LC-MS analysis of basic pharmaceuticals (beta-blockers and anti-ulcer agents) in wastewater and surface water

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Given the concern for assessing environmental exposure due to the presence of pharmaceuticals and their potential impact, most studies carried out in this field have been on the development of analytical methods. Beta-blockers and anti-ulcer agents are pharmaceuticals with amine functionalities and basic sites in the molecules that can cause difficulties in their analysis. This review compiles the advances presented in the current literature as approaches to solving the difficulties in the analysis of basic pharmaceuticals by using liquid chromatography with mass spectrometry systems and sample treatment for complex matrices. We also compile environmental findings for beta-blockers and anti-ulcer agents in this review.

Keywords: Anti-ulcer agent; Beta-blocker; Liquid chromatography; Mass spectrometry; Surface water; Wastewater

Abbreviations: CID, Collision-induced dissociation; cLC-MS^2, Capillary-column-switching liquid chromatography coupled to tandem mass spectrometry; CRM, Consecutive reaction monitoring; EDTA, Ethylenediaminetetraacetic acid; ESI, Electrospray ionization; HLB, Hydrophilic-lipophilic balance; HPLC, High-performance liquid chromatography; IP, Identification point; K_{ow}, Octanol-water coefficient; LC, Liquid chromatography; LOD, Limit of detection; LOEC, Lowest observed effect concentration; MANOVA, Multifactor analysis of variance; MCX, Mixed phase-cation exchange; MRM, Multiple-reaction monitoring; MS, Mass spectrometry; ODS, Octadecylsilane; PBD-ZrO_2, Polybutadiene-coated zirconia; PFP, Pentafluorophenyl; pKa, Dissociation constant; POCIS, Polar organic chemical integrative sampler; PTFE, Polytetrafluoroethylene; QTRAP, Triple quadrupole with linear trap MS; Rs, Resolution factor; SIM, Single-ion monitoring; SPE, Solid-phase extraction; STP, Sewage treatment plant; TOF, Time-of-flight; UPLC, Ultra-performance liquid chromatography.

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1. Introduction

Most current research concerned with pharmaceuticals in the environment has been in the development of sensitive and selective analytical methods able to determine pharmaceutical residues in different environmental compartments. Since the first findings of clofibric acid, ibuprofen, nicotine and caffeine in natural water and wastewater in Kansas City and in the Berlin area (Stan and Linkerhäger), several monitoring studies have been carried out to evaluate their occurrence in the environment.

The main interest of water-quality experts then focused on the most likely therapeutic groups and locations (sewage-treatment plants, STPs). Initially, antibiotics and growth steroids were discovered in the run-off from livestock facilities, which fed into streams and rivers and caused potential damage to aquatic organisms, relating to bacterial resistance and endocrine-disruption processes in the reproduction functions of fish, amphibians, and reptiles.

The dangers of other human and veterinary pharmaceuticals, if any, have not been comprehensively studied. To improve and to complement existing knowledge, a growing number of publications are dedicated to the development of toxicity studies and environmental risk assessment [1]. For example, further attention has been given to the therapeutic groups that can act on specific receptors but can also act as non-selective blocking receptors. Many of these receptors might also be present in other mammals, vertebrates and some invertebrates.

Beta-blockers, extensively used for the treatment of hypertension, angina, and

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other disorders of the cardiovascular system, include selective and non-selective pharmaceuticals (e.g., metoprolol is a selective blocker of β1-adrenergic receptors while propanolol is non-selective blocker that acts on β1- and β2-adrenergic receptors and can act as a serotonin receptor antagonist and as a potent membrane-stabilizing agent).

Until now, the ecotoxicity of some beta-blockers (metoprolol, atenolol, propanolol) has been analyzed with various phytoplankton, zooplankton and fish species. Hazard characterization and classification, according to the mode of action, does not seem to be sufficiently clear. The results obtained indicate the detection of acute toxicity towards phytoplankton and zooplankton species (*Synechococcus leopolensis* and *Daphnia magna*), in particular, due to the activity of propanolol [2–5]. Sub-chronic effects of propanolol and metoprolol have also been documented in the growth, reproduction and physiology of *D. magna*, showing a higher toxic action in the case of propanolol, related to growth and fecundity (LOECs 0.4 mg/L and 0.1 mg/L) [6]. Regarding other species, the presence of β-adrenergic receptors in fish (*O. mykiss*) has been proved and suggested in amphibians, but the effect provoked due to the exposure of beta-blockers has not been evaluated [7,8].

Another pharmaceutical group of concern, due to its occurrence in the environment, comprises the anti-ulcer agents. However, the available data concerning the impact of anti-ulcer agents is scarce and refer only to the effects of ranitidine. To date, ranitidine has been considered as not being harmful to aquatic organisms, according to European Union (EU) guidelines [1]. Also, there is no evidence of the presence of target receptors related to anti-ulcer agents. Histamine antagonists act on H2-receptors in the gastric system. Related studies have only demonstrated the presence of H3-histamine

