Analytical methods for tracing pharmaceutical residues in water and wastewater

D. Fatta, A. Nikolaou, A. Achilleos, S. Meriç

The focus of environmental analysis and determination of chemicals in the environment has recently been extended from the more classical pollutants to new contaminant categories including pharmaceuticals. This is attributed mainly to the development of new analytical techniques including liquid chromatography with tandem mass spectrometry (LC-MS^2) and gas chromatography with mass spectrometry (GC-MS), which enable the determination of such compounds down to the ng/l level. This article reviews the most recent developments and applications within water and wastewater environmental matrices.

Keywords: Analytical method; Determination; Environmental analysis; Pharmaceutical residue; Wastewater; Water

D. Fatta*, A. Achilleos
University of Cyprus,
Department of Civil and
Environmental Engineering,
Laboratory of Environmental
Engineering,
GAIA, 75, Kallipoleos, 1678,
Nicosia, Cyprus

A. Nikolaou
University of the Aegean,
Faculty of Environment,
Department of Marine
Sciences, University Hill,
81100 Mytilene,
Greece

S. Meriç
University of Salerno,
Department of Civil
Engineering,
84084 Fisciano (SA),
Italy

*Corresponding author.
Tel.: +35 7 22 892275;
Fax: +35 7 22 892295;
E-mail: dfatta@ucy.ac.cy

1. Introduction

Among the various compounds considered as emerging pollutants, pharmaceuticals at ng/l levels are of particular concern because of both their ubiquity in the aquatic environment and their health effects.

Pharmaceutical residues have been detected in many environmental matrices worldwide (e.g., in waters, wastewaters, sediments and sludges). Those compounds depending on hydrophilicity can enter the aquatic environment or remain adsorbed on solid particles (Fig. 1). The most important sources of such compounds in the environment are households, wastewater treatment plants, hospitals, industrial units and intensive animal-breeding farms [1]. So far, an important negative impact is that continual sub-lethal levels of antibiotic residues in aquatic environments have led to the emergence of antibiotic-resistant strains of bacteria [2].

The application of advanced measurement technologies (e.g., gas chromatography with mass spectrometry (GC-MS) and GC with tandem MS (GC-MS^2) or liquid chromatography with MS (LC-MS) and LC with tandem MS (LC-MS^2)) to environmental analysis has allowed the determination of a broader range of compounds, including pharmaceuticals, and has therefore permitted more comprehensive assessment of environmental contaminants. LC-MS^2 is becoming more commonly used in pharmaceuticals analysis because of its high sensitivity and its ability to confirm compounds (as compared with conventional LC with ultraviolet (UV) or fluorimetric detection). LC-MS^2 allows separation and detection of compounds having the same molecular mass but different product ions, even if they co-elute. MS^2 detection is therefore preferred for increased analytical sensitivity and selectivity in complex matrices, such as wastewaters [3]. Table 1 shows that, from the various studies reviewed in this article, GC-MS was used in 17 studies, LC-MS^2 in 12, high-performance LC with diode-array detection (HPLC-DAD) in two, HPLC-fluorescence in two, and GC-MS^2 and LC-MS in one each.

As mentioned by Petrovic et al. [36], both GC-MS and LC-MS methods have some drawbacks. Prior to GC-MS analysis, derivatization of polar pharmaceuticals is necessary, performed using highly toxic and carcinogenic diazomethane or, less frequently, acid anhydrides, benzyl halides and alkylchloroformates. This step can also affect the accuracy of the method.

Ternes [37] directly compared GC-MS and LC-electrospray ionization (ESI)-MS^2, and showed that only LC-(ESI)-MS^2 allows the analysis of extreme polar compounds (e.g., β-blockers atenolol and sotalol) due to an incomplete derivatization of the

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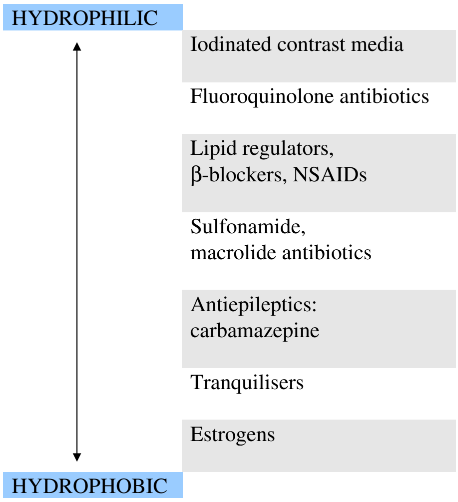

Figure 1. Level of hydrophilicity and hydrophobicity of pharmaceutical compounds.

functional groups. Further, the relative standard deviation (RSD) using LC-(ESI)-MS^2 was found to be lower. However, when analyzing highly contaminated samples, such as sewage, suppression of electrospray ionization is likely to occur, so, to guarantee accurate, reproducible data, either an efficient clean-up step has to be included in sample preparation or an appropriate surrogate standard has to be spiked prior to enrichment by solid-phase extraction (SPE).

Farré et al. [38] compared LC-(ESI)-MS and GC-MS (after derivatization with BF_3-MeOH) for monitoring some acidic and very polar analgesics (salicylic acid, ketoprofen, naproxen, diclofenac, ibuprofen and gemfibrozil) in surface water and wastewater. The results showed a good correlation between methods, except for gemfibrozil, for which derivatization was not completely achieved in some samples. In general, the limits of detection (LODs) achieved so far with LC-MS^2 methods are slightly higher than those obtained with GC-MS methods [3]; however, LC-MS methodology showed advantages in terms of versatility and sample preparation being less complicated (i.e. derivatization is not needed).

In order to propose structures and to obtain information about unknown ions, exact mass analysis can be performed in tandem in-time instruments, which are, typically, ion-trap mass spectrometers (e.g., two-dimensional and three-dimensional quadrupole ion traps and Fourier transform ion cyclotron resonance [3]). These instruments are able to record a complete mass spectrum of each pulse of ions introduced into the trapping volume, so the sensitivity they achieve is extremely high [39]. However, since a triple quadrupole does not perform with exactly the same set of physical principles as an ion trap, for specific applications, both instruments may provide different product ions. In a triple quadrupole, an isolated precursor ion is directed into a collision cell and accelerated through an inert gas via a voltage offset, meaning that any ion that enters the collision cell (precursor ion) or is formed in the collision cell (product ion) can be fragmented. A triple quadrupole is therefore selective in isolating precursor ions. In an ion trap, fragmentation is performed at a resonant frequency, which is specific for the isolated precursor ion, meaning that any fragment ion is unable to fragment further, so the MS^2 process on an ion trap is selective in both isolating and activating precursor ions.

An approach to increasing selectivity and avoiding false positive findings is to use time-of-flight MS (TOF-MS) [3]. Comparing the power of TOF-MS and triple-quadrupole MS in analyzing several pharmaceutical compounds (including antimicrobials) in wastewaters, Benotti et al. [40] concluded that the overall sensitivity of LC-TOF-MS, operated in accurate-mass mode, often approached that obtained by the triple quadrupole operated in selected reaction monitoring (SRM) mode. One of the most important drawbacks of using LC-TOF-MS for quantitative measurements is the significantly lower effective linear dynamic range compared to that provided by quadrupole instruments. To confirm proposed analyte identities in complex matrices, the new quadrupole-orthogonal acceleration TOF-MS (Q-TOF-MS) is of great interest because of the accurate masses provided for both parent and product ions and the possibility of providing a full-scan product-ion spectrum. For confirmation purposes, triple-quadrupole instruments can be used; however, Q-TOF-MS is preferred, since one LC run screens and confirms analytes because the relevant ions can be extracted from the MS^2 spectra and provide accurate masses for product ions. Recently, Stolker et al. [41] compared the performances of triple-quadrupole MS and Q-TOF-MS in screening and confirming pharmaceutical residues, including chloramphenicol, erythromycin and sulfamethoxazole in surface water, drinking water and groundwater. They concluded that, with both techniques, fully satisfactory results can be obtained, but the LODs of LC-triple-quadrupole-MS are slightly better. Concerning the MS-ionization mode, besides Pfeifer et al. [42] and Horimoto et al. [43,44], who employed atmospheric pressure chemical ionization (APCI), most authors have preferred the ESI mode, since it is excellent for both polar and non-polar compounds and for compounds with poor thermal stability.

