439-14-5	284	2.19	4.9–5.7	<1%	
	Fluoxetine	54910-89-3	309	3.82	8.7–9.5	15%	Norfluoxetine
	Levodopa	59-92-7	197	2.39	2.32		
	Lorazepam	846-49-1	321	2.42	0.03		
	Oxazepam	604-75-1	286	2.20	1.7, 11.6		
	Paroxetine	61869-08-7	329	3.95	9	<1%	
	Phenytoin	57-41-0	252	2.47	8.33		
	Primidone	125-33-7	218	0.91	9.25		
	Sertraline	079617-96-2	306	5.29	8.9	<1%	Desmethylsertraline
	Venlafaxine	93413-69-5	277	3.28	9.9		Desmethylvenlafaxine
	Zolpidem	82626-48-0	307	3.85	6.2		
Antihistaminic	Cetirizine	83881-51-0	388	0.01	2.5–8.2	<30%	
	Loratadine	79794-75-7	382	5.20	<0		Desloratadine
	Prednisone	53-03-2	358	1.46	15	2–5%	Prednisolone
	Salbutamol	18559-94-9	239	0.64	10.3		
	Terbutaline	76095-16-4	376	0.07	3.75		
Angiotensin	Irbesartan	138402-11-6	428	4.20	4.7	>90%	
	Losartan	114798-26-4	422	4.01	5.5–3.15	4%	
	Valsartan	137862-53-4	435	3.90	4.9	80%	
β-Blocker	Acebutolol	37517-30-9	336	1.71	9.2	10–17%	
	Atenolol	29122-68-7	266	0.36	9.6	40–50%	
	Betaxolol	63659-18-7	307	2.81	9.4		
	Bisoprolol	66722-44-9	325	1.87	14.4		
	Doxazosin	74191-85-8	451	2.09	6.93	5%	
	Labetalol	36894-69-6	328	3.09	9.45		
	Metoprolol	37350-58-6	267	1.80	9.7	5–10%	
	Nadolol	42200-33-9	309	0.71	9.67	25%	
	Propanolol	525-66-6	259	3.00	9.49	1–4%	
	Sotalol·HCl	3930-20-9	272	0.24	9.55	>75%	
Cardiac	Amlodipine	88150-42-9	409	3.00	8.6		
	Clopidogrel	113665-84-2	322	3.68	4.61		
	Diltiazem	42399-41-7	414	2.20	8–9	2–4%	
	Enalapril maleate	76095-16-4	376	0.07	3.75		
	Furosemide	54-31-9	331	2.03	4–10		
	Hydrochlorothiazide	58-93-5	298	−0.1	7.9	95%	
	Lisinopril	83915-83-7	405	−1.1	2.5		
	Warfarin	81-81-2	308	2.70	5	<1%	
Hormones	Estrone	53-16-7	270	3.13	10.3		
	β-Estradiol	50-28-2	272	4.01	10.4		
	Estriol	50-27-1	288	2.45	10.4		
	Ethynyl estradiol	57-63-6	276	3.70	10.5		
	Progesterone	57-83-0	314	3.87	<0		
	Tamoxifen	10540-29-1	371	6.30	8.5		

[a] Percentage of parent compound excreted unchanged.

equipped with a quaternary pump system (Milford, MA, USA) using an Acquity BEH C_{18} column (100 mm × 2.1 mm i.d., 1.7 μm particle size) preceded by a Vanguard precolumn BEH C_{18} (5 mm × 2.1 mm i.d., 1.7 μm particle size) both supplied by Waters (Waters Corp., Milford, MA, USA). Separation was performed with a binary mobile phase at a flow rate of 0.8 mL/min. For the positive ionization mode, the optimized separation conditions were as follows: solvent (A) acetonitrile with 0.1% formic acid; solvent (B) 10 mM formic acid/ammonium formate (pH 3.5). The gradient elution was: 0–0.1 min, 5% A; 0.1–1.5 min, 5–20% A; 1.5–2.0 min, 20–30% A; 2.0–5.0 min, 30–45% A; 5.0–7.0 min 45–80% A; 7.0–7.5 min 80% A; 7–8 min return to initial conditions; 8.0–9.0 min, equilibration of the column. Analysis in negative ionization mode was performed by using acetonitrile:methanol solution (90:10) (A) and water (B). The gradient elution was: 0–1.5 min, 0–40% A; 1.5–5.0 min, 40–50% A; 5.0–7.0 min, 50–70% A; 7.0–8.0 min return to initial conditions; 8.0–9.0 min, equilibration of the column. The sample volume injected was 10 μL for both modes.

The UPLC instrument was coupled to a 3200 Qtrap hybrid triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) with a turbo Ion Spray source. Acquisition was performed in selected reaction monitoring (SRM) mode and the protonated or deprotonated molecular ion of each compound was chosen as the precursor ion.

Compound-dependent parameters, declustering potential (DP), entrance potential (EP), collision energy (CE), collision cell entrance potential (CEP), and collision cell exit potential (CXP), were optimized by direct infusion of individual standard solutions of each compound at 0.15 mg/L. A summary of the optimum values is displayed in Table 2. Additionally, IDA experiments were performed with SRM as a survey scan and an enhanced product ion (EPI) scan at three different energies (CE: 25, 45, 55 eV or −30, −50, −60 eV), as dependent scans, for those compounds showing poor

Table 2
LC–MS/MS parameters established for the SRM acquisition mode.