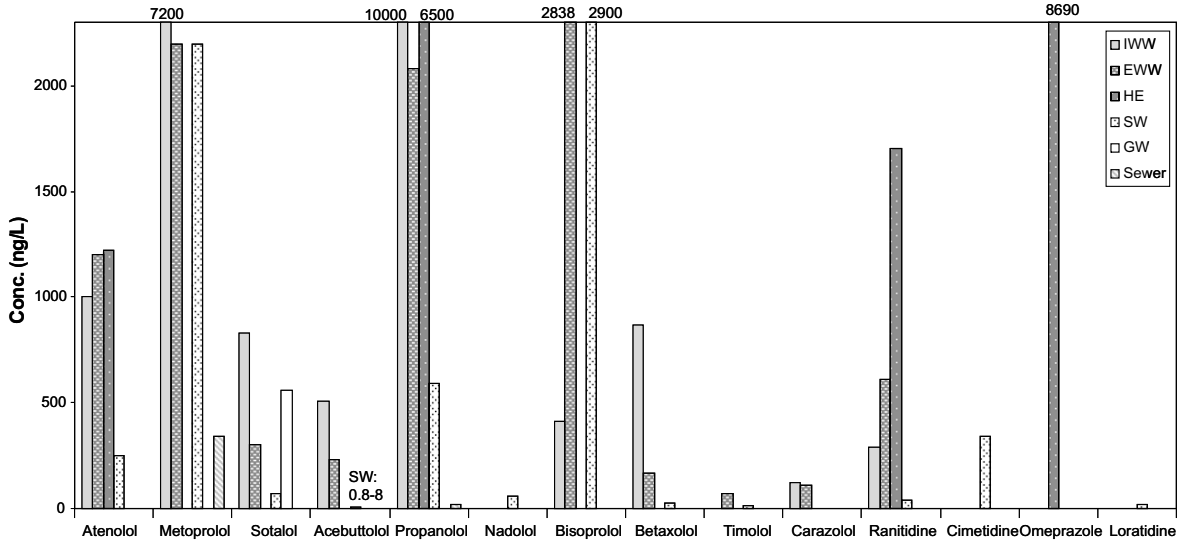

Figure 1. Occurrence of beta-blockers and anti-ulcer agents in wastewater (IWW, Influent wastewater; EWW, Effluent wastewater; HE, Hospital effluent), surface water (SW), groundwater (GW) and sewer water. Reported data in European and American countries. **IWW:** atenolol-range conc. below limit of detection (bld) (42)-1000 ng/L (Finland, Croatia, Spain; [15–17]); metoprolol-range conc. 980–7200 ng/L (Finland; [15,22,23]); sotalol-range conc. 120–830 ng/L (Finland, Croatia; [15,16]);acebutolol-range conc. 390–510 ng/L (Finland; [16]); propanolol-range conc. 80–10000 ng/L (Croatia, Germany, France, USA., Canada, UK.; [4,16–19,22,23,30]); bisoprolol-conc. 410 ng/L (Germany; [22]); betaxolol-range conc. 190–870 ng/L (Germany; [22]);carazolol-range conc. 19–120 ng/L (Germany; [22,27]); ranitidine-range conc. bld-290 ng/L (Croatia; [16]). **EWW:** atenolol-range conc. bld (10)-1200 ng/L (Finland, Croatia, Spain; Italy, Canada, USA, Greece, France, Sweden, Denmark; [15–21]); metoprolol-range conc. bld (1)-2200 ng/L (Finland, Germany, Canada, Norway, USA, France, Greece, Italy, Sweden, Denmark; [15,19,21–27]); sotalol-range conc. bld (60) -300 ng/L (Finland, Croatia; [15,16]); acebutolol-range conc. 80–230 ng/L (Finland; [15]); propanolol-range conc. 10–2080 ng/L (Norway, Croatia, Germany; [16,22,25,27]); bisoprolol-conc. 140–2838 ng/L (Germany, France; [22,23,27]); betaxolol- conc. 170 ng/L (Germany; [22]); timolol- range conc. <25–70 ng/L (Germany; [22]); carazolol-range conc. 70–110 ng/L (Germany; [22,27]); ranitidine-range conc. bld(36)-610 ng/L (Croatia, Italy [16,18]). **HE:** atenolol-range conc. 100–1200 ng/L (Spain; [14]); propanolol-range conc. 200–6500 ng/L (Spain; [14]);ranitidine-range conc. 400–1700 ng/L (Spain; [14]); omeprazole-range conc. 2750–8690 ng/L (Spain; [14]). **SW**: atenolol-range conc. bld(9)-250 ng/L (Finland-Vantaa, Spain-Ebro, Italy-Lambro and Po; [15,16]); metoprolol-range conc. <3–2200 ng/L (Finland-Vantaa; Germany-Rhine and Main; The Netherlands, Canada [15,22,27–29]);sotalol-range conc. <3.9–70 ng/L (Finland-Vantaa Spain-Ebro, Canada; [15,16,29]); acebutalol-range conc. <0.8–8 ng/L (Finland-Vantaa [15]); propanolol-range conc. <10–590 ng/L (UK, Germany-Rhine and Main, Canada; 22,27,29,30]); nadolol-range conc. <50–60 ng/L (Germany; [24,27]); bisoprolol-range conc. <25–2900 ng/L (The Netherlands; [27,28]); betaxolol-conc. 28 ng/L (Germany; [27]); timolol-range conc. <3–10 ng/L (Germany; [24]); ranitidine-range conc. bld-38.5 ng/L (Spain-Ebro, Italy-Lambro and Po, USA; [16,31,32]); cimetidine-range conc. bld-338 ng/L (Spain-Ebro, Italy-Lambro and Po, USA; [31]); loratadine-range conc. bld-20 ng/L (Spain-Ebro; [16]). **GW:** sotalol-conc. 560 ng/L (Germany; [13]). **Sewer**: metoprolol-conc. 340 ng/L (Norway; [25]); propanolol-conc. 20 ng/L (Norway; [25]).

Table 1. Physico-chemical properties of beta-blockers and anti-ulcer agents and removal by sewage-treatment plants (STPs)

Pharmaceuticals	MW (g/mol)	Water sol (mg/L)	log Kow	Ref.	pKa	Excreted unchanged	Metabolites excreted	STP removal (%)	Ref.
Atenolol	266.3	13300 (25°C)	0.16[e]–0.46[t]	[24]	9.6	40–50% (o.a.)	–	<10	[21]
Metoprolol	267.4	4780	1.69–1.88[t]	[24]	9.7	5–10% (o.a.)	3 metabolites[*]	67% 83% <10%	[21,24]
Sotalol	272.4	137000	0.24		9.55	>75	–	n.a.	
Acebutolol	336.4	259	1.71		9.2	10–17% (o.a.) 30–40 (iv.a.)	diacetolol acetolol	n.a.	
Propanolol	259.3	70	3.48[e]–3.03[t] 3.585	[24,33]	9.49	1–4% (o.a. and iv.a.)	8 metabolites[*]	95%	[22]
Nadolol	309.4	8330	0.71	[24]	9.67	24.6% (urine)	–	n.a.	
Bisoprolol	325.4	2240	1.69					65%	[22]
Betaxolol	307.4	451	3.265	[24,33]	9.4			80%	[22]
Timolol	316.4	2740	1.761	[24,33]	9.21	20%	inactive metabolites[***]	n.a.	
Carazolol	298.0	–	–		–				
Ranitidine	314.4	24700 25°C	0.27	[24,33]	8.2	70%	N-oxide S-oxide N-desmethyl metabolities	29%	[33]
Cimetidine	252.3	5000	0.20	[33]	7.1	75% (iv.a.) 48% (o.a.)	Sulfoxide	n.a	[33]
Omeprazole	345.4	82.3	1.16	[33]	3.97 8.8 (anpholyte)	5%	5'-hydroxyomeprazole omeprazole sulfone		[33]
Loratadine	382.9	0.011	3.64	[33]					
Lansoprazole	369.4	0.97	1.73	[33]	8.73	5%	5-hydroxylansoprazole lansoprazole sulfone		[33]

o.a., Oral administration.
iv.a., Intravenous administration.
[*]Metabolites of metoprolol: 3 main metabolites (85% excretion) formed by oxidative deamination, O-dealkylation and subsequent oxidation, and aliphatic hydroxylation).
[**]Metabolites of propanolol: 4-hydroxypropranolol, naphthoxylacetic acid, n-deisopropylpropranolol; 1-(alpha-naphthoxy)-2,3-propyleneglycol; ring hydroxylated 1-(alpha-naphthoxy)-2,3-propyleneglycol; alpha-naphthoxyacetic acid; alpha-naphthol and 1,4-dihydroxynaphthalene.
[***]Metabolites of timolol, formed by ring cleavage ethanolamine and glycine products.
[e]Experimental.