The sample-preparation procedure is an important step in analysis. In the case of pharmaceuticals containing acidic groups in their structure and existing largely in their ionized form at neutral pH, acidification of water samples is necessary [45]. The presence of natural organic matter in the samples may reduce the extraction efficiencies. In general, the water samples are filtered through 0.45-μm or 0.2-μm glass-fiber filters. Several techniques have been developed and optimized, with SPE being the most frequent. Also solid-phase

Table 1. Analytical methods for the determination of pharmaceuticals in water and wastewater

Pharmaceuticals	**Extraction**	**Derivatization**	**Solvent(s) (Elution solvent)**	**Column (Mobile phase/ temperature program)**	**Detection**	**LOD (ng/l)**	**Ref.**
				In water matrices			
Diclofenac Ibuprofen Ketoprofen Indomethacine Naproxen Fenoprofen Clofibric acid Bezafibrate Gemfibrozil Etofibrate Fenofibrate Fenofibric acid Carbamazepine Pentoxifylline Diazepam	SPE (RP-C18)	Pentafluorobenzyl bromide	Acetone (Acetone)	DB-35 (65°C for 2 min, 30°C/min to 180°C, 5°C/min to 300°C, held for 12 min)	GC-MS	3.3–9.6	[4]
Phenazone Dimethylaminophenazone Propyphenazone Metoprolol Propranolol Atenolol Bisoprolol Sotalol Pindolol Betaxolol Salbutamol Clenbuterol Terbutaline Ifosfamide Cyclophosphamide Simvastatin	SPE (PPL Bond-Elut)	–	Methanol (Methanol)	Nucleosil 120-3-C18 (Solvent A: 20 mM ammonium acetate in Milli-Q water, pH 6.8 Solvent B: 20 mM ammonium acetate in acetonitrile-methanol, 2:1 v/v)	LC-MS^2	2.3–13	[4]
Iopamidol Iopromide Iomeprol Amidotrizoic acid	SPE (LiChrolut EN)	–	Methanol Acetonitrile (Methanol, Acetonitrile)	Nucleosil 120-3-C18 (Solvent A: 2 mM ammonium formiate in Milli-Q water, pH 7.0 Solvent B: 2 mM ammonium formiate in acetonitrile-methanol, 2:1 v/v)	LC-MS^2	2.3–4.8	[4]
Sulfamethoxazole	SPE (Isolut ENV+)	–	Acetonitrile Water Triethylamine (Acetonitrile, Water, Triethylamine)	Nucleosil 120-3-C18 (Solvent A: 20 mM ammonium acetate in Milli-Q water, pH 6.8 Solvent B: 20 mM ammonium acetate in acetonitrile-methanol, 2:1 v/v)	LC-MS^2	1–3.2	[4]

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Table 1 (*continued*)

Pharmaceuticals	Extraction	Derivatization	Solvent(s) (Elution solvent)	Column (Mobile phase/ temperature program)	Detection	LOD (ng/l)	Ref.
Sulfadiazine Sulfadimidine Sulfamerazine Ronidazole Metronidazole Furazolidone Trimethoprim Dapsone							
Chloramphenicol Virginiamycin Oleandomycin Erythromycin Anhydro-erythromycin Roxithromycin Clarithromycin Spiramycin Tylosin	SPE (Isolut ENV+)	–	Acetonitrile Water Triethylamine (Acetonitrile, Water, Triethylamine)	Nucleosil 120-3-C18 (Solvent A: 20 mM ammonium acetate in Milli-Q water, pH 6.8 Solvent B: 20 mM ammonium acetate in acetonitrile-methanol, 2:1 v/v)	LC-MS^2	1.8–4.5	[4]
Amoxicillin Oxacillin Cloxacillin Dicloxacillin Nafcillin Penicillin G Penicillin V	SPE (Isolut ENV+)	–	Acetonitrile Water Triethylamine (Acetonitrile, Water, Triethylamine)	Nucleosil 120-3-C18 (Solvent A: 2 mM ammonium formiate in Milli-Q water, pH 7.0 Solvent B: 2 mM ammonium formiate in acetonitrile-methanol, 2:1 v/v)	LC-MS^2	3.6–6.5	[4]
Gemfibrocil Clofibric acid Clofibrat Etofibrat Etofyllinclofibrat Diclofenac Ibuprofen (hydroxy- and carboxy-ibuprofen) Ketoprofen Indomethacin Fenoprofen Sarkosin-N-(phenylsulfonyl) (SPS)	SPE (LiChrolut-EN)	"On column" reaction with trimethylsulfonium hydroxide (TMSH) and trimethylanilinium hydroxide (TMAH), and "pre-column" with diazomethane	Methanol Ethyl acetate (Methanol, ethyl acetate)	HP Ultra-2 (50°C for 1 min, 40°C/min to 170°C, 1 min, 4°C/min to 250°C, 15.6 min 40°C/min to 280°C, 4.3 min)	GC-MS	3–12	[5]

Ibuprofen Hydroxy-Ibuprofen Carboxy-Ibuprofen Clofibric acid Diclofenac Triclosan	SPE Oasis HLB [Bakerbond SDB-1, LiChrolut EN, Chromabond HR-P, Isolut Env+, Chromabond EASY, abselut Nexus also tested]	Methyl chloromethanoate	Acetone Ethyl acetate Hexane Methanol Toluene (Methanol)	HP-5MS (353 K (80°C) [2 min] → (7 K/min) → 533 K (260°C) [10 min])	GC-MS	0.05–0.38*	[6]
Paracetamol Ibuprofen Salbutamol Mefenamic acid Propranolol	SPE (LiChrolut ENV+)	MSTFA (N-methyl-N-trimethylsilyl-trifloroacetamide)	Methanol Hexane (Methanol)	DB5MS (50°C for 7.5 min, 30°C/min to 270°C, held for 10 min)	GC-MS	2–4*	[7]
N,*N*-bis(3,3-dimethyl-2-oxetanyl)-3,3-dimethyl-2-oxetanamine Ibuprofen Aspirin Galaxolide Tonalide Cyclophosphamide Triclosan 1,1′-Sulfonylbis 4-chloro-benzene 4-Formylaminoantipyrine 4-Acetylaminoantipyrine Carbamazepine Codeine Pentoxyfylline Diazepam	SPE (Oasis)	–	Dichloromethane Acetonitrile (Acetonitrile, Dichloromethane)	HP5MS (90°C for 1 min, 10°C/min to 120°C, 3.5°C/min to 200°C, 5°C/min to 315°C, held for 11 min)	GC-MS	30*	[8]
Ibuprofen Naproxen Ketoprofen Diclofenac Carbamazepine Clofibric acid	SPE Oasis HLB [RP-C18 (Supelclean ENVI-18 SPE), PS-DVB (polystyrene-divinylbenzene, LiChrolut EN) also tested]	Large-volume on-line derivatization tetrabutyl-ammonium salts	Methanol Acetone Ethyl acetate (Methanol) (Acetone-ethyl acetate) (Acetone-methanol)	DB5-MS (70°C for 4 min, 10°C/min to 300°C, held for 4 min)	GC-MS	1–8	[9]
Ibuprofen Paracetamol Phenazone Carbamazepine	SPME SPME fiber coating: polyacrylate, Carbowax-DVB [poly(dimethyl)-siloxane-di-vinylbenzene and C18 also tested]	–	Hexane Acetone	HP5-MS (70°C for 2 min, 10°C/min to 250°C, 5 min, increased to 280°C and held for 10 min)	GC-MS	200–50,000	[10]
Ibuprofen Paracetamol Phenazone Carbamazepine	SPE LiChrolut-EN and LiChrolut-RP-C	BSTFA	Hexane Acetone Methanol (Acetone, Methanol)	HP5-MS (70°C for 2 min, 10°C/min to 250°C, 5 min, increased to 280°C and held for 10 min)	GC-MS	–	[10]
Piroxicam Ketorolac	LPME Accurel Q3/2 polypropylene tubular membranes	–	Methanol 1-octanol	Luna Phenyl-Hexyl (MeOH/water gradient: Solvent A: 20% MeOH Solvent B: 95% MeOH, both containing 10mM tri-*n*-butylamine and 0.5% acetic acid)	LC-MS^{2}	0.5–42	[11]