	Rt (min)	Q1	DP (V)	Quantification		Confirmation		Ion ratio
				Q3	CE (V)	Q3	CE (V)	(±SD)
Terbutaline	0.78	226	41	152	21	107	39	2.1 ± 0.3
Salbutamol	0.81	240	26	148	25	222	17	1.7 ± 0.2
Sotalol	0.84	273	26	255	19	213	25	1.4 ± 0.3
Atenolol	1.00	267	51	145	33	190	25	1.7 ± 0.2
Atenolol-d_7 (IS)	1.01	274	41	145	33	–	–	–
Lisinopril	1.21	406	51	84	47	246	33	15 ± 0.7
Fluoxetine	1.46	310	26	44	27	148	13	1.5 ± 0.2
Metoprolol	1.81	268	61	116	25	72	31	2.4 ± 0.1
Primidone	1.81	219	36	91	39	162	17	1.1 ± 0.1
Desmethylvenlafaxine	1.98	264	31	58	39	246	19	2.7 ± 0.2
Acebutolol	2.05	337	51	116	29	56	49	1.3 ± 0.1
Zolpidem	2.32	308	59	235	45	236	33	1.6 ± 0.2
Zolpidem-d_6 (IS)	2.33	314	21	286	33	–	–	–
Labetalol	2.41	329	36	311	21	91	59	2.3 ± 0.3
Venlafaxine	2.46	278	31	58	35	260	19	2.9 ± 0.1
Bisoprolol	2.48	326	51	116	25	74	37	3.1 ± 0.4
Carbamazepine epoxide	2.49	253	31	180	33	210	19	1 ± 0.2
Desloratadine	2.50	311	51	259	29	294	27	1.0 ± 0.1
Enalapril maleate	2.61	377	41	234	27	91	83	1.8 ± 0.2
Chlordiazepoxide	2.63	300	41	227	33	283	21	1.8 ± 0.1
Propanolol	2.64	260	46	116	25	183	25	1.4 ± 0.3
Prednisolone	2.73	361	26	343	15	147	29	18 ± 0.1
Bromazepam	2.76	316	51	182	43	209	33	1.4 ± 0.1
Norfluoxetine	2.78	296	76	134	11	105	33	18 ± 4
Doxazosin	2.80	452	56	344	41	247	51	1.1 ± 0.3
Betaxolol	2.81	309	51	116	27	55	43	1.5 ± 0.2
Carbamazepine	3.05	237	41	194	27	192	31	4.2 ± 0.6
Carbamazepine-d_2 (IS)	3.05	239	41	196	27	–	–	–
Diltiazem	3.08	415	46	178	33	109	81	1.7 ± 0.1
Nadolol	3.22	310	46	254	23	201	31	4.8 ± 0.4
Paroxetine	3.30	330	56	70	43	192	27	1.2 ± 0.1
Paroxetine-d_6 (S.)	3.31	336	46	76	51	–	–	–
Oxazepam	3.36	287	56	104	45	77	83	1.0 ± 0.01
Oxazepam-d_5 (IS)	3.36	292	51	246	29	–	–	–
Lorazepam	3.51	321	46	275	29	303	21	1.2 ± 0.1
Lorazepam-d_4 (S.)	3.52	325	46	279	31	–	–	–
Amlodipine	3.64	409	21	238	19	294	17	1.3 ± 0.3
Alprazolam	3.68	309	61	281	31	205	51	11.4 ± 2
Alprazolam-d_5 (IS)	3.69	314	71	235	45	–	–	–
Desmethylsertraline	3.93	292	16	159	35	275	17	16 ± 0.5
Chlorpromazine	4.05	319	46	86	29	58	49	1.2 ± 0.1
Chlorpromazine-d_3 (S.)	4.05	322	41	89	31	–	–	–
Sertraline	4.10	306	26	159	39	275	19	1.2 ± 0.2
Norethindrone	4.26	299	51	109	41	91	71	1.3 ± 0.1
Diazepam	4.67	285	56	193	39	154	35	1.3 ± 0.04
Diazepam-d_5 (IS)	4.68	290	61	198	41	–	–	–
Warfarin	4.70	309	36	163	20	251	25	2.0 ± 0.1
Loratadine	5.31	383	56	337	31	267	45	1.5 ± 0.2
Progesterone	6.05	315	46	109	37	97	29	1.1 ± 0.2
Tamoxifen	6.17	372	61	72	39	70	63	8.8 ± 0.3
Clopidogrel	6.25	322	36	212	23	155	45	1.4 ± 0.1
Negative ionization mode								
Ldopa	0.50	196	−35	135	−22	109	−30	14 ± 0.4
Hydrochlorothiazide	1.09	296	−55	78	−44	205	−28	1.4 ± 0.2
Estriol	1.65	287	−85	143	−70	145	−50	1.3 ± 0.2
Prednisone	1.74	357	−35	327	−14	123	−32	5.6 ± 0.9
Furosemide	1.82	329	−35	285	−20	205	−28	1.0 ± 0.1
Furosemide-d_5 (IS)	1.83	334	−35	290	−18	–	–	–
Phenytoin	1.90	251	−45	902	−26	208	−22	2.5 ± 0.3
Cetirizine	2.09	387	−59	75	−38	101	−34	6.5 ± 0.5
Losartan	2.29	421	−50	127	−42	179	−32	1.2 ± 0.1
Losartan-d_3 (IS)	2.30	424	−55	179	−32	–	–	–
Estradiol	2.42	271	−85	145	−54	143	−82	1.6 ± 0.2
Ethynyl estradiol	2.70	295	−80	145	−52	143	−72	1.4 ± 0.4
Irbesartan	2.73	427	−50	193	−36	121	−94	7.1 ± 0.6
Estrone	2.76	269	−75	145	−46	143	−80	1.8 ± 0.2
Valsartan	2.82	434	−45	179	−32	350	−26	1.7 ± 0.1

DP: Declustering potential (V); CE: collision energy (V); SD: standard deviation; IS: internal standard; S.: surrogate.

fragmentation. All data were acquired and processed using Analyst 1.4 software.

2.5. Quantitation and quality parameters

Quantitation was carried out using the SRM acquisition mode. Two transitions per compound were selected and ion ratios and LC retention times were set in order to accurately identify each compound (Table 2).

The reproducibility of the method was evaluated from run-to-run experiments by extracting and injecting six replicates in 1 day of wastewater matrices spiked with a pharmaceuticals standard mixture at a concentration of 165 ng/L. Since it was not feasible to find a wastewater matrix completely free of the target pharmaceuticals, the wastewater with lowest content of pharmaceuticals (TOC = 18 mg C/L) was selected. For repeatability, extraction and analysis were performed on five successive days on both matrices at the same spiked levels. The precision of the method (in terms of peak areas) was expressed as the relative standard deviation (%RSD).