Table 2. Methods developed for analyzing beta-blockers and anti-ulcer agents by LC-MS systems

Compounds	Sample pre-treatment	Recovery					LC conditions		MS system			LOD (ng/L)		Ref.
		Technique	Sorbent/Elution solvent	Clean-up step	Matrix	Recovery (%)	Column	Mobile phase	Interface (polarity)	Analyzer	Operation mode	Matrix	LOD (ng/L)	
Multi-residue analysis of neutral and basic pharmaceuticals (acebutolol, atenolol, metoprolol sotalol)	Filtration (0.45-μm filter) pH adjustment to 10 with NaOH 2M	SPE	Oasis HLB*/MeOH	–	IWW EWW SW GW	64[e] –108[e] 78[e]–123[i] 62[e]–115[i] 76[e]–119[i]	C18	water (AcH, 1%)/ACN	ESI (+)	TQ	MRM	IWW EWW SW GW	6.4–49 2.1–21 0.8–11 0.4–6.5	[15]
β-blockers (atenolol, metoprolol, propanolol)	Filtration, 1.6 and 0.7-μm glassfiber filters	SPE	Oasis HLB*/ MeOH	Drying column of anhydrous precombus-ted $NaSO_4$	IWW EWW	74[i] –106[i] 67[i]–87[i]	Chirobiotic V vancomycin-based chiral column	water (TEAA 0.1%, pH 4)/ MeOH	ESI (+)	hybrid QTRAP	MRM	IWW EWW	17–110 4.4–17	[19]
Multi-residue method for pharmaceutical residues (atenolol, sotalol, metoprolol, propanolol, ranitidine, famotidine, loratadine, lansoprazole)	Filtration, glassfiber filter and nylon memb-rane filters 1, 0.7 and 0.45 μm for WW and well water	SPE	Oasis®HLB*/MeOH		EWW IWW SW	44–114 38–97 40–115	C18	water(NH_4Ac 5mM/ HAc, pH 4.7)/ACN-MeOH(2:1 v/v)	ESI (+)	TQ	MRM	EWW IWW SW	3–60 4–42 2–18	[16]
Selected pharmaceuticals (metoprolol, propanolol)	Filtration with GF/C glassfiber filters 1.2-μm exclusion size, adjust-ment of pH to 7 with SO_4H_2	SPE	Oasis®HLB*/ MeOH	–	HE	70–100[e]	Symmetry-Shield RP18	water (NH_4Ac 2.5mM)/MeOH	ESI (+)	TQ	MRM	–	–	[25]
Selected pharmaceuticals (propanolol)	Filtration with GF/C glassfiber filters 0.45-μm exclusion size, adjust-ment of pH to 3 with HCl	SPE	Phenomenex Strata X Varian Bond Elut C_{18}	–	SW	41–45	C18	water (NH_4Ac 40 mM, pH 5.5)/MeOH	ESI (+)	TQ	CRM	SW	10	[30]
Multi-residue method for pharmaceuticals (atenolol, omeprazole, ranitidine)	Filtration with GF/D glassfiber filters 2.7 μm and adjustment of pH to 1.5–2	SPE	Oasis®MCX*/MeOH, 2% ammonia solution in MeOH/0.2 % NaOH in MeOH	–	EWW	49–106	C8	water (formic acid 0.1 %, pH2)/ACN	ESI (+)	TQ	MRM	EWW	1.06–1.57	[18]

Multi-residue method for pharmaceutical residues (atenolol, propanolol, ranitidine)	Filtration with glass-fiber filters 0.7 μm and adjust-ment pH to 7	SPE	Oasis®HLB*/ MeOH	–	HE	44.8–97	C18	water (formic acid, 0.1%)/ACN	ESI (+)	TQ	MRM	HE	8–32	[14]
Pharmaceutical residues (atenolol, propanolol)	Filtration with GF/C glass-fiber filters and adjust-ment of pH to 4	SPE-on line	C18 monolithic silica column/ 10% v/v ACN in water	–	WW SW	50 (flow rate of 8 ml/min)	Chromolithic Performance RP-18e and Chromolithic zero dead volume column coupler	water/MeOH	ESI (+)	MS	SIM	–	–	[35]
Pharmaceutical residues in water bisoprolol, metoprolol)	Filtration, adjustment of pH to 3 with HCl	SPE	Oasis®MCX/ MeOH-ammonia 95:5 (v/v)	–	SW DW GW	87–97	C18	water (NH_4Ac 2mM)/ MeOH (NH_4Ac, 2 mM)	ESI (+)	TQ TOF	MRM exact mass	SW DW GW	5	[28]
Analysis of small molecules in water (bisoprolol, metoprolol)	–	Column for switching LC system	C18/water (0.1 % formic acid)	–	SW GW DW	13–77	C18	water/ MeOH (formic acid, 0.1%)	ESI (+)	TQ	MRM	DW-BS	50	[36]
Analysis of pharmaceuticals (atenolol, sotalol, metoprolol, propanolol, ranitidine, famotidine, loratadine, labetalol)	Filtration glass-fiber filters and nylon-membrane filters 1 μm and 0.45 μm for WW and surface water	SPE	Oasis®HLB/ MeOH	–	–	–	C18, 1.7 μm for UPLC system	water (NH_4Ac 5mM/ AcH, pH 4.8/ACN-MeOH (2:1, v/v)	ESI (+)	TOF	exact mass	IWW	15–200	[17]
Analysis of pharmaceutical residues (metoprolol, propanolol, atenolol, bisoprolol, sotalol, pindolol, betaxolol)	Adjustment of pH to 7	SPE	PPL Bond Elute/ MeOH	–	TW SW	67–96 44–81	C18	water (NH_4Ac 20 mM, pH 6.8)/ACN-MeOH (NH_4Ac 20 mM (2:1, v/v)	ESI (+)	TQ	MRM	SW	8–17	[13]
Analysis of lipid regulator agents and beta-blockers (atenolol, sotalol, metoprolol, betaxolol)	Filtration 0.45-μm nylon-memb-rane filter	SPE	C18/MeOH	–	UW	52–89	C18	water/ACN	ESI (+)	TQ	MRM	EWW	17–750	[40]