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Table 1 (*continued*)

Pharmaceuticals	Extraction	Derivatization	Solvent(s) (Elution solvent)	Column (Mobile phase/ temperature program)	Detection	LOD (ng/l)	Ref.
Clofibric acid Naproxen Bezafibrate Fenoprofen Ibuprofen Diclofenac Indomethacin							
			In wastewater matrices				
Metoprolol Nadolol Propranolol	SPE Empore SDB-XC extraction disks	N-Methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA)	Hexane Acetone Methanol (Methanol)	DB5-MS (160°C for 1 min, 10°C/min to 290°C, for 10 min)	GC-MS	1–10	[12]
Ibuprofen Naproxen Ketoprofen Tolfenamic acid Diclofenac	SPE Oasis HLB	*N*-Methyl-*N*(*tert.* butyldimethylsilyl) trifluoroacetamide (MTBSTFA)	Methanol Ethyl acetate Hexane (Ethyl acetate)	BP5 (50°C for 1 min, 10°C/min to 180°C, for 7 min, 10°C/min to 230°C, for 25 min, 20°C/min to 250°C, for 5 min)	GC-MS	20–50 *	[13]
Phenazon Clofibric acid Propyphenazone Ibuprofen Diclofenac	SPE C18	Pentafluorobenzyl bromide (PFBBr) (triethylamine as catalyst)	Acetone Methanol Toluene (Methanol)	HP-5MS (100°C for 1 min 30°C/min to 150°C for 1 min, 3°C/min to 205°C 10°C/min to 260°C for 23 min)	GC-MS	0.6–20	[14]
Carbamazepine Clofibrate Phenazone Aminopyrine	C2/ENV+	–	Acetonitrile Methanol (Methanol)	RP-C6 (Solvent A: 95% H_2O; 5% CH_3CN; 0.1% HCOOH Solvent B: CH_3CN; 0.1% HCOOH)	LC-MS	–	[15]
Clofibric acid Diclofenac Fenofibrate	SPE RP C18	Diazomethane	Methanol Dichloromethane (Methanol, Dichloromethane)	HP-5 (80°C for 8 min, 5°C/min to 280°C 3°C/min to 300°C, held for 5 min)	GC-MS	–	[15]
Fenoprofen Flurbiprofen Gemfibrozil Ibuprofen Ketoprofen Naproxen							
Acetylsalicylic acid Ibuprofen	SPE	Photo-derivatization	Methanol	Nova- Pak C18	HPLC-fluorescence	2,000–120,000	[16]

Ketoprofen Naproxen Bezafibrate Diclofenac-sodium salt Tolfenamic acid Carbamazepine 10,11- Diazepam	Oasis HLB	Acetonitrile Ethyl acetate (Ethyl acetate) dihydrocarbamazepine	(Solvent A: MeOH-Milli-Q Water 70:30, v/v, acidified with 0.2% formic acid Solvent B: MeCN–Milli-Q water 70:30, v/v or 50:50, v/v, both containing 0.2% formic acid)				
Sulfamethazine Sulfadiazine Sulfaguanidine Trimethoprim Oxytetracycline Enrofloxacine Penicillin G/ procaine	SPE Oasis HLB	–	Methanol (Methanol)	LiChrosphere 100 CN (Solvent A: oxalic acid 0.01 M Solvent B: acetonitrile)	HPLC-diode array (DAD)	0.1–40	[17]
Carbamazepine Diazepam	SPE Oasis HLB	–	Hexane Ethyl acetate (Ethyl acetate)	BP5 (50°C for 1 min, 10°C/min to 180°C, for 7 min, 10°C/min to 230°C, for 25 min, 20°C/min to 250°C, for 5 min)	GC-MS	18.9–22.2	[18]
Diclofenac Ibuprofen Naproxen Roxithromycin Sulfamethoxazole Iopromide	SPE Oasis HLB	Silylation with *N*-methyl-*N*-(*tert*.-butyldimethylsilyl) trifluoroacetamide (MTBSTFA)	Hexane Ethyl acetate (Ethyl acetate)	BP5 (50°C for 1 min, 10°C/min to 180°C, for 7 min, 10°C/min to 230°C, for 25 min, 20°C/min to 250°C, for 5 min)	GC-MS	0.5–16.7	[18]
Clofibric acid Bezafibrate Gemfibrocil Fenofibrate Atenolol Sotalol Metoprolol Betaxolol	SPE Isolute C18	–	Methanol Acetonitrile (Methanol)	RP-18 (Solvent A: acetonitrile Solvent B: HPLC-grade water)	LC-MS^2	0.017–1.25*	[19]
Ibuprofen Salicylic acid Gemfibrozil Naproxen Ketoprofen Diclofenac Indomethacin	SPE Oasis MAXSPE	Pentafluoroprop-ionic acid anhydride (PFPA) *N* – *t*-butyldimethylsilyl-*N*-methyltrifluoro-acetamide (MTBSTFA)	Methanol Hexane Dichloromethane (Methanol)	Restek Rtx-5Sil MS (70°C for 1 min, 30°C/min to 190°C, 5°C/min to 260°C, 15°C/min to 300°C, held for 5 min)	GC-MS	10	[20]

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Table 1 (*continued*)

Pharmaceuticals	**Extraction**	**Derivatization**	**Solvent(s) (Elution solvent)**	**Column (Mobile phase/ temperature program)**	**Detection**	**LOD (ng/l)**	**Ref.**
Clofibric acid Ibuprofen Ketoprofen Mefenamic acid Diclofenac	ENVI-18 reverse phase packed tube	Pentafluorobenzyl bromide (triethylamine as catalyst)	Methanol Diethyl ether Toluene (Methanol)	RTX-5 (80°C for 1 min, 30°C/min to 150°C, 3.5°C/min to 280°C, held for 30 min)	GC-MS	5–15	[21]
Clarithromycin Erythromycin	SPE Oasis MCX and Lichrolut EN	–	Acetone Methanol	Luna C8 (Positive ion mode: Solvent A: formic acid 0.1% in Milli-Q water, pH 2 Solvent B: acetonitrile)	LC-MS^2	0.1–5.2*	[22]
Spiramycin			Ethyl acetate	(Negative ion mode: Solvent A: triethylamine, pH 8, 0.05% in water Solvent B: acetonitrile)			
Lincomycin Ciprofloxacin Ofloxacin Amoxycillin Sulfamethoxazole Oleandomycin Tilmicosin Tylosin Oxytetracycline Cyclophosphamide Methotrexate Ibuprofen Salbutamol Atenolol Enalapril Diazepam Carbamazepine Furosemide Hydrochlorothiazide 17α-Ethinylestradiol Omeprazole Ranitidine Bezafibrate Clofibric acid Demethyl diazepam 17β-Estradiol Estrone			Acetonitrile (Methanol, Ethyl acetate)				