The limits of detection (LOD) were defined as the lowest analyte concentration with a signal-to-noise (S/N) ratio of 3, and the limits of quantification (LOQ) were defined as the concentration with S/N ratio of 10 and an imprecision lower than 20%. Instrumental limits of quantification (IQL) were determined by direct injection of standards at low concentrations. LOD and LOQ values of the method were determined by spiking wastewater samples with mixtures of standard compounds at low concentrations. Since drug-free matrices could not be found, the lowest TOC wastewater sample was previously extracted by SPE in order to obtain cleaner wastewater matrix. Pharmaceuticals were then added to these extracts at decreasing concentrations (from 0.01 to 60 ng/L), extracted by SPE and finally injected into the LC–MS/MS system.

The linearity of the method was studied over the established working concentration range of 0.015–140 μg/L; three replicates were analyzed for each concentration level. Calibration was performed using alprazolam-d_5, atenolol-d_7, carbamazepine-d_2, diazepam-d_5, furosemide-d_5, losartan-d_3, oxazepam-d_5 and zolpidem-d_6 labeled compounds as internal standards. In Table 2, the compounds quantified with each deuterated standards are displayed. Linearity was expressed as the squared correlation coefficient ($r^2 > 0.998$) and a weighing factor $1/x$ was used.

2.6. Matrix effects and recoveries

Evaluation of the ionic suppression or enhancement due to sample matrix or coeluting compounds was performed by using the method suggested by Matuszewski et al. [20]. One standard solution containing internal standards and surrogate (A_0) was prepared in LC–MS water at a concentration of 80 μg/L for each compound. The same amount (0.8 ng/L in sample) was added to wastewater matrices before (A_{Ex}) and after (A_{nEx}) SPE extraction and a non-spiked wastewater sample (A_{nsp}) was also used. The standard solution represents the 100% response value, the relative difference between A_0 and A_{nEx} shows the effect of the sample matrix and A_{Ex} provides information regarding the loss of signal related to the extraction process. Each sample was prepared in triplicate and the procedure was repeated with three additional wastewaters (two influent and two effluent samples from urban and industrial sources) to verify the absence of variations between matrices. Peak areas obtained for each compound were used for calculation.

$$\text{matrix effect}\,(\%) = 100 \times \left(1 - \left(\frac{A_0 - (A_{nEx} - A_{nsp})}{A_0}\right)\right)$$

According to this calculation, a negative (−) value indicates matrix suppression and a positive (+) one indicates matrix enhancement.

In order to compare the behavior of the SPE sorbents, recoveries were calculated as the percentage of the mean relative peak areas obtained when both analytes and internal/surrogate standards were added after (A_{Ex}) and before (A_{nEx}) SPE.

3. Results and discussion

3.1. Chromatographic separation

In order to optimize chromatographic separation, different mobile phases and additives were tested. The use of buffers such as ammonium formate/formic acid or ammonium acetate/acetic acid at different concentration levels was evaluated for the aqueous phase, whilst methanol, acetonitrile and mixtures of both solvents with formic acid were tested for the organic phase.

Initially, a mobile phase consisting of acetonitrile (A) and water (B) was selected and a mixed standard solution of the target compounds at a concentration of 500 μg/L was used. A linear gradient from 5% to 90% of A in 10 min and a flow rate of 500 mL/min were selected as starting conditions. For compounds acquired under positive ionization mode, the chromatographic separation showed poor responses in terms of peak shapes and efficiencies. The addition of formic acid (0.05–0.1%) to both aqueous and organic phases improved signal response due to the protonation of the basic compounds. However, for compounds such as terbutaline or enalapril, with pK_a values above 3–4, slight modifications in the acidic content provoked significant modifications in their responses, indicating the necessity of using a buffer. A mobile phase consisting of an ammonium formate buffer solution (pH: 3.5) or ammonium acetate buffer solution (pH: 4.5) and acetonitrile with 0.1% formic acid was evaluated. In general, an improvement in resolution and peak shapes was observed for most of the compounds when ammonium formate buffer was used. The effect of the mobile phase ionic strength in the separation was then evaluated by increasing the buffer concentration from 2 mM to 20 mM. It was observed that an increase in the concentration to 10 mM improved peak shapes, resolution and efficiencies. Finally, the use of methanol instead of acetonitrile was evaluated. Separations obtained showed lower efficiencies and a worsening in chromatographic resolution when this solvent was used. Therefore, acetonitrile was maintained as the organic solvent.

Once the mobile phase composition was established the gradient elution was optimized in order to improve chromatographic resolution and to reduce total analysis time. First, flow rate was increased from 500 mL/min to 800 mL/min in order to reduce retention times and to increase peak heights by narrowing them. For enalapril, a notable worsening in peak shape was observed, yielding the appearance of a bimodal peak. This effect has been previously reported by Trabelsi et al. [21] who reported that an increase in flow rates yields two peaks corresponding to the *cis–trans* isomers of enalapril, which has little time to isomerize. In order to improve peak shape, temperature was increased from 40 °C to 50 °C, since as described by these authors, an increase in temperature yields to an improved peak shape due to the acceleration of the conformational changes between both isomers. Under these conditions, enalapril peak shape was improved whilst the total analysis time was reduced by 1 min and an increase from 30% to 50% in peak responses was achieved. However, a general worsening in chromatographic resolution was obtained due to the higher flow rate; thus, the gradient elution profile was next optimized. Curved gradients were used since they enable resolution in eluted peaks to be improved, by avoiding time gaps throughout the chromatogram.