(*continued on next page*)

Table 2 (*continued*)

Compounds	Sample pre-treatment	Recovery					LC conditions		MS system			LOD (ng/L)		Ref.
		Technique	Sorbent/Elution solvent	Clean-up step	Matrix	Recovery (%)	Column	Mobile phase	Interface (polarity)	Analyzer	Operation mode	Matrix	LOD (ng/L)	
					TW SW IWW EWW	40–76 42–63 18–34 42–52							18 550 750	
Analysis of pharmaceutical Compounds in surface- and ground-water (ranitidine, cimetidine)	Filtration, 0.7-μm glass-fiber filters	SPE	Oasis®HLB*/ MeOH-MeOH pH 3.7 (trifluoroacetic acid)	Filtration, 0.2 μm PTFE syringe filter	SW	52–54	C18	water (NH_4COOH, 10 mM/HCOOH pH 3.7)/ACN	ESI (+)	MS	SIM	SW	6.7–10	[41]
Analysis of pharmaceutical compounds in wastewater and rivers (omeprazole)	Glass-fiber filters Adjust-ment to pH 7.0–7.5	SPE	Isolute C18/ MeOH	–	IWW EWW SW GW	64–120* 57–94* 81–97* 88–91*	C18	water (NH_4Ac 20 mM)/ACN	ESI (+)	TQ	MRM	IWW EWW River	10–100 25 10	[42]
Analysis of pharmaceutical compounds in treated sewage effluents (omeprazole)	–	Integrative sampler	POCIS/MeOH	Glass gravity-flow chromatography columns. Concentration and filtration.	UW	95	C18	water (NH_4Ac 1 mM, AcH 0.1%, MeOH, 1%)/MeOH (NH_4Ac, 1 mM, AcH, 0.1%, water 2%).	ESI (+)	ITMS	MS/MS	–	–	[37]
Organic contaminants in wastewater effluents (cimetidine, ranitidine)	–	Integrative sampler SPE	POCIS/MeOH Oasis®HLB/ MeOH-MeOH with trichloro-acetic acid	Glass gravity-flow chromato-graphy columns. Concentra-tion and filtration.	–	–	C-18	water (NH_4COOH/ HCOOH 10 mM, pH 3.7)/ACN	ESI (+)	MS	SIM	–	–	[38]

Polar organic contaminants in the aquatic environment (cimetidine, ranitidine)	Glassfiber filters	SPE	Oasis®HLB/ MeOH- 0.1% TFA in MeOH	Millipore ultrafree-MC 0.45 µm filters	–	–	C-18	water (5 mM HCOOH 5 mM/ NH_4COOH, pH 3.7)/ACN	ESI (+)	MS TQ TOF	SIM MRM exact mass	–	–	[43]
Multi-residue method for pharmaceutical residues (atenolol, propanolol, ranitidine, omeprazole)	Filtration with glassfiber filters 0.7 µm and adjustment pH to 7	SPE	Oasis®HLB[*]/ MeOH	–	HE	16.3–90.4	C18	Water (0.1% formic acid)/ACN	ESI (+)	TOF	Exact mass	HE	6–20	[34]

SPE, Solid phase extraction.
IWW, Influent wastewater; EWW, Effluent wastewater; SW, Surface water; GW, Groundwater; TW, Tap water; UW, Ultrapure water; HE, Hospital effluent.
CRM, Consecutive reaction monitoring.
POCIS, Polar Organic Chemical Integrative Sampler.
[e]External calibration.
[i]Internal calibration.
[*]Corrected recoveries with surrogate standard.

receptors in the central nervous system of zebrafish (*Danio rerio*) [9].

Given the interest in assessing environmental exposure caused by the presence of pharmaceuticals and their potential impact, the initial trend in this field has been the development of different analytical strategies. Pharmaceuticals with amine functionalities and basic sites in the molecule, such as beta-blockers and anti-ulcer agents, entail analytical difficulties. We review the previous studies in the literature as approaches to solving the analysis of basic pharmaceuticals and the related problems in the treatment of complex environmental matrices.

2. Detection of beta-blockers and anti-ulcer agents

The compounds studied are commonly used pharmaceuticals in USA and Europe, and many of them have been found to be ubiquitously present in STPs as well as in streams and rivers (Fig. 1). Table 1 shows various physico-chemical data (e.g., molecular weight, water solubility, octanol-water coefficient (K_{ow}), dissociation constant (pKa)) and pharmacodynamic data (e.g., the proportions of the parent compound that are typically excreted and the major metabolites excreted). Most of the beta-blockers and anti-ulcer agents are basic in nature, with pKa values in the range 7.1–9.7, and, at a neutral pH, exist largely in their ionized form. Water is an extremely important medium for transporting organic compounds in the environment.

Log K_{ow} is an indicator of the lipophilicity of the compound. A high K_{ow} is typical of hydrophobic compounds, whereas a low K_{ow}signifies a compound soluble in water. K_{ow} also affects sorption of the compound (e.g., a low K_{ow} reduces the affinity between the compound and the soils, sediments and dissolved organic material). The majority of the target compounds are hydrophilic pharmaceuticals, so they are more likely to partition to the dissolved phase, leading to enhanced bioavailability of the compounds in the environment. The log K_{ow} values of the target compounds ranged between 0.16 for atenolol and 3.6 for loratadine, and their water solubility ranged between 0.011mg/L for loratadine and 13 7000 mg/L for sotalol. Atenolol, sotalol, nadolol, ranitidine and cimetidine were highly-soluble compounds, and propanolol, betaxolol, loratadine and lansoprazole were the least-soluble compounds of the target pharmaceuticals.

Table 1 shows that the proportion of excreted parent compound is very different, depending on the compound. An excreted proportion of 40–75% parent compound can be considered relatively high [10], as was found in the cases of beta-blocker sotalol and anti-ulcer agents ranitidine and cimetidine. Ranitidine, in particular, is metabolized in the liver to its N-oxide, S-oxide and

desmethyl forms, although 70% of the drug is excreted in urine unchanged. However, beta-blockers metoprolol and propanolol, once consumed, are extensively metabolized in the liver and are, to a large extent, excreted in the form of various metabolites with very little unchanged compound (10% or less).