Diclofenac Ibuprofen Ketoprofen Naproxen Carbamazepine Caffeine	SPE Oasis HLB	–	Acetonitrile Methanol Hexane Ethyl acetate (Ethyl acetate)	RP-18 (Solvent A: methanol Solvent B: acetonitrile Solvent C: a 50mM potassium dihydrogen phosphate solution)	HPLC-DAD HPLC-fluorescence	3,000–160,000*	[23]
Carbamazepine Mefenamic acid Indomethacine Ibuprofen Naproxen Diclofenac Ketorolac Acetaminophen Codeine Fluoxetine Paroxetine Atenolol Propranolol Trimethoprim Metronidazole Erythromycin Ranitidine	SPE Oasis HLB	–	Ethyl acetate Acetonitrile Methanol (Methanol)	C8 ZB-5MS (Positive ion mode: Solvent A: acetonitrile Solvent B: formic acid 0.1% in MilliQ water) (Negative ion mode: Solvent A: acetonitrile Solvent B: MilliQ Water)	LC-MS^2	3.8–47	[24]
Sulfaguanidine Sulfacetamide Sulfadiazine Sulfathiazine Sulfapyridine Sulfamerazine Sulfamethazine Sulfamethoxazole Sulfadimethoxine Sulfasalazine	SPME with Carbowax/ divinylbenzene (CW/DVB) fiber [polydimethyl-siloxane, carbowax-templated resin, polyacrylate, and PDMS/ DVB also tested]	–	Acetonitrile Methanol	C18 (Solvent A: 20mM aqueous ammonium acetate, 0.1% formic acid, pH 3 Solvent B: 20mM ammonium acetate in 2:1 CH_3CN:MeOH)	LC-MS^2	9,000–55,300	[25]
				In both water and wastewater matrices			
Clarithromycin Erythromycin Roxithromycin Sulfamethazine Sulfamethoxazole Trimethoprim	lyophilization	–	Ethanol Methanol Hexane	RP-8 (Tetracyclines: Solvent A: 20 mmol/l oxalic acid in water Solvent B: acetonitrile Penicillins: Solvent A: 10 mmol/l ammonium acetate in water Solvent B: acetonitrile Other antibiotics: Solvent A: 10 mmol/l ammonia acetate in water Solvent B: acetonitrile)	LC-MS^2	Tetracyclines 50* other antibiotics 20*	[26,27]

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Table 1 (*continued*)

Pharmaceuticals	Extraction	Derivatization	Solvent(s) (Elution solvent)	Column (Mobile phase/ temperature program)	Detection	LOD (ng/l)	Ref.
Chloramphenicol Chlortetracyline Doxycycline Oxytetracyline Tetracycline Cloxacillin Dicloxacillin Methicillin Nafcillin Oxacillin Penicillin G Penicillin V							
Acetylsalicylic acid Clofibric acid Fenofibric acid Bezafibrate Ibuprofen Diclofenac Ketoprofen Fenoprofen Indometacine Naproxen Meclofenamic acid Tolfenamic acid	SPE RP-C18 Lichrolut	Derivatization with N-Methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA)	Hexane Methanol Acetone (Methanol)	HTI-5 (50°C for 2 min, 16°C/min to 180°C, 4°C/min to 290°C, held for 7 min)	GC-MS	50–250 for sewage 10–25 for surface water 1–25 for drinking water	[28]
Carbamazepine Gemfibrozil Ibuprofen Diclofenac Ketoprofen Naproxen Clofibric acid	SPE Oasis HLB	Direct for neutrals, Diazomethane for acidic compounds	Ethyl acetate Methanol Dichloromethane Acetone (Ethyl acetate, acetone)	RTX-5MS (90°C for 1 min, 15°C/min to 150°C, for 15 min, 5°C/min to 200°C, for 5 min, 15°C/min to 290°C, held for 6 min)	GC-MS	1–10	[29]
Sulfamethoxazole Acetyl-sulfamethoxazole Trimethoprim Erythromycin Paracetamol Ibuprofen	SPE Strata X	–	Methanol Dichloromethane (Methanol)	Luna C18 (mobile phase of water, methanol and 40mM ammonium acetate in water, adjusted to pH 5.5 by the addition of formic acid)	LC-MS^2	10–50	[30–32]

Mefenamic acid Diclofenac Clofibric acid Propranolol Lofepramine Tamoxifen Ofloxacin Ciprofloxacin Norfloxacin Sulfamethoxazole Sulfamethazine Lincomycin Tylosin tartrate Oxytetracycline Penicillin G1/2-benzathine salt Penicillin V-potassium salt Trimethoprim	SPE Oasis HLB	Dextropropoxyphene –	Methanol (Methanol)	RP-18e	LC-MS^2	10–20	[33]
Ibuprofen Naproxen Ketoprofen Diclofenac	SPE Strata X	MSTFA (N-methyl-N-(trimethylsilyl) trifluoroacetamide)	Methanol Toluene (Methanol)	HP-5- MS (2 min at 100°C, 4°C/min to 180°C, 10°C/min to 230°C, held for 20 min, 20°C/min to 270°C held for 7 min)	GC-MS	2–6	[34]
Acetylsalicylic acid Ibuprofen Gemfibrozil Fenoprofen Naproxen Ketoprofen Diclofenac	SPE LC-18	BF3/MeOH	Methanol Light petroleum Methyl tert-butyl ether Acetone Hexane Dichloromethane (Methanol)	CP-SIL 8CB-MS (50°C for 0.75 min, 20°C/min to 120°C, 2°C/min to 200°C, 9°C/min to 290°C, held for 10 min)	GC-MS^2	0.1–1	[35]

microextraction (SPME), liquid-phase microextraction (LPME) and lyophilization have been applied [26,37,46]. Of the 32 studies reviewed in this article (Table 1), sample extraction of water and wastewater was achieved using SPE in 28, SPME in two (in one study both SPE and SPME were applied), LPME in one and lyophilization in two.

SPE sorbents (e.g., ENV+, Oasis HLB, Strata-X, Lichrolut C18, and Lichrolut EN) have been assessed for preconcentration as well as for clean-up of pharmaceuticals in water samples. These were employed most because they give better recovery of both polar and non-polar compounds and have greater capacity than alkyl-bonded silicas.

SPE is typically performed manually, but there are some significant disadvantages with this approach:

(i) manual (off-line) SPE is time-consuming as well as labor-intensive and costly, which compromises productivity;

(ii) exposure to hazardous or infectious matrices (such as sewage) involves safety issues; and,

(iii) the recovery of the analyte can vary from batch to batch, causing reproducibility problems.

By automating the process, these problems can be eliminated, with the following benefits:

(i) direct injection of untreated samples;

(ii) automatic sample clean-up and/or analyte enrichment;

(iii) elimination of conventional manual sample-pretreatment steps;

(iv) faster procedures;

(v) methods are less prone to errors, resulting in better reproducibility;

(vi) reduction of health risks; and,

(vii) samples can be run unattended (e.g., overnight or over the weekend).

A review on the current aspects and future prospects for automating SPE was published by Rossi and Zhang [47]. However, only a few studies have so far used automated procedures for extraction (e.g., accelerated solvent extraction (ASE) [48], on-line coupled continuous flow liquid membrane extraction (CFLME) with a C18 precolumn system [49], or sequential injection analysis (SIA) with a lab-at-valve (LAV) approach for on-line liquid–liquid micro-extraction [50]). These studies focused on the determination of organic pollutants (e.g., polyaromatic hydrocarbons (PAHs) and bisphenol A). However, there has not yet been any application reported for pharmaceuticals.

This article aims to provide an up-to-date review of analytical methods applied for the determination of pharmaceutical residues in water and wastewater matrices.

2. Analytical methods

Table 1 shows various studies that have been performed in recent years for the determination of various categories of pharmaceuticals. Fig. 2 shows the typical procedure followed so far for the analysis of pharmaceuticals in aqueous matrices. Table 1 also sets out the various compounds analyzed and information on LODs and limits of quantitation (LOQs) during analysis. The most important findings in respect to the analytical procedure are set out below.