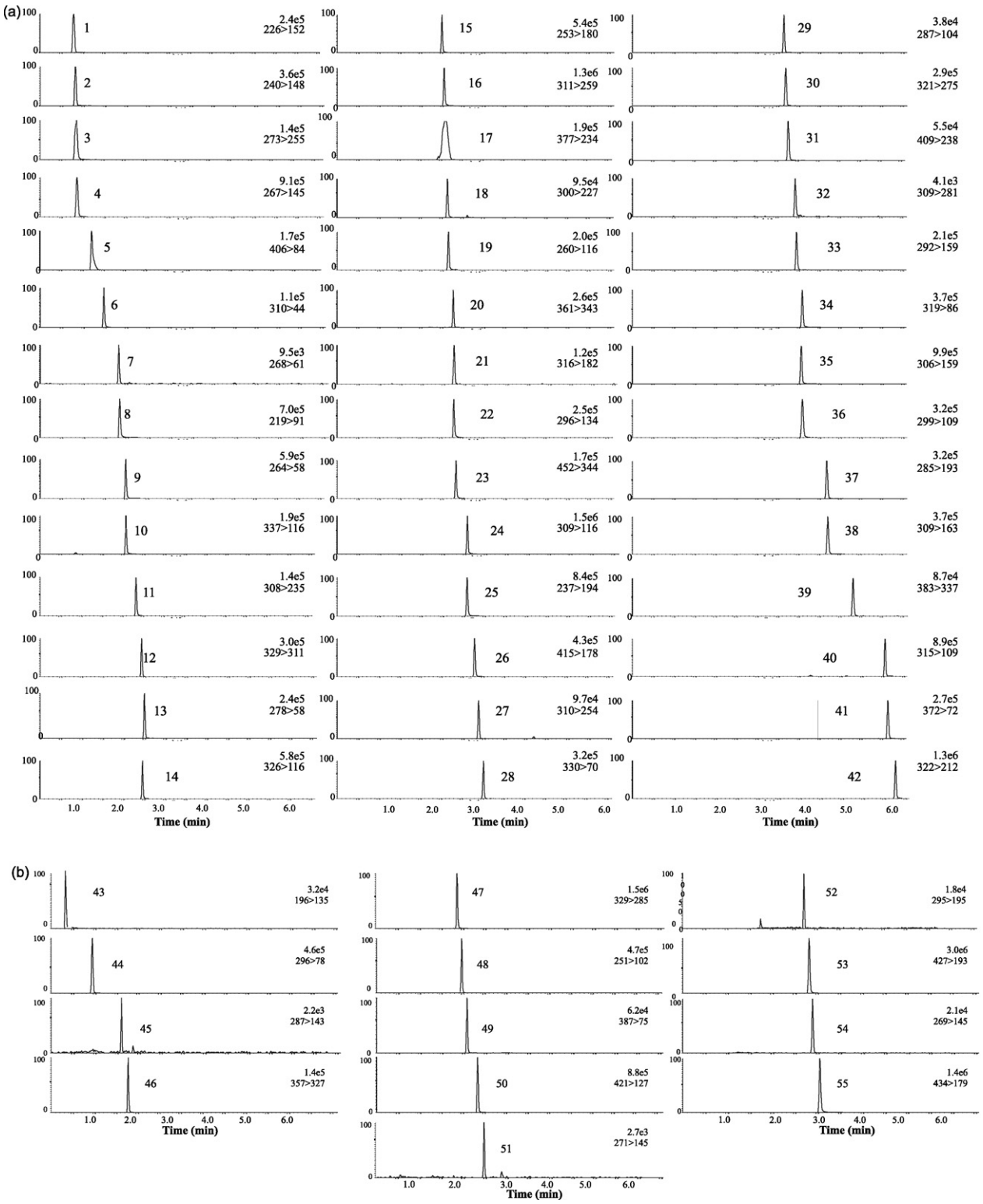

Fig. 1. Total ion chromatogram of a standard mixture (1.5 μg/L) in SRM acquisition mode. (a) Positive electrospray ionization; (b) negative electrospray ionization. 1: Terbutaline, 2: salbutamol, 3: sotalol, 4: atenolol, 5: lisinopril, 6: fluoxetine, 7: metoprolol, 8: primidone, 9: desmethylvenlafaxine, 10: acebutolol, 11: zolpidem, 12: labetalol, 13: venlafaxine, 14: bisoprolol, 15: carbamazepine epoxide, 16: desloratadine, 17: enalapril, 18: chlordiazepoxide, 19: propanolol, 20: prednisolone, 21: bromazepam, 22: norfluoxetine, 23: doxazosin, 24: betaxolol, 25: carbamazepine, 26: diltiazem, 27: nadolol, 28: paroxetine, 29: oxazepam, 30: lorazepam, 31: amlodipine, 32: alprazolam, 33: desmethylsertraline, 34: chlorpromazine, 35: sertraline, 36: norethindrone, 37: diazepam, 38: warfarin, 39: loratadine, 40: progesterone, 41: warfarin, 42: clopidogrel, 43: ldopa, 44: hydrochlorothiazide, 45: estriol, 46: prednisone, 47: furosemide, 48: phenytoin, 49: cetirizine, 50: losartan, 51: estradiol, 52: ethynyl estradiol, 53: irbesartan, 54: estrone, 55: valsartan.