Anti-ulcer agents omeprazole and lansoprazole are also excreted in an unaltered form in the same low proportion. They are prodrugs, which are rapidly converted at low pH to reactive metabolites. This reveals the importance of studying the degradation products of the pharmaceuticals. Most efforts in environmental analysis have focused on the detection of parent compounds, while the analysis of metabolites and transformation products has been limited to only a few groups of compounds. However, metabolites and early-degradation products can also be of environmental concern. They can occur in higher concentrations and be even more toxic than the parents compounds.

Table 1 shows that even pharmaceuticals that have been reported to have a very low proportion of the excreted parent compound have also been encountered in the environment, in some cases at relatively high concentration, as with metoprolol and propanolol. The excreted metabolites and unaltered parent compounds can be transformed further in STPs. Studies show that many of these compounds survive biodegradation, eventually being discharged into receiving waters. Metabolic conjugates can even be converted back to the free forms of their parent. Removal efficiencies in STPs depend on the chemical characteristics of the drug structure. The efficiencies of various STPs also vary for the same compound, not only due to the treatment technology employed, but also because the treatment effectiveness may fluctuate according to other factors (e.g., the time of day or even the season).

Fig. 1 shows that beta-blockers and anti-ulcer agents are abundant in influent and effluent wastewaters. Atenolol, metoprolol, propanolol, sotalol and ranitidine have been found at high levels. Despite only 10% of propanolol being excreted unchanged, this beta-blocker is found to occur ubiquitously in the STPs, and at the highest concentrations of all the studied compounds, with a maximum concentration of 10 μg/L in the influent and 2.08 μg/L in the effluent. It has also been found at a concentration of 6.5 μg/L in hospital effluents. In the literature, the reported reduction value for propanolol in the STPs is approximately 95%. However propanolol has frequently been detected in surface waters at concentrations of 10–590 ng/L.

Metoprolol is reported to be the major beta-blocker found in surface waters in the concentration range 3–2200 ng/L. Some authors calculated a removal rate of less than 10% for this compound [11]. However, other authors observed a reduction of 67% to 83% for metoprolol [12]. Investigations of influent and effluent samples from different STPs have shown that atenolol is not significantly removed during sewage treatment and that this compound has also been found in the concentration range 9–250 ng/L in the surface waters of different countries. Sacher et al. [13] detected beta-blocker sotalol at a concentration of 560 ng/L in groundwater samples from Germany. This data is not surprising, since the drug is highly polar and it seems likely that it would leach through the sub-soil and therefore appear in groundwater aquifers.

Anti-ulcer agents ranitidine and omeprazole have been found at μg/L concentrations in hospital effluents. Omeprazole is one of the most consumed pharmaceuticals in hospitals [14] but it is only occasionally detected in hospital effluents and has not previously been detected in STPs or surface waters, probably because of its low stability and poor recoveries. For these compounds, it would be more suitable to analyze their principal active metabolites.

3. Extraction methods

Table 2 shows a survey of analytical methods, developed for the determination of the target basic pharmaceuticals in aqueous environmental matrices. Before extracting target analytes from a water matrix, the sample is filtered to subtract the suspended matter, usually with 0.45-μm or 0.7-μm glass-fiber or nylon-membrane filters. Prefiltering will not affect the determination of these compounds, since these basic pharmaceuticals are predominantly distributed in water in the dissolved phase. Several papers reported a sample pH adjustment, with values ranging from acid to alkaline pH (2, 3, 4, 7, 10) depending on the solid-phase extraction (SPE) sorbent used and if the analytical method included several groups of pharmaceuticals. In most cases, extraction and preconcentration were performed by off-line solid-phase extraction.

Cross-linked polymer Oasis HLB (hydrophilic-lipophilic balance) has been the adsorbent most widely employed. This sorbent provides the best conditions for the extraction of compounds with a wide range of polarity [14,16,34].

In many of the analytical methods described in the literature, the target compounds are analyzed simultaneously with other pharmaceuticals (often with quite different physico-chemical characteristics) in a multiresidue method. This simultaneous analysis of several groups of compounds generally requires a compromise in the selection of experimental conditions, which, in some cases, means not obtaining the best performance for each one of the compounds. The potential of cross-linked polymers (Oasis HLB) for extracting acidic, neutral and basic compounds from water over a wide range of pHs has been demonstrated [14,16,18,34]. However, for

this type of analysis, some difficulties can be expected (e.g., low retention of the most polar compounds).

The effect of pH on extraction efficiency has been studied [14–16,34]. The results showed that the extraction recovery for the majority of the compounds was higher at neutral pH. The co-extraction of matrix components was significantly reduced at pH 7, compared to extraction in acidic conditions, and an increase in pH led to a reduction in the extraction efficiency of some analytes [14,34]. In these studies, the authors attributed the low recoveries obtained for some basic compounds with high polarity and water solubility, such as omeprazole or ranitidine, to poor retention of the polymeric sorbent, as a result of an inappropriate pH adjustment of the samples before extraction.

Recoveries of 92% and 77% for ranitidine and omeprazole, respectively, were achieved at the basic pH of 8.5 [34]. Vieno and co-workers [15], applied for the SPE analysis of neutral and basic pharmaceuticals, Oasis HLB sorbent with pH values adjusted to 4, 7.5 and 10, and obtained very good recovery results for all beta-blocker compounds at pH 10. For most compounds, pH did not have a pronounced effect on the recovery, with the exception of beta-blockers atenolol and sotalol, which were poorly recovered at low pH. Both compounds were recovered at fairly high yield at basic pH.

Most of the beta-blockers and anti-ulcer agents have weak bases with pKa values in the range 7.1–9.7 (Table 1), so they occur at higher pH conditions, mainly as uncharged compounds, which are more readily adsorbed to the sorbent. Oasis MCX, a mixed reversed-phase-cation-exchange cartridge, has been used [18,28]. Drugs bearing amino groups, which are positively charged at acidic pH, are bound by the cation exchanger, while neutral and acidic compounds are retained by the polymeric phase. Good recoveries were achieved with this sorbent for bisoprolol and metoprolol [28], as well as atenolol and ranitidine, although omeprazole gave a 50% recovery, probably because of the poor stability in solution of omeprazole, which was greatly affected by pH and salinity [18].

Precautions necessary to enhance the recovery of polar compounds (e.g., beta-blockers or anti-ulcers agents) are silanization of all glassware coming into contact with either the water sample or the extract and the use of other container materials (e.g., PTFE) or the addition of EDTA, in order to minimize the surface adsorption of analytes [18,30,35].