A typical analytical method for the determination of pharmaceuticals in wastewater includes the use of

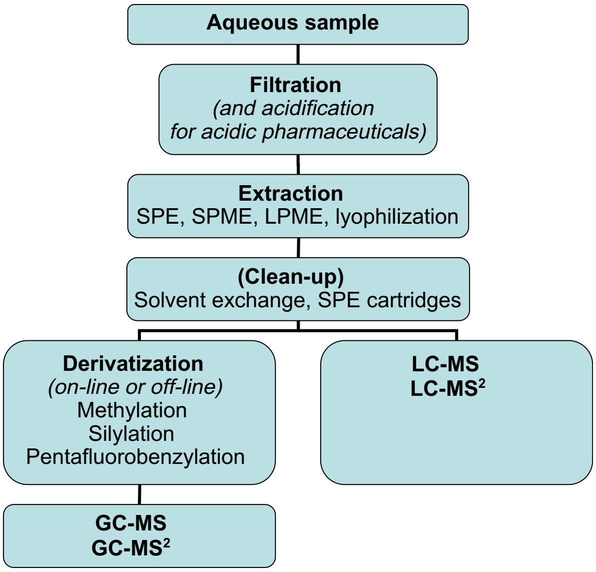

Figure 2. Typical procedure followed for the analysis of pharmaceuticals in aqueous matrices.

octadecylsilica, polymeric, or hydrophilic-lipophilic balanced (HBL) supports for on-line SPE of water samples, with either disks or, most frequently, cartridges at low pH (typically, pH = 2) [51].

The selection of an appropriate solid phase is a difficult task, as the recoveries obtained for some compounds can be low. This problem is more evident in the case of methods that simultaneously determine several classes of pharmaceuticals, as, in those cases, there needs to be a compromise between the solid phases providing the best recoveries for each class of compounds.

As shown in Table 1, Weigel et al. [6] monitored acidic, neutral and basic pharmaceuticals and estrogens in surface water using seven polymeric SPE sorbents. The highest recoveries (70–100%) were obtained with styrene-methacrylate and styrene-N-vinylpyrrolidone co-polymers. Oasis HLB was chosen for extraction of acidic and neutral drugs analyzed by GC-MS.

Lin et al. [9] reported that the HLB was the most effective for SPE-GC-MS optimization, with recoveries in the range 50–108% (RSD range 1–10%) in spiked tap water. According to the authors, the injection-port derivatization technique provided the advantage of avoiding the handling of hazardous derivatizing agents.

Möder et al. [10] tested different SPME fiber coatings [polyacrylate, poly(dimethylsiloxane–di-vinylbenzene) (PDMS-DVB), C18 and Carbowax-DVB]. The results showed that some coatings, such as polyacrylate and Carbowax-divinylbenzene, are the most effective (optimal extraction time 30 min).

A hollow-fiber LPME method using Accurel Q3/2 polypropylene (PP) tubular membranes, as an alternative to SPE, was used prior to LC-MS^2 (recoveries in the range 82–111%, with a low precision range 3.4–32% RSD) [11].

Lee et al. [20] used a cartridge with anion-exchange groups for extraction (Oasis MAXSPE) in sewage for acidic pharmaceuticals, and it provided better selectivity than that provided by C18 groups. Elution of acids into two separate groups produced an extract with less coextractives in each fraction, which is beneficial for complex matrices, such as sewage samples (mean recoveries 87–110% in fortified distilled water at two spiking levels of 10 μg/l and 0.01 μg/l with SD < 7% and recoveries 75–115% in wastewater).

Tauxe-Würsch et al. [21] used the A ENVI-18 reverse-phase packed tube for extraction in sewage samples with pentafluorobenzyl bromide triethylamine derivatization following a SiOH cartridge conditioned with toluene (recoveries 68–91% with RSD < 16%).

Lyophilization has scarcely been reported as an alternative to SPE for preconcentrating pharmaceutical residues [26,37,46]. Ternes [37] reported recoveries using lyophilization slightly higher (54–108%) than for SPE (15–120%) for a set of antibiotics.

Following sample preconcentration, the analytical technique typically includes either GC or LC. The selection needs to take into account that GC can provide better LODs, but, in many cases of pharmaceuticals, it requires the additional step of derivatization. Several derivatizing agents have been tested for a number of pharmaceuticals (Table 1). Moreover, some compounds are thermolabile and decompose during GC analysis (e.g., carbamazepine forms iminostilben as degradation product) [51].

Jux et al. [5] reported that the LODs with TMSH (trimethylsulfonium hydroxide) were in the same range or lower than those with diazomethane or TMAH (tetramethyl ammonium hydroxide) in river water using the SPE-GC-MS method. The recoveries were in the range 75–135% when using TMSH for derivatization. With TMSH, small molecular byproducts are not detected, as they remain in the solvent peak of the chromatogram. As a byproduct, TMAH produces aniline, which can interfere with the determined compounds with similar retention times.

Huggett et al. [12] determined some β-blocking pharmaceuticals in wastewater using a method combining SPE (derivatization with MSTFA, N-Methyl-N-(trimethylsilyl)-trifluoro-acetamide) with GC-MS. The recoveries for metoprolol, nadolol and propranolol were 92% ± 9%, 85% ± 10%, and 94% ± 8%, respectively.

Rodriguez et al. [13] reported that butyldimethylsilylation was preferred to trimethylsilylation for forming more stable derivatives after SPE with Oasis HLB cartridges and GC-MS detection in sewage.

Sacher et al. [4] reported six analytical methods using SPE-GC-MS (after derivatization of the acid compounds) or HPLC-ESI-MS^2 in water and wastewater (recoveries 75–125% for bezafibrate and pentoxifylline, which could be attributed to the presence of very low concentration levels of these compounds). However, low recoveries were observed for iopamidol, iopromide, iomeprol and amidotrizoic (<50%, in some cases even <10%), probably due to the extremely high polarity and water solubility of these compounds (RSD of a five-fold analysis <10%).

Acidic and neutral pharmaceuticals were extracted and analyzed by SPE-GC-MS and LC-MS (recoveries >60%) with the exceptions of gemfibrozil (46%), clofibrate (33%), sulfamethoxazole (40%) and fluoroquinolones enoxacin, norfloxacin and ciprofloxacin (34–35%) [15].

Hirsch et al. [27] determined various antibiotics in sewage-treatment-plant effluents and river-water samples using preconcentration with lyophilization and analysis by HPLC-MS.

Hernando et al. [19] used SPE-LC-MS^2 in wastewater (recoveries >60% in spiked effluent except for betaxolol (52%), RSD range 3.7–18.5%). Lower recoveries (18–62%)

obtained in influent-wastewater samples were probably due to matrix effects.

Castiglioni et al. [22] developed a method of two extraction steps using SPE-HPLC-MS^2 analysis in urban wastewater (recoveries >70%, RSD < 8% with the exception of amoxycillin (36%), erythromycin (50%), spiramycin (56%), tylosin (64%), ciprofloxacin and ofloxacin (30%), hydrochlorothiazide (56%), and sulphamethoxazole (65%)).

Gomez et al. [24] developed a multi-residue analytical method using SPE at pH 7 (selected after tests at pH values 2, 4 and 7) and LC-MS^2 with multiple reaction monitoring (MRM) for hospital-wastewater analysis (recoveries >75% with the exception of ranitidine (45%), probably due to its high polarity and water solubility). Overall variability <9% was obtained in spiked hospital effluent.

An SPE-HPLC-MS^2 method was used in wastewater and in surface-water samples (recoveries 45–120% with the exception of mefenamic acid (24%)). However, low recovery for lofepramine (4.2%) indicated that this method was not suitable for all environmental monitoring programmes [31,32].

Brown et al. [33] determined various antibiotics in effluent from hospitals, dairies, municipal wastewater and surface water using SPE-HPLC-MS^2 (recoveries 20–180%).