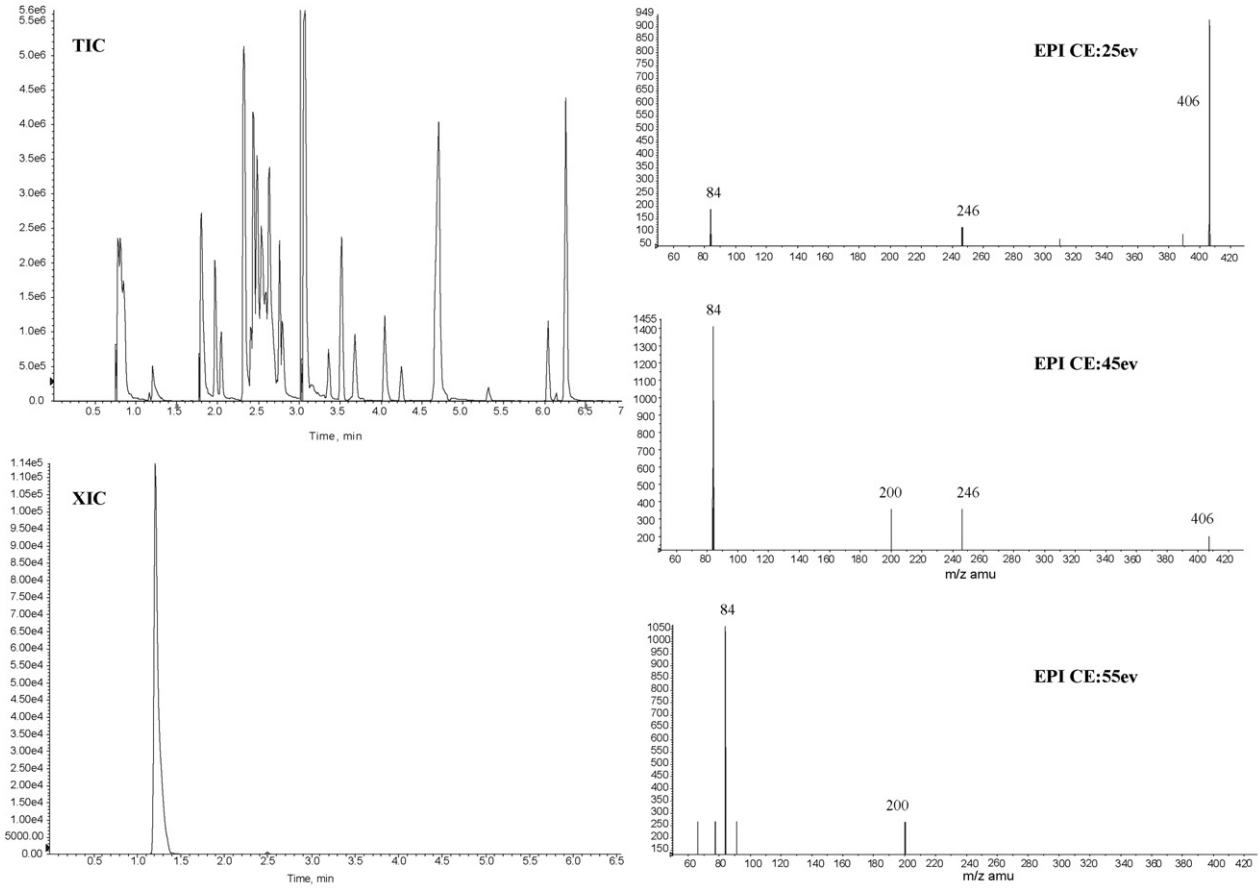

Fig. 2. IDA experiment performed for lisinopril in a wastewater influent sample. Total ion chromatogram (TIC) of all SRM transitions. Extracted ion chromatogram (XIC) for 406>84 transition and MS/MS acquisition (EPI) at three collision energies (25, 45 and 55 eV).

Considering the chromatographic separation previously achieved with linear elution, two concave gradients ($n > 6$) were used. A f_9 curve was selected for compounds eluting from 0 to 2 min. The steepness of this gradient, in the first part of the chromatogram, is slower than in a linear one ($n = 6$) and results in an improvement in resolution of the early eluting peaks, such as terbutaline, salbutamol, atenolol and sotalol. However, a partial coelution between the later eluted compounds metoprolol and primidone was obtained since elution in this part of the gradient is faster than in the linear one and results in a lower resolution. In order to increase resolution for compounds eluting between 2 and 4 min, without losing efficiency between the last eluted peaks (4–7.5 min), a slighter curved gradient was selected (f_7) for the second part of the chromatogram (from 2 to 7.5 min). Acceptable separation was obtained for these compounds, although partial coelution for the compounds eluting from 2.4 to 3.1 min could not be avoided. This coelution did not represent a major drawback since different m/z were acquired for these compounds and no ionic suppression was observed when individual and mixed standard solutions were infused into the MS system. Under these conditions, the 42 pharmaceuticals were separated in less than 6.5 min (Fig. 1a).

For compounds acquired under negative ionization mode, acetonitrile (A) and water (B) were maintained as mobile phase. A 10% of methanol was added to the organic phase in order to slightly increase the retention of the first eluted compounds. No modifiers were added to the aqueous solution since satisfactory responses in terms of efficiency and peak shapes were obtained. Flow rate was fixed as for the positive mode at 800 mL/min and gradient elution was also optimized by using a curved gradient in order to obtain the best resolution together with shorter analysis times. In this case a convex gradient (f_4) profile was used since it provided a faster elution for the first eluting compounds (Ldopa and hydrochlorothiazide), whilst improving resolution for the partially coeluting compounds irbesartan, estrone and valsartan. Under these conditions, the 13 pharmaceuticals were separated in less than 4.0 min. Gradient was then extended to 70% of organic content in order to clean the column and to avoid further contaminations. In Fig. 1b, the extracted ion chromatograms from a standard mixture are displayed.

3.2. Mass spectrometry conditions

Ionization parameters were optimized by infusing individual standard solutions at a concentration of 150 μg/L. Thirteen drugs were found to give higher responses in negative ionization mode, whilst for the others the positive ionization mode gave better responses.

Since high flow rates were used during chromatographic separation (800 mL/min), high source temperature and gas flow rates were mandatory in order to improve desolvation efficiency and analyte ionization. Therefore, source temperature was set up to 660 °C, whilst curtain gas was fixed at 35 psi and desolvation gases at 40 and 45 psi. Declustering potential was optimized for each compound in order to obtain maximum response for the protonated ($[M+H]^+$) or deprotonated ($[M-H]^-$) molecular ion and to prevent in-source fragmentation or adducts.

Data acquisition was performed in SRM mode. Collision energies and collision cell entrance and exit potentials were optimized in order to obtain the two most sensitive transitions. The most intense one was used for quantification, whilst the other was used

Table 3
Validation parameters of the method in wastewaters.