Bones and co-workers [35] have developed a fully automated methodology for the on-line SPE and analysis of pharmaceutical residues in water samples, using a micro-reversed-phase monolithic silica column. This methodology allows for very rapid trace enrichment from large volume samples (500 ml) with minimal sample handling. The columns can be washed and conditioned on-line with no sample carryover and used repeatedly for up to eight extractions each. Acceptable recoveries of >70% were obtained for the majority of pharmaceuticals investigated. Nevertheless, there was little or no recovery of a number of the more polar analytes (e.g., beta-blocker atenolol) from the extraction column, due to insufficient retention of these polar compounds on the sorbent. In an attempt to increase the retention of the more polar pharmaceuticals, the authors investigated pH values in the range 3–7 (the silica-based column is stable only within this range). The recovery of beta-blocker propanolol increased as the pH was increased, from 40% at pH 4 to 71% at pH 7. No appreciable recovery was observed for very polar analytes (e.g., atenolol). These analytes may require more extreme alkaline conditions in order to exhibit retention on the C_{18} phase.

Pitarch et al. [36] also investigated the potential of capillary-column-switching liquid chromatography coupled to tandem mass spectrometry (cLC-MS^2) for the trace determination of drugs in environmental water samples. In order to improve the efficiency of the miniaturization process in LC, column-switching systems are used to overcome the limited injection volumes. In this on-line approach, a short capillary column, typically 1 cm in length, was used as a first column, enabling relatively high flow rates during trapping of analytes. Besides fully automated analysis, the methodology enabled the determination of pharmaceuticals at the sub-ppb level, consuming only 25 μl of sample and in a short analysis time (less than 20 min). For three out of five compounds tested, including beta-blocker bisoprolol, the mean recoveries were in the range 70–105%. Less metoprolol was recovered (mean recovery 18%), which the authors explained by the presence of a matrix effect on the ionization at this low concentration level. Recoveries of various types of water samples were studied (surface water, groundwater and drinking water) and the differences between the different types of water were low.

Using a passive in situ sampling device to analyze pharmaceuticals in environmental water was reported recently [37,38]. Interestingly, these authors developed the Polar Organic Chemical Integrative Sampler (POCIS), which integratively concentrates trace levels of complex mixtures of hydrophilic environmental contaminants, enables the determination of their time-weighted average water concentrations, and provides a method of estimating the potential exposure of aquatic organisms to the complex mixture of waterborne contaminants. According to Alvarez [38], who studied the comparison of the passive sampler to standard water-column sampling for organic contaminants, out of a total of 96 targeted analytes, 24 were identified on the water-column samples and 32 were identified in the POCIS extracts. Review of the data generated by both sampling methods indicated that the passive sampling method has

advantages over traditional water-column sampling regimes. Most conventional environmental-pollutant-screening techniques for water matrices use grab sampling coupled with SPE. Grab samples give an incomplete picture of overall concentrations of pollutants. Among the strengths of the POCIS are its capacity to handle large volumes of water for several days or weeks and its ability to detect episodic changes in environmental-contaminant concentrations, which are often missed with conventional grab samples. Thus, according to Alvarez [38], the POCIS increases method sensitivity, is simple to use, and helps with ecological risk assessments not easily obtainable with traditional methods. The recoveries for the pharmaceuticals from the laboratory experiments reported [39] using the POCIS with Oasis HLB sorbent were greater than 86%; among the pharmaceuticals studied was the anti-ulcer agent omeprazole.

4. Liquid-chromatography analysis

The chromatography of basic pharmaceuticals has traditionally been performed using bonded C18-silica phases, especially, in the pharmaceutical and biotechnological industries, and, over recent years, in environmental analysis [13–16,28–30,36,40]. Basic pharmaceuticals, such as beta-blockers or anti-ulcer agents, are hydrophilic compounds and, for their chromatographic analysis, C8 or C18 columns are used, with buffer salts and various additives (ion-pairing agents or ion-countering agents) often needed to provide an adequate retention of analytes in the stationary phase.

The presence of amine functionalities in basic pharmaceuticals can produce tailed peaks, and therefore poor peak efficiency, due to secondary interactions with the unreacted silanols. Other sources, including sample overload, slow detector response or slow adsorption-desorption kinetics, can also cause detection of tailed peaks. Because of energetic surface heterogeneity, possible ion-exchange types of interactions, with high energetic adsorption sites in the column, can lead to slow sorption-desorption of solute molecules from the strong sites compared to the weak sites, so this phenomenon could further increase band tailing. Guiochon and co-workers [44,45] have shown that kinetic tailing, due to slow desorption from strong sites, may exist in addition to non-linear tailing and contribute to asymmetrical peaks appearing.

When this effect is associated with interactions with accessible residual silanols, the use of ion-pairing and ion-suppressing agents in the mobile phases helps to decrease these secondary interactions. Ion-pairing reagents have been widely applied, facilitating peak-shape improvements by diminishing the silanophilic interactions in the stationary phase. Inorganic mobile phase additives are effective in blocking the silanol-active sites using a high buffer concentration ($Na^+ < K^+ < NH_4^+ <$ triethylammonium < dimethyloctylammonium) [46]. The pH of the mobile phase can also be a determining factor. At pH below 3, since the pKa of normal silanols is in the range 5–7, the majority of silanol sites should be in neutral form, so the interactions with the protonated basic compounds should be minimized. The result is that low pHs may cause early elution of basic pharmaceuticals in the chromatogram.

The effect of the concentration of volatile salts (ammonium acetate, ammonium citrate and sodium phosphate) has not had a significant influence on retention factors when conventional columns (4.6-mm i.d.) are used, while it is notable in micro-columns (0.3-mm and 1.0-mm i.d.). In this case, the retention of analytes decreases with increasing concentration of electrolytes. Multi-factor analysis of variance (MANOVA) carried out by Vervoort and co-workers to evaluate the influence of various variables (modifier, stationary phase, buffer, buffer pH and buffer concentration) on the resolution, peak symmetry and retention of basic pharmaceuticals showed that miniaturization by simply downscaling dimensions can result in varying selectivity and peak shapes for basic pharmaceuticals [47]. This study also indicated that the chromatographic performance for the separation of basic pharmaceuticals, at pH 3, is similar in the selected columns (4.6-mm, 0.3-mm and 1-mm i.d.) using volatile mobile-phase additives (ammonium acetate, ammonium citrate) or even non-volatile electrolytes (sodium phosphate). The peak symmetry appears to be affected by the parameters studied, and modifier ammonium citrate shows the best symmetry.