Balakrishnan et al. [25] monitored sulfonamide antibiotics in wastewater using SPME-LC-MS^2 analysis (recoveries 59.2–112%, RSD 11.1–23.5% with the exception of sulfathiazole and sulfamethazine (29% and 39.8%, respectively)). These high recoveries compared to those obtained by SPE were probably due to the reduced matrix effects. However, the method yielded lower precision.

Gonzalez-Barreiro et al. [16] performed simultaneous determination of acidic and neutral pharmaceuticals in wastewater based on the combination of HPLC and photochemically-induced fluorimetry (recovery range 90–115%). The determination of acetylsalicylic acid was problematic, while ibuprofen had high LODs and tolfenamic acid could not be determined by fluorescence detection (FD).

Six pharmaceuticals were extracted from wastewater by SPE-HPLC-DAD, also using on-line FD for confirmation of ibuprofen and naproxen (recoveries 71–103%, RSD 15.1%) [23].

Babic et al. [17] reported on SPE-HPLC-DAD for pharmaceuticals in wastewater (recoveries 68.3–97.9%, RSD < 8.4% except for sulfaguanidine with recovery 11.3%).

Verenitch et al. [35] optimized an SPE-GC-MS^2 method for acidic pharmaceuticals in wastewater and surface water (recovery range 55–128%).

GC was reported to provide slightly better LODs, while LC can provide better accuracy for some compounds (Fig. 4a and b). However, GC requires derivatization and some compounds, as already mentioned, are thermolabile and decompose during GC analysis. As seen in Table 1, LODs using LC-MS^2 are slightly higher than those obtained with GC-MS in recent studies (e.g., the LOD range for ibuprofen and related pharmaceuticals obtained using LC-MS^2 was 3.8–47 ng/l [24], while that obtained using GC-MS was 0.5–16.7 [18] and 3.3–9.6 [4]). Of course, there are also some exceptions (e.g., in the case of ibuprofen and related compounds, LODs were in the range 0.5–42 ng/l using LC-MS^2 [11], while LOD levels of the order of mg/l using GC-MS [10]). However, versatility and less complicated sample preparation make LC-MS^2 very suitable for environmental analysis.

Fig. 3 shows various analytical methods applied for the most common pharmaceutical compounds, while Fig. 4 presents examples of GC-MS, LC-MS and LC-MS^2 chromatograms of some pharmaceuticals.

3. Extraction and matrix effects

In the following paragraphs, we discuss the various problems encountered so far with respect to the analytical procedures applied.

3.1. Optimizing SPE

Castiglioni et al. [22] optimized the extraction methods for different groups of pharmaceuticals using SPE cartridges as follows:

- Oasis MCX, tested at pH 1.5–2.0 and 3.0 for all the compounds, and at pH 7.0 or 7.5 for omeprazole;
- Lichrolut EN, tested at pH 3.0, 5.0, 7.0 and 9.0 for all the compounds;
- Bakerbond C18, tested at pH 8.0 and 9.5 for the extraction of amoxicillin; and,
- Oasis HLB, tested at pH 7.0 for omeprazole and pH 8.5/9.0 for amoxycillin.

Oasis MCX was selected to be used at pH 1.5–2.0 for the extraction of acidic, basic and neutral compounds at low pH values, since the cation-exchanger binds the basic compounds, which are in the ionized form, and the reversed phase can retain both acidic and neutral compounds. The elution conditions for Oasis MCX were optimized using consecutively 2 ml methanol, 2 ml 2% ammonia and 2 ml 0.2% sodium hydroxide.

Lichrolut EN can extract polar organic compounds through hydrophobic interactions at pH 7.0. Recoveries of about 70% were obtained in mineral water. The addition of EDTA to the samples enhanced the recovery of amoxycillin, methotrexate, omeprazole and oxytetracycline.

3.2. Applying SPME instead of SPE

SPME was used to quantify sulfonamides accurately in fortified wastewater and was demonstrated to be a viable

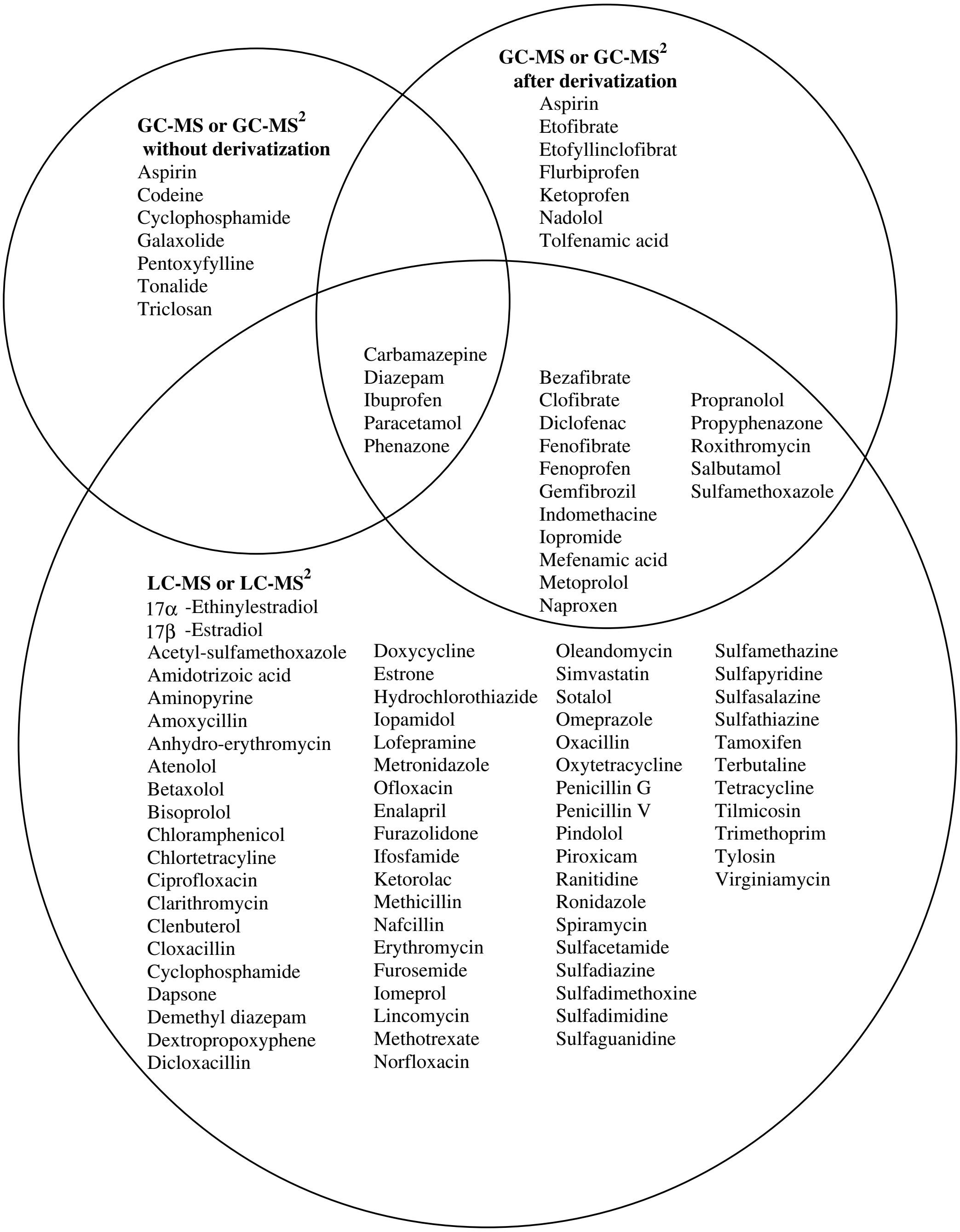

Figure 3. Analytical methods applied for the most common pharmaceuticals in water and wastewater.

technique for overcoming the matrix effects that block LC-MS^2 analyses of environmental samples according to the calibration equations obtained for SPE and SPME. However, with the exception of sulfasalazine, SPE was found to be a more sensitive technique for the determination of sulphonamide antibiotics, because SPME is an equilibrium technique, which merely samples the solution, whereas SPE is a quantitative extraction in which the entire solution is passed through a sorbent cartridge for compound uptake [23]. However, SPME has four advantages over SPE:

- it is rapid, requiring only 50 min to process sample, compared to 5 h for SPE;
- it uses 100 times less organic solvent than SPE;
- it requires a much lower sample volume than SPE (25 ml instead of 500 ml); and,
- it has re-usable fibers instead of single-use SPE cartridges [19].

