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Review

Liquid chromatography–(tandem) mass spectrometry of selected emerging pollutants (steroid sex hormones, drugs and alkylphenolic surfactants) in the aquatic environment

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Abstract

Among the various compounds considered as emerging pollutants, alkylphenolic surfactants, steroid sex hormones, and pharmaceuticals are of particular concern, both because of the volume of these substances used and because of their activity as endocrine disruptors or as causative agents of bacterial resistance, as is the case of antibiotics. Today, the technique of choice for analysis of these groups of substances is liquid-chromatography coupled to mass spectrometry (LC–MS) and tandem mass spectrometry (LC–MS–MS). In the last decades, this technique has experienced an impressive progress that has made possible the analysis of many environmental pollutants in a faster, more convenient, and more sensitive way, and, in some cases, the analysis of compounds that could not be determined before. This article reviews the LC–MS and LC–MS–MS methods published so far for the determination of alkylphenolic surfactants, steroid sex hormones and drugs in the aquatic environment. Practical considerations with regards to the analysis of these groups of substances by using different mass spectrometers (single quadrupole, ion trap and triple quadrupole instruments, etc.), interfaces and ionization and monitoring modes, are presented. Sample preparation aspects, with special focus on the application of advanced techniques, such as immunosorbents, restricted access materials and molecular imprinted materials, for extraction/purification of aquatic environmental samples and extracts are also discussed.
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Keywords: Reviews; Environmental analysis; Liquid chromatography–mass spectrometry; Pollutants; Alkylphenols; Endocrine disrupting compounds; Steroids; Sex hormones; Pharmaceuticals

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1. Introduction

For decades, environmentalists have centered on the study of chemicals whose presence in the environment has been regulated through the various lists of criteria or priority pollutants included in the different legislations.

The development of new and more sensitive methods for both detecting chemicals and determining their biological effects has, however, shifted the attention of the scientific community towards new, unregulated contaminants that were previously undetected or had not been considered as a risk. This is the case of the so-called emerging contaminants. Emerging contaminants are defined as newly identified or previously unrecognised pollutants [1], and include products used in everyday life, such as surfactants and surfactant residues, pharmaceuticals and personal care products, gasoline additives, plasticizers, etc. (see Table 1).

For most of these so-called ‘‘emerging contaminants’’, occurrence, risk assessment and ecotoxicological data are not available and, therefore, it is difficult to predict what health effects they may have on humans and aquatic organisms.

A recent study conducted by the USGS (United States Geological Survey) to report some of the first monitoring data on pharmaceuticals and other emerging organic wastewater contaminants [2,3] has revealed detergent metabolites and steroids as the groups of compounds presenting the highest concentrations in the aquatic environment, and steroids again and nonprescription drugs as the compounds most frequently found (see Fig. 1).

Due to their estrogenic activity, both detergents and steroids have been included in diverse preliminary lists of endocrine disrupting compounds (EDCs) [4,5], and nonylphenols (NPs) and octylphenols (OPs), degradation products of the widely used alkylphenol ethoxylate surfactants (APEOs), have been, in fact, listed as priority hazardous substances in the field of water policy by the European Community Water Framework Directive 2000/60/EC and the final European Union decision No. 2455/2001/EC.

The risks derived from the presence of these two groups of substances in the aquatic environment have been pointed out in various monitoring programs that, integrating both chemical and biological analyses, have identified both steroids and APEOs [6–9] as the compounds responsible for the induction of estrogenic effects, such as feminization and hermaphroditism, in aquatic organisms.

However, of all the emerging contaminants, antibiotics are probably the biggest worry because of the potential for antibiotic resistance [2]. The increasing use of these drugs in livestock, poultry production, and fish farming during the last 5 decades has caused a genetic selection of more harmful bacteria, which is a matter of great concern.

The technique of choice for analysis of the above mentioned groups of emerging pollutants is LC–MS and LC–MS–MS. Before the advent of LC–MS, many of these polar compounds were difficult and sometimes impossible to measure.