Other silica stationary phases with cyano (CN), pentafluorophenyl (PFP) groups have also been investigated in the chromatographic analysis of basic pharmaceuticals as alternatives to reversed phases C18 and C8 in avoiding the addition of ion-pairing and ion-suppressing agents. Signal suppression may be produced in MS detection (MSD) when an electrospray-ionization (ESI) interface is used. ESI interfaces have been the technique of choice in most methods developed for the analysis of beta-blockers. It has been reported that the addition of buffer in the mobile phase (at a concentration level of about 100 mM) decreases the ESI-MS signal. This effect is observed when the concentration level is about 100 mM, which is the buffer concentration often required to decrease peak tailing in the analysis of basic compounds [48].

One option is the use of stationary phases with cyano (CN), pentafluorophenyl (PFP) groups. These columns have shown an adequate retention of basic compounds, which are eluted under isocratic conditions using mobile phase with 90% acetonitrile [49], with which the ESI-MS signal increases, since most organic solvents (e.g.,

Table 3. Mass-spectra information of beta-blockers and anti-ulcer agents

Compounds	Molecular formula	Precursor ion m/z	Precursor ion	Exact Mass	Product ion		Product ion		Ref.
Atenolol	$C_{14}H_{22}N_2O_3$	267.3	$[M+H]^+$		190.2	$[M-H_2O-C_3H_7NH]^+$	145.2	$[190-CO-NH_3]^+$	[13]
					145	$[190-CO-NH_3]^+$	190	$[M-H_2O-C_3H_7NH]^+$	[16]
									[14]
									[15]
									[18]
									[40]
Metoprolol	$C_{15}H_{25}NO_3$	268.4	$[M+H]^+$		116.1	[(N-isopropyl-N-2-hydroxypropylamine)+H]$^+$	74.1		[13]
			$[M+H]^+$		159	$[C_8H_{17}NO_2]^+$	133	$[C_6H_{15}NO_2]^+$	[16]
					133	$[C_6H_{15}NO_2]^+$	191	$C_{12}H_{15}O_2$	[36]
					190.9	$C_{12}H_{15}O_2$	98	[(N-isopropyl-N-propenamine)+H]$^+$	[28]
							159	$[C_8H_{17}NO_2]^+$	[15]
									[40]
Bisoprolol		326.6			116.3	[(N-isopropyl-N-2-hydroxypropylamine)+H]$^+$	56.1		[13]
							74		[36]
									[28]
Acebutolol	$C_{18}H_{28}N_2O_4$	336.8			116.0	[(N-isopropyl-N-2-hydroxypropylamine)+H]$^+$			[13]
									[15]
Sotalol		273.4	$[M+H]^+$		213.1	$C_9H_{19}NO_3S$	133.1		[13]
					255	$C_{12}H_{19}NO_3S$	213	$C_9H_{19}NO_3S$	[16]
		254.8			132.9				[15]
					255	$[M-H_2O+H]^+$	213	$[M-C_3H_9N+H]^+$	[40]
Propanolol	$C_{16}H_{21}NO_2$	260.2	$[M+H]^+$		183.3	$[M-H_2O-C_3H_7NH]^+$	116.1	$[M-C_{10}H_7O]^+$	[13]
									[16]
									[14]
		260.0			182.9				
Pindolol	$C_{14}H_{20}N_2O_2$	250.1			56.2		72.0		[13]
Betaxolol		308.3			55.2		56.2		[13]
					116	[(N-isopropyl-N-2-hydroxypropylamine)+H]$^+$	98	[(N-isopropyl-N-propenamine)+H]$^+$	
		308			133	$[C_6H_{15}NO_2]^+$	159	$[C_8H_{17}NO_2]^+$	[40]
Nadolol	$C_{17}H_{27}NO_4$	310			254	[M-tert-butyl$^+$+2H]$^+$	201		[13]
Timolol	$C_{13}H_{24}N_4O_3$	317			261	[M-tert-butyl$^+$+2H]$^+$	244	[M-tert-butylamine+H]$^+$	[13]
Carazolol		299			116		222		[13]
Ranitidine	$C_{13}H_{22}N_4O_3S$	315	$[M+H]^+$		176	$[M-C_8H_{12}NO]^+$	130	$[M-C_8H_{12}NO-NO_2]^+$	[14]
									[16]
									[18]
									[43]
									[17]
									[34]
Omeprazole	$C_{17}H_{19}N_3O_3S$	346	$[M+H]^+$		136	$[M-H_3CO-(C_7H_4N_2)-SO-CH_2]^+$	197	$[M-H_3CO-C_7H_4N_2]^+$	[42]
					151		198		[18]
					214				[34]
Cimetidine	$C_{10}H_{16}N_6S$	253	$[M+H]^+$		158				[43]
Famotidine	$C_8H_{15}N_7O_2S_3$	338	$[M+H]^+$		189		259		[16]
									[17]
Lansoprazole	$C_{16}H_{14}F_3N_3O_2S$	370	$[M+H]^+$		252		205		[16]
									[17]

(*continued on next page*)

Table 3 (*continued*)

Compounds	Molecular formula	Precursor ion m/z	Precursor ion	Exact Mass	Product ion	Product ion	Ref.
Loratadine	$C_{22}H_{23}ClN_2O_2$	383	$[M+H]^+$		337	259	[16] [17]

acetonitrile and methanol) have low surface tension and high volatility, so there is more efficient desolvation in the ESI interface. The use of phases and operational conditions is presented as a reliable alternative for the pharmaceutical industry, where fast analysis is necessary [49]. Isocratic elution is desirable for the fast cycle times required for high-throughput analysis.

To achieve adequate chromatographic resolution in a reduced analysis time, several approaches have used short columns [50], high mobile-phase flow-rate, HPLC columns filled with particles of a small size (<2μm), or, more recently, ultra-performance liquid chromatography (UPLC) [17] or nano-liquid chromatography (nano-LC) [51].

The advantages of down-scaling dimensions in chromatography were first demonstrated by the introduction of the micro-column for separating ribonucleotides [52]. High efficiency, reduced volumes of mobile phases or small amounts of packing materials make the results of that these techniques attractive. By contrast, due to the small volume of sample injected, the sensitivity could be limited, but that can be solved applying an on-column focusing method, two-dimensional LC or coupling with MS.