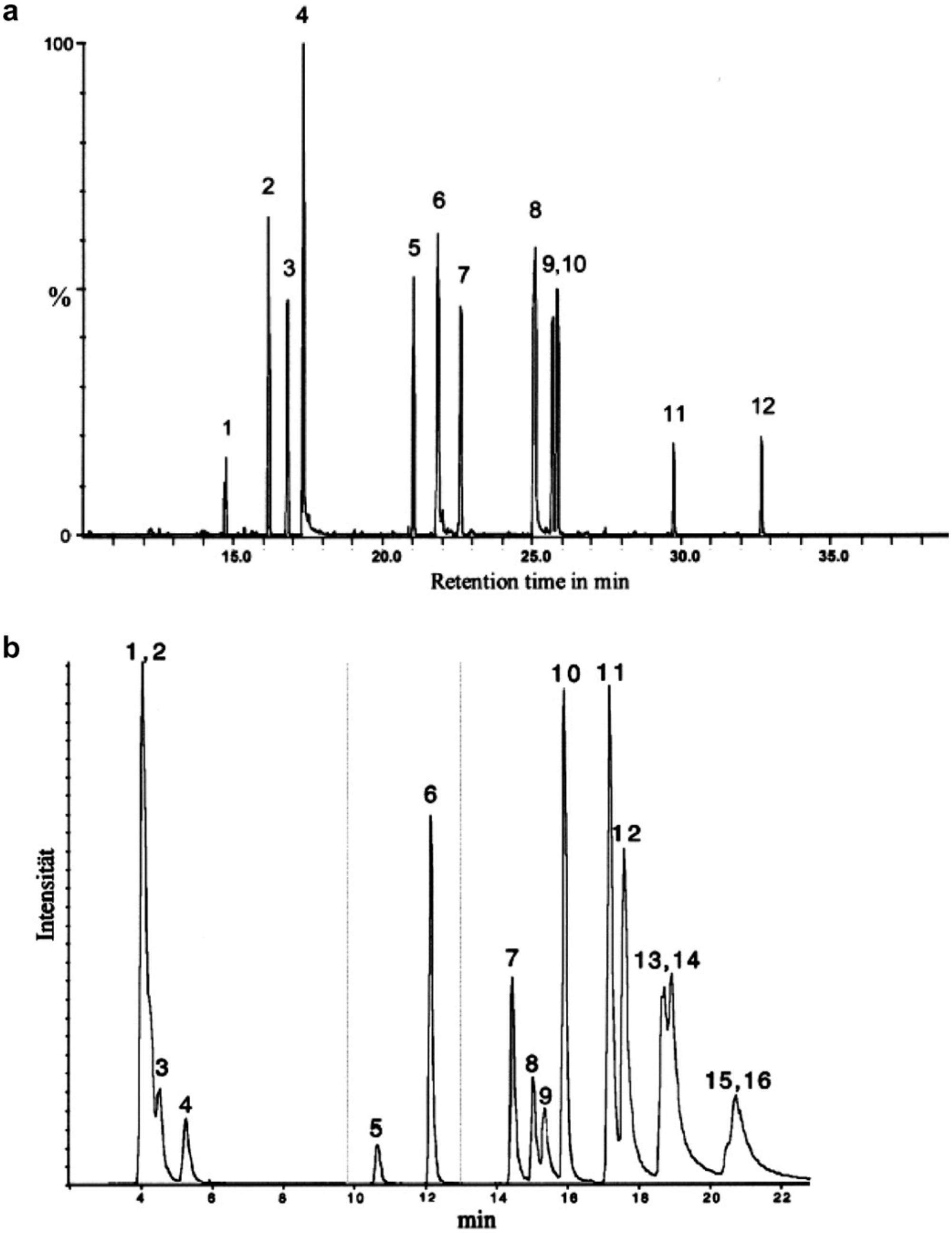

Figure 4. (a) GC-MS chromatogram of a standard solution of pharmaceuticals. 1: O-HCH (internal standard); 2: Terbutalin; 3: Clenbuterol; 4: Salbutamol; 5: Metoprolol; 6: Timolol; 7: Propranolol; 8: Nadolol; 9: Bisoprolol; 10: Betaxolol; 11: Fenoterol; and, 12: Carazolol (adapted from [37] with permission). (b) LC-MS chromatogram of a standard solution of pharmaceuticals. 1: Atenolol; 2: Salbutamol; 3: Terbutalin; 4: Sotalol; 5: Fenoterol; 6: Nadolol; 7: Timolol; 8: Metoprolol; 9: Clenbuterol; 10: Celiprolol; 11: Bisoprolol; 12: Carazolol; 13: Propranolol; and, 14: Betaxolol (adapted from [37] with permission). (c) LC-MS^2 extracted MRM chromatograms for target pharmaceuticals in spiked STP effluents. 1: Clofibric acid; 2: Bezafibrate; 3: Gemfibrocil; 4: Atenolol; 5: Sotalol, 6: Metoprolol; 7: Betaxolol; and, 8: Fenofibrate (adapted from [19] with permission).

3.3. Applying LPME instead of SPE

To check matrix effects during extraction, ultrapure water, and treated and untreated wastewater were spiked with the analytes at the 5 µg/L level, extracted by LPME and analyzed by LC-MS^2 (93% ± 35% mean recoveries for acidic pharmaceuticals in treated wastewater and 123% ± 45% in the raw wastewater) [51]. No loss of sensitivity was observed, and quantification could be performed without standard addition and just internal standard calibration. However, samples had to be filtered prior to extraction. Three-phase LPME can be also performed: the analyte is first extracted into an organic solvent that