In the last decades, LC–MS and LC–MS–MS have experienced impressive progress, both in terms of technology development and application. Interface designs have changed considerably and have become much more sophisticated and efficient. Today, the interfaces most widely used for the LC–MS analysis of steroids, drugs, and surfactants, and of organic

Table 1
Emerging compound classes

Compound class	Examples
Pharmaceuticals	
Veterinary and human antibiotics	Trimethoprim, erytromycine, lincomycin, sulfamethaxozole
Analgesics and anti-inflammatory drugs	Codein, ibuprofene, acetaminophen, acetylsalicilyc acid, diclofenac, fenoprofen
Psychiatric drugs	Diazepam
Lipid regulators	Bezafibrate, clofibric acid, fenofibric acid
β-Blockers	Metoprolol, propanolol, timolol
X-ray contrasts	Iopromide, iopamidol, diatrizoate
Steroids and hormones (contraceptives)	Estradiol, estrone, estriol, diethylstilbestrol
Personal care products	
Fragrances	Nitro, polycyclic and macrocyclic musks,
Sun-screen agents	Benzophenone, methylbenzylidene camphor
Insect repellants	*N,N*-Diethyltoluamide
Antiseptics	Triclorsan, Chlorophene
Surfactants and surfactant metabolites	Alkylphenol ethoxylates, alkylphenols (nonylphnol and octylphenol), alkylphenol carboxylates
Flame retardants	Polybrominated diphenyl ethers (PBDEs), Tris(2-chloroethyl)phosphate
Industrial additives and agents	Chelating agents (EDTA), aromatic sulfonates
Gasoline additives	Dialkyl ethers, methyl-t-butyl ether (MTBE)
Disinfection byproducts	Iodotrihalomethanes, bromoacids, bromoacetonitriles, bromoaldehydes, cyanoformaldehyde, bromate

pollutants in general, in the aquatic environment, are electrospray (ESI) and atmospheric pressure chemical ionisation (APCI). ESI is particularly well suited for analysis of polar compounds whereas APCI is very effective in the analysis of medium- and low-polarity substances.

On the other hand, these atmospheric pressure ionisation (API) technologies have been interfaced with a variety of mass analysers, including single and triple quadrupole, orthogonal-acceleration time-of-flight (oaTOF), ion trap, and sector-field MS instruments. For analysis of the compound classes here reviewed, single quadrupole mass spectrometers, and to a lesser extent ion trap and MS–MS instruments, have been used.

LC–MS and LC–MS–MS have been mostly applied in the selected ion monitoring (SIM) mode and in the selected reaction monitoring mode (SRM), respectively, to the determination of target analytes. LC–MS–MS offers very good sensitivity and selectivity in the trace analysis of environmental pollutants. Additional benefits are that analytes do not have to be fully resolved to be identified and quantitated, as is required using conventional photodiode array detection (PDA), and that chemical derivatization is not needed, as in gas chromatography–mass spectrometry (GC–MS). However, in the case of very complex matrices, such as wastewater and sludge, even when using SRM detection, both false negative results, due to matrix ionisation suppression effects, and false positive results, due to insufficient selectivity, can be obtained [10].

To avoid false positives, the following confirmation criteria are normally employed when using LC–MS–MS [11,12]:

1. LC retention time must be within 1–2% of the retention time of the standard compound
2. the relative abundances of at least two selected

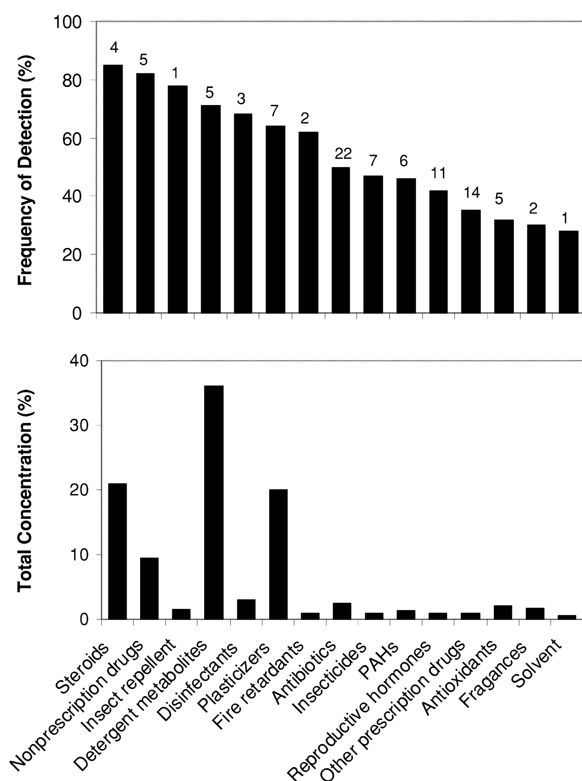

Fig. 1. Frequency of detection (A) and percent of total measured concentration (B) of organic wastewater contaminants by general use category. Number of compounds in each category shown above bar. From Kolpin et al. [3].

precursor ion-product ion transitions must be within 20% of the ions ratios obtained for the standards.

Compound identification is also possible in the full scan mode and after collision induced dissociation with single quadrupole and ion trap instruments, and