To improve the efficiency of miniaturization in LC, column-switching systems have been used for analyzing low volumes of samples [36]. For coupling to MS systems, nano-flow ESI interfaces have been developed using capillary tips of small i.d.

A recent study evaluated the nano-ESI and ESI interfaces in terms of sensitivity and efficiency. The nano-ESI interface coupled to an ''ion-trap'' spectrometer provided the best sensitivity in analyzing beta-blockers. The reduced sensitivity achieved by ESI was attributed to the presence of the sheath liquid that resulted in significant dilution of the sample. The stability of the nano-ESI spray was also greater. In addition, the use of fused-silica capillaries of 75 μm i.d., packed with C18-silica-modified particles (without free silanol groups) was advantageous, since better resolution, peak symmetry and efficiency were achieved in the LC analysis of beta-blockers [51].

Chiral HPLC methods have been used extensively for separating pharmaceutical steroisomers in formulations or biological samples [53]. In environmental analysis, chiral analysis can provide useful information on pharmaceuticals. Pharmaceutical steroisomers may have different fates and toxicological effects (e.g., the S-enantiomers of beta-blocker propanolol have higher chronic toxicity to fathead minnows than its antipode [54]). One current publication described the development of an analytical method for quantifying beta-blocker stereoisomers in wastewater, using a chiral stationary-phase column [19]. The resolution factor (Rs) obtained for the analyzed beta-blockers (atenolol, metoprolol and propanolol) was sufficient to quantify enantiomer composition in influent and effluent wastewater [19]. The sensitivity achieved by MS^2 should allow the detection of trace concentrations of beta-blockers in wastewater (limit of detection (LOD) in the range 2–17 ng/L) [19]. The direct coupling of a reversed-phase chiral column to an ESI interface is compatible, but some favorable factors in chiral separation with respect to modifiers and organic solvents need to be compromised, so non-volatile modifiers used for improving chiral selectivity may adversely affect the ionization process.

Conventional organic solvents, such as hexane, are generally not suitable for use with an ESI interface. Also to be considered in chiral separation are matrix effects, in particular, in the analysis of pharmaceutical formulations, for which isocratic elution is usually preferred, and when the retention-time window for the enantiomers is more than 25% of the total chromatographic run time. A post-column infusion system is proposed as an effective solution for minimizing matrix interferences.

5. Mass-spectrometry detection

Given the advantages that MSD provides in selectivity and sensitivity, this technique has been introduced for the analysis of different pharmaceutical residues in complex matrices such as environmental samples. The most common analyzer used for the determination of beta-blockers and anti-ulcer agents has been the triple quadrupole, which has been mainly applied for confirmative and quantitative purposes by the selection of, at least, two multiple reaction monitoring (MRM) transitions.

The criteria for the confirmation of pharmaceutical residues are usually based on the detection of a second MRM transition but also on the MRM ratio, which is compared with the MRM ratio observed in the standard,

so the MRM ratio obtained in a sample should be similar to the standard, within ±10%. With this information, sufficient identification is provided for confirmation of pharmaceutical residues in accordance with the concept of identification points (IPs) defined in EU guidelines, as 4 IPs can be achieved using the triple-quadrupole mass analyzer. This technique has also shown enough sensitivity to detect trace-concentration levels of pharmaceuticals in different environmental matrices (e.g., LODs have been reported in the range 5–20 ng/L, in effluent wastewater, or 0.8–12 ng/L in surface water). The complexity of the matrix affects the sensitivity. Analyses performed with groundwater, surface water, effluent and influent wastewater have reported LODs that indicate greater sensitivity in groundwater, compared with that obtained in influent wastewater, by a factor of 8. In the case of wastewater samples, enhanced sensitivity could be achieved by pre-concentrating samples (LODs of 1 ng/L) [18]. However, one drawback is that the pre-concentration of matrix interferences could lead to signal enhancement or signal suppression during analyses.

In general, ESI has been the technique of choice for analysis of pharmaceuticals. Beta-blockers and anti-ulcer agents show amine-containing side chains in the molecules, and protonation on the N-atom is favored in the ESI process. If the ionization process of the molecule is followed by a fragmentation step, these processes could involve the energetically favorable elimination of neutral molecules (e.g., amines, alkenes and water). Such processes shorten this side chain and contract ring systems to which these chains are bonded. The result is an MS-fragmentation pattern that is characteristic for beta-blockers (e.g., with metoprolol, bisoprolol, acebutolol, betaxolol, carazolol and propanolol, the $[M + H]^+$ ion underwent in-source fragmentation to give a common signal at m/z 116, which corresponds to $[(\text{N-isopropyl-N-2-hydroxypropylamine})+H]^+$.

Anti-ulcer agents, which have been selected in publications relating to the environment, involve histamine H_2-receptor antagonists, also known as H_2-blockers (cimetidine, ranitidine), and the benzimidazole enzyme-inhibitor proton pumps (omeprazole, lansoprazole). Table 3 summarizes MS information, showing fragment ions reported in available literature.

Few papers have been dedicated to the analysis of beta-blockers and anti-ulcer agents based on MSD and using the single-ion monitoring (SIM) operating mode [35,38]. In these works, sensitivity comparable to that obtained by a triple-quadruple mass analyzer has been reported, in particular for anti-ulcer agents. However, the analysis of complex matrices can be resolved in a suitable way using a triple-quadrupole analyzer due to the enhanced selectivity that is provided in MRM mode.

The QTRAP system is a hybrid analyzer that can operate in both modes (i.e. triple quadrupole or ion trap (IT)). The selectivity of the QTRAP system in triple-quadrupole mode was also probed in a recent publication, in which Nikolai et al. reported the sensitivity achieved with LODs obtained in effluent and influent wastewater at low ng/L level operating in MRM mode for the analysis of beta-blockers in environmental samples [19].

Until now, data have rarely been published on the use of a time-of-flight (TOF) mass analyzer for analyzing pharmaceuticals in water samples. The selectivity of this system, based on the exact-mass measurements, has been demonstrated by the analysis of complex matrices. Multi-residue methods have been developed to make possible the determination, in full-scan mode, of different pharmaceuticals in wastewater samples. Another advantage of using the TOF system is its capability to provide accurate mass determination for product ions generated by in-source collision-induced dissociation (CID). In this way, the MS information obtained should also meet the requirements for IPs to identify residues in analyzing environmental samples. However, the greater resolving power is limited by lower sensitivity when compared with a triple quadrupole or an IT mass analyzer. The LODs obtained in analyzing beta-blockers and anti-ulcer agents have been reported in the range 50–200 ng/L [17,34].

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