	LOQ (ng/L)	Recovery		Run-to-run (%RSD)[a]	Day-to-day (%RSD)[a]	Signal sup./enh.
		R (%)	%RSD			
Terbutaline	0.02	75	4	5.5	7.7	−19
Salbutamol	0.02	70	4	5.7	8.9	−24
Sotalol	0.02	87	5	6.9	8.3	−49
Atenolol	15	70	5	5.2	8.4	−5
Lisinopril	20	75	1	5.7	8.7	−40
Fluoxetine	15	62	4	7.7	8.9	−44
Metoprolol	0.02	74	5	1.2	3.2	−9
Primidone	1.5	89	5	3.2	5.9	−22
Desmethylvenlafaxine	0.02	73	4	5.1	11.3	−18
Acebutolol	0.02	66	3	4.5	8.1	−26
Zolpidem	0.2	71	2	1.9	5.3	−26
Labetalol	15	80	4	2.8	8.6	−34
Venlafaxine	1.5	72	4	1.7	6.9	−9
Bisoprolol	0.02	65	3	5.3	9.3	−14
Carbamazepine epoxide	0.02	71	4	1.7	6.5	−10
Desloratadine	0.02	61	3	4.2	5.1	−18
Enalapril	0.2	101	3	3.4	5.4	−16
Chlordiazepoxide	1.5	71	1	4.7	8.9	−21
Propanolol	0.02	70	4	4.2	8.2	−11
Prednisolone	0.2	99	4	3.1	4.0	−36
Bromazepam	15	73	3	4.8	8.4	−7
Norfluoxetine	1.5	93	3	4.0	6.2	−38
Doxazosin	0.02	72	3	5.4	8.0	−19
Betaxolol	0.02	60	3	6.8	8.4	−16
Carbamazepine	1.5	71	3	3.7	4.7	−5
Diltiazem	5	97	2	2.2	5.9	6
Nadolol	15	60	4	7.1	8.3	−23
Paroxetine	15	86	4	5.7	7.9	−41
Oxazepam	0.02	75	4	4.1	4.6	−20
Lorazepam	1.5	101	4	3.3	5.4	−24
Amlodipine	0.02	74	3	4.0	8.2	−28
Alprazolam	15	110	5	5.1	7.2	−35
Desmethylsertraline	1.5	101	1	3.7	5.6	−26
Chlorpromazine	1.5	55	4	6.1	7.8	−14
Sertraline	15	60	3	4.2	6.1	−8
Norethindrone	0.02	106	3	3.4	6.6	−32
Diazepam	1.5	88	3	1.8	6.5	−24
Warfarin	0.02	96	4	5.0	8.3	−27
Loratadine	15	61	4	2.0	5.8	−29
Progesterone	1.5	82	1	4.0	6.6	−23
Tamoxifen	0.02	110	3	2.6	4.0	−32
Clopidogrel	0.2	78	5	4.5	9.2	−23
Ldopa	10	101	5	5.6	6.9	−19
Hydrochlorothiazide	50	86	4	4.9	8.9	−49
Estriol	5	79	4	6.3	8.1	−30
Prednisone	10	80	4	3.8	8.4	−23
Furosemide	20	110	4	3.4	8.5	−21
Phenytoin	0.2	99	3	2.6	3.3	−16
Cetirizine	10	111	5	3.1	7.3	−19
Losartan	0.02	81	5	4.9	5.2	−15
Estradiol	10	82	4	2.9	4.3	−32
Ethynyl estradiol	0.2	78	4	3.0	5.5	−27
Irbesartan	0.02	95	5	2.6	8.4	−16
Estrone	10	85	4	1.6	3.2	−35
Valsartan	0.02	97	4	2.7	4.7	−18

R: recovery of the SPE step; RSD: relative standard deviation.
[a] Run-to run precision calculated for $n = 6$; Day-to-day precision calculated for $n = 30$.

for confirmation purposes, achieving the four identification points established in the EU Guidelines [22] regarding mass spectrometric detection. Two additional criteria were established in order to confirm a positive finding in real samples; the relative abundances between the quantification and confirmation transition had to fall within the tolerance range established in the EU Guidelines [22] and the retention time had also to fit within ±2% of that of the reference standard.

However, for some compounds (prednisolone, lisinopril, desmethylsertraline and Ldopa) the confirmation transition proved not to be intense or robust enough. Therefore, an alternative strategy was followed, consisting in performing an IDA experiment during SRM acquisition (survey scan). The IDA scan intensity threshold for all the SRM transitions was set to 1000 cps to assure the detection of small peaks; when a transition matched this criteria level, three EPI (enhanced product ion) spectra were acquired at three different collision energies for the precursor ion. Dynamic exclusion time, which defines the time for which a transition is excluded after acquiring an EPI scan, was set to 6 s in order to handle coeluting peaks with different signal intensities. For those compounds with no confirmation transition, EPI spectra were compared with those obtained when the standard was directly infused. In Fig. 2, an example of an IDA experiment for lisinopril is displayed.

Table 4
Drug concentrations (ng/L) in influent and effluent samples from six WWTPs (NE-Spain) (September 2008).