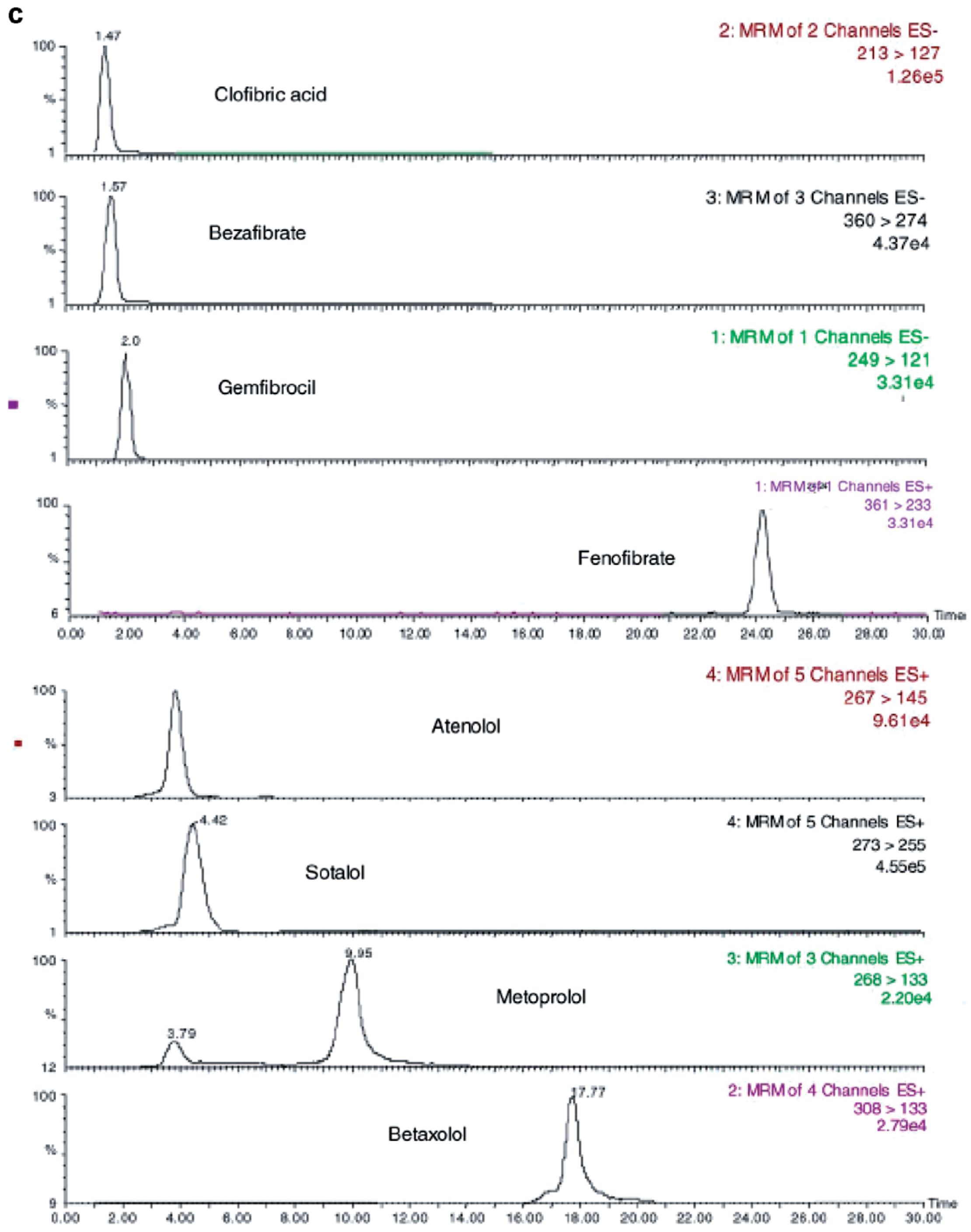

Figure 4. (*continued*)

impregnates the walls of the membrane, and then back-extracted into an aqueous acceptor solution adjusted to the adequate pH, depending on the acidic properties of the analytes [52,53]. When an isotopically-labelled internal standard was used, an RSD of 21% was obtained for bisphenol A [54]. This relatively low precision of LPME may be attributed to the fact that fiber preparation, conditioning and arrangement, as well as the handling of very small extract volumes, (20 μl) have to be done manually. Furthermore, variations in the wall thickness and pore size of the membranes may occur [55].

3.4. Minimizing matrix effects

The most direct means of obtaining maximum sensitivity and signal reproducibility is through the reduction of matrix components prior to the LC-MS^2 analysis, applying a selective extraction and improved sample clean-up. Many efforts have been described in the literature for this purpose, by optimizing the sample-preparation steps [11,19,24,25]. A simple solution to this problem can be the dilution of the extract from complex matrices. In spiked hospital wastewater, the dilution 1:2 was sufficient to avoid the decrease in the analyte signal in all compounds analyzed in ESI negative, and for compounds analyzed in ESI positive, except for the compounds with severe ion suppression: erythromycin, atenolol, paroxetine and fluoxetine (ion suppression was completely eliminated for these compounds at 1:5, 1:7 and 1:10 dilution, but a decrease of sensitivity was also observed) [20].

4. Conclusions

Several advanced analytical methods have been developed and optimized in recent years for water and wastewater matrices, aimed at obtaining better precision and sensitivity, in order to quantify accurately pharmaceuticals present at trace concentrations in the aquatic environment. Different alternative methods and materials of extraction, derivatization and clean-up have been investigated, in order to minimize the matrix effects during the GC-MS, GC-MS^2, LC-MS or LC-MS^2 analysis that follows. Simultaneously, minimizing sample-preparation time and cost, as well as minimizing the amount and the handling of toxic reagents, are objectives of newly proposed methods.

Currently, SPE is the most popular, well-established sample-preparation technique, with which the best sensitivity is obtained. Alternative techniques (e.g., SPME and LPME) have been applied more recently due to several advantages that they have over SPE in terms of speed, ease of sample handling and minimizing solvent use. However, the precisions obtained have been lower, indicating the need for these techniques to be further optimized.

Despite the current availability of advanced detection instrumentation, the rapid, accurate and perhaps simultaneous determination of a large number of pharmaceuticals in complex environmental matrices continues to constitute a major and fascinating challenge for researchers not only because of the diversity of chemical properties of the pharmaceutical compounds, but also because of the generally low concentrations (usually ppb or ppt levels) and the complexity of matrices.

Further improvements are needed in order to lower LODs. Moreover, increasing and intensive scientific research is needed to assess the impact of pharmaceuticals, in order to establish limits for their presence in wastewater discharges or drinking water. Current knowledge concerning their impacts on the environment refers to information on individual compounds, and this needs to be extended to more complex environmental mixtures with the help of improved analytical methods. Technological advances in analytical capabilitites will allow us to learn more about the presence, the fate and the bioavailability of pharmaceutical compounds in the water cycle and in soil matrices.

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Despo Fatta received her first degree from the National Technical University of Athens in 1993 (Diploma in Chemical Engineering), her M.Sc. in Environmental Management from the European Association for Environmental Management and Education in 1995 (University of Athens, JRC Ispra), and her Ph.D. from the National Technical University of Athens in 1999. She has been a Lecturer at the Department of Civil and Environmental Engineering at the University of Cyprus since 2003. Her principal research interests are in the field of environmental pollution-monitoring techniques (water quality, xenobiotics in water and wastewater), wastewater treatment (e.g., advanced chemical oxidation and anaerobic digestion), environmental hazard analysis and risk assessment, and life-cycle analysis of products and processes. She has authored or co-authored a number of refereed papers, technical manuals and reports and has been Guest Editor for *Desalination Journal*.

Anastasia Nikolaou obtained her B.Sc. (1997) and Ph.D. degree (2002) in the Department of Environmental Studies, University of the Aegean, Mytilene, Greece. She has been a Lecturer in the Department of Marine Sciences of the University of the Aegean (Mytilene, Greece) since 2005. Her research interests include analytical methods for the determination of toxic priority substances in water and sediment, water quality and treatment or disinfection by-products, investigation of the fate and the toxicity of organic compounds in the environment. She has participated in many related national and international projects, authored or co-authored a number of refereed papers and technical reports and has been the Editor of two books and Guest Editor for *Desalination Journal*.

Antigoni Achilleos received her first degree from the National Technical University of Athens in 1999 (Diploma in Chemical Engineering), and currently is a Ph.D. student at the University of Cyprus in the Department of Civil and Environmental Engineering. Her principal research interests are in the field of determination of pharmaceuticals, such as ibuprofen, carbamazepine and diclofenac, in sewage water-treatment plants, and the application of advanced oxidation processes for their removal.

Sureyya Meriç holds B.S., M.Sc. and Ph.D. degrees in Environmental Engineering from Istanbul Technical University (Turkey). She has been working in this field for 19 years. Her post-doc studies were in ecotoxicology. She has developed expertise in chemical and biological treatment of wastewater, re-use, water-treatment and disinfection by-products, toxicity, activated sludge modeling, inhibition, industrial pollution control, water quality and management, aquatic and sediment-toxicity monitoring, impact assessment of priority-emerging pollutants, xenobiotics and their removal by advanced oxidation processes, and groundwater remediation. She has been investigator or co-ordinator of many national and international projects, a member of environmental organizations and committees, and Guest Editor for *Desalination Journal*.