ng/L	WWTP 1		WWTP 2		WWTP 3		WWTP 4		WWTP 5		WWTP 6		Max	90th perc.[a]	Median
	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.			
Terbutaline	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Salbutamol	–	–	8	3	6	1	–	–	–	–	–	–	8	8	5
Sotalol	–	–	139	103	177	168	22	11	–	–	22	–	177	171	103
Atenolol	189	66	3699	2850	2390	9929	1063	549	1084	497	3016	220	3699	2999	1046
Lisinopril	3	–	306	132	216	113	122	79	59	–	59	7	306	225	96
Fluoxetine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Metoprolol	173	113	3113	398	96	351	273	407	71	160	638	222	638	406	248
Primidone	–	–	224	217	305	–	132	120	–	–	–	–	305	273	217
Desmethylvenlafaxine	5	2	–	–	–	–	–	–	–	–	–	–	5	5	4
Acebutolol	45	35	1	11	11	50	3	6	2	4	–	–	50	46	9
Zolpidem	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Labetalol	–	–	480	309	432	155	300	291	271	–	–	–	480	451	300
Venlafaxine	–	14.3	414	249	197	197	326	372	120	36	144	100	414	376	197
Bisoprolol	–	–	292	114	200	94	103	59	39	–	39	–	292	227	98
Carbamazepine epoxide	880	179	4026	1987	1523	191	3715	2377	1764	69	1910	631	4026	3581	1774
Desloratadine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Enalapril	155	–	6774	–	6960	41	1955	–	566	–	2757	–	6960	6848	1955
Chlordiazepoxide	274	140	5937	29395	2635	1027	5480	3510	2607	127	2621	939	5937	5283	2621
Propanolol	–	–	–	0.7	–	8	11	17	–	–	–	–	17	15	10
Prednisolone	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Bromazepam	–	–	3662	1554	2623	15542	1288	335	797	–	1463	104	3662	2831	1288
Norfluoxetine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Doxazosin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Betaxolol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Carbamazepine	–	–	113	175	85	110	68	81	–	4.96	26	20	175	125	81
Diltiazem	–	–	23	23	–	12	12	4	14	–	0.17	–	23	23	12
Nadolol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Paroxetine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Oxazepam	1218	324	83	143	330	149	573	31	21	30	31	27	1218	550	146
Lorazepam	–	–	289	135	502	532	164	150	–	4	45	32	532	508	150
Amlodipine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Alprazolam	–	–	–	–	–	–	–	–	–	–	4	1	–	–	–
Desmethylsertraline	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Chlorpromazine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sertraline	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Norethindrone	165	252	–	–	–	–	–	–	–	–	–	–	252	244	209
Diazepam	–	–	–	–	–	–	49	–	–	–	–	–	49	49	49
Warfarin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Loratadine	330	–	–	–	–	–	–	–	–	–	–	–	330	330	330
Progesterone	–	–	–	–	5	–	–	–	–	–	–	–	–	5	5
Tamoxifen	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Clopidogrel	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LDOPA	1117	128	1543	580	2581	1374	2888	556	–	–	1620	–	2888	2642	1374
Hydrochlorotiazide	252	15	3879	1789	2346	1329	1926	1673	1524	–	3524	1120	3879	3559	1731
Estriol	9.0	–	1.6	1.3	1.9	0.5	0.45	–	–	–	1.1	–	9.0	4.7	1.3
Prednisone	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Furosemide	201	–	1223	490	1314	560	1226	612	506	–	505	–	1314	1244	560
Phenytoin	6	–	93	120	35	129	55	170	40	67	43	36	170	129	–
Cetirizine	1213	–	2461	546	3596	419	692	367	211	142	237	125	3596	2461	419
Losartan	11	–	557	739	433	583	569	533	88	56	484	82	739	583	484
Estradiol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ethynyl estradiol	–	–	31	–	34	7	–	–	–	–	–	–	35	33	30
Irbesartan	271	208	1176	2225	1839	1672	1313	2976	3175	1694	1508	870	3175	2901	1590
Estrone	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Valsartan	2236	355	11388	9529	22886	17459	11693	1350	5900	405	8127	606	22886	16882	7013

Inf.: Inffluent; Eff.: effluent; Max.: maximum.
[a] Percentile calculation according to NIST [25].

In order to obtain enough data points (10–15) across the narrow peaks of the UPLC chromatogram, rapid data acquisition should be performed by using rapid analyzers and adjusting the dwell times. For the positive ionization mode, dwell times were reduced to 5 ms but not enough points per peak were obtained for the 93 transitions monitored. Therefore, two time segments were established with 48 and 45 transitions for each one, thus achieving then at least 12 points per peak. For the negative mode, one unique segment time was used since by adjusting dwell times to 15 ms, enough points per peak were obtained for the 28 transitions monitored.

3.3. Solid-phase extraction

The performance of four different sorbents was evaluated for the 55 studied compounds. For basic compounds, highest recoveries were obtained with Oasis MCX and WCX cartridges. β-Blockers such as metoprolol, sotalol, bisoprolol or atenolol gave better recoveries (70–90%) with WCX sorbent, whilst for propanolol, doxazosin or labetalol, MCX proved to be more adequate. On the other hand, Oasis MAX cartridges were more effective for compounds such as enalapril, warfarin or angiotensin agents, which exhibited acidic properties. However, to extract all the compounds included in

this study, hydrophobic based bonded phase of Oasis HLB cartridges, capable of non-polar interactions, were selected since they showed acceptable recoveries higher than 55% for all the compounds (Table 3).

The addition of methanol to the washing solutions after sample loading was tested in order to remove interferences from the samples. However, when methanol was added, significant losses in recoveries were observed. Therefore, a rinsing solution of 100% of water was selected.

Prior to SPE extraction, wastewater samples were filtered through glass microfiber GF/A filters (Whatman, England). However, a loss in recoveries was observed for several compounds when this step was performed. Angiotensin agents, sertraline and several β-blockers were more affected by filtration, probably due to their higher hydrophobic properties which could favor their partial retention onto the filter. This problem was solved by rinsing filters with 5 mL of methanol after filtration and collecting the filtrate together with the samples.

3.4. Quality parameters and quantification

Instrumental LOQs obtained ranged from 0.1 to 50 pg injected. The highest in-column injected values were for hydrochlorotiazide (50 pg), lisinopril (40 pg) and furosemide (50 pg), whilst the lowest IQLs were obtained for the β-blockers sotalol (0.1 pg), metoprolol (0.1 pg) and propanolol (0.2 pg). In order to evaluate the performance of the SPE-UPLC/QqLIT method for the analysis of water samples, limits of detection and quantification, run-to-run and day-to-day precision were studied for each compound in wastewater matrices. The LOQs determined ranged from 0.02 ng/L to 50 ng/L. These values turned out to be lower (over 10-folds) than those obtained when similar MS instruments were used to determine some of these compounds [8,19,23]. For instance LOQs previously reported [17] for metoprolol (14 ng/L) or for furosemide (160 ng/L) are more than 10 times lower in this method (0.02 ng/L and 20 ng/L, respectively). This may be due to the high efficiency and sensitivity provided by the UPLC system compared to the use of conventional narrow-bore columns. Run-to-run precision, calculated as %RSD, was lower than 7%, whilst higher values ranging from 3% to 11% for day-to-day precision were obtained.

Concentrations of target compounds were calculated by using the standard calibration curves. Dilution of the samples analyzed proved unnecessary, since all the concentrations calculated fell within the linear dynamic ranges established (0.015–140 μg/L). A summary of the results obtained is displayed in Table 3.

3.5. Matrix effects and recoveries

One of the main disadvantages of electrospray mass spectrometry is that it is highly susceptible to matrix components. As a consequence, signal suppression (or enhancement) can take place due to several factors, such as a change in spray droplet properties caused by the presence of nonvolatile or low volatile solutes which interfere with the efficiency of droplet formation [24] and ion evaporation that affects the amount of charged ions getting the detector. The signal suppression observed for the analyzed pharmaceuticals spiked in wastewater samples after SPE extraction is displayed in Table 3. As can be observed relative low values were obtained for most of the studied compounds, which could be related to the use of UPLC since the sharper chromatographic peaks obtained allow to increase resolution between both target compound peaks and matrix components [25]. Signal enhancement was only detected for diltiazem (+6%), whilst for the other analytes signal suppressions ranging from 5 to 50% were obtained. Slight suppression was observed for several compounds such as atenolol, propanolol, metoprolol, venlafaxine, carbamazepine and its metabolite, sertraline or bromazepam. On the other hand, highest matrix effects were observed for tamoxifen, valsartan, prednisolone, sotalol, fluoxetine and its metabolite, lisinopril, paroxetine, alprazolam, estrone and hydrochlorothiazide with values ranging from 30 to 50%. Additionally, comparisons to evaluate the effect of different wastewater samples into signal enhancement or suppression were performed yielding to slight variations. For instance differences between the values obtained for influent matrices were lower than 3% whilst values lower than 8% were obtained when influent and effluent matrices were compared.

The suppression/enhancement effects were corrected by means of surrogates and internal standards. However, since no isotopically labeled standards were available for each compound or therapeutic group, some compounds were subject to a certain level of inaccuracy.

3.6. Environmental application

The occurrence of pharmaceuticals in influents and effluents from six WWTPs located in Catalonia (NE, Spain) was evaluated using the multi-residue method described above. A summary of the results is displayed in Table 4 and Fig. 3, the total ion chromatograms obtained from influent and effluent wastewater samples is displayed. In the total sample set collected, 31 out of 55 target compounds were found. Among them, clopidogrel, irbesartan, levodopa and zolpidem have been analyzed for the

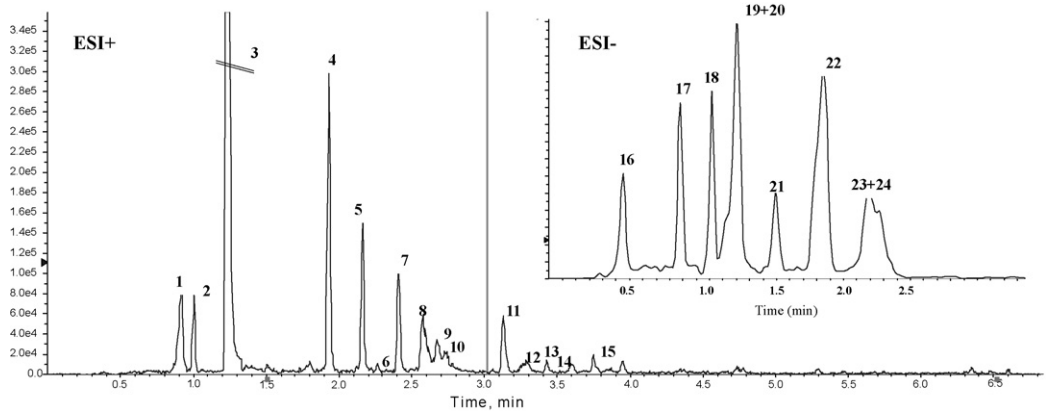

Fig. 3. Total ion chromatogram of a real influent wastewater sample. (1) Sotalol; (2) atenolol; (3) lisinopril; (4) metoprolol; (5) venlafaxine; (6) bisoprolol; (7) carbamazepine epoxide; (8) enalapril; (9) chlordiazepoxide; (10) bromazepam; (11) carbamazepine; (12) diltiazem; (13) oxazepam; (14) lorazepam; (15) alprazolam; (16) Ldopa; (17) hydrochlorothiazide; (18) estrone; (19) furosemide; (20) phenytoin; (21) cetirizine; (22) losartan; (23) valsartan; (24) irbesartan.

first time in wastewater samples. Maximum concentrations at the μg/L level were found for the antihypertensives valsartan (23 μg/L) and enalapril (7 μg/L). High concentrations were also detected for chlordiazepoxide (6 μg/L), atenolol (4 μg/L), the metabolite of the antiepileptic drug carbamazepine (4 μg/L), irbesartan (3.2 μg/L) and Ldopa (2.9 μg/L). Despite the different operational ways of the WWTPs selected, the different flow rates of raw wastewaters and the varying size of treated population, some compounds exhibit similar recalcitrant properties throughout all wastewater treatments. Carbamazepine epoxide, metoprolol, atenolol, venlafaxine, oxazepam, chlordiazepoxide, irbesartan and valsartan were found in all the wastewater effluents sampled.

In order to normalize these values, influent and effluent concentrations were multiplied by the measured flow rates. Above 40 g/day × 1000 inhabitants of pharmaceuticals were calculated to be entering the six WWTPs measured, whilst a total amount of 17 g/day × 1000 inhabitants persists treatment and is discharged into surface water resources.

4. Conclusions

A fast method has been developed for the analysis of 55 pharmaceuticals in environmental samples. For the first time, the separation of this number of compounds has been achieved in less than 9 min (6.3 min positive ionization mode and 2.7 min negative ionization mode). The use of UPLC technology has made possible this fast separation, with an improved sensitivity and a reduction in matrix effects, together with a significant cost reduction in terms of time and solvent consumption. Moreover, the acquisition performed by using the QqLIT instrument provided additional confirmation information by means of IDA experiments without losing sensitivity since SRM acquisition mode was used for quantification. Therefore, the benefits of UPLC combined with QqLIT acquisition modes for trace determination of pharmaceuticals in the environmental field have been demonstrated.

Finally, the applicability of the method developed was evaluated by analyzing wastewater samples obtained from several WWTPs ($n = 6$) in NE-Spain. The results achieved showed the frequent occurrence of 31 of the studied analytes with relatively high concentrations at the μg/L concentration level or in the low ng/L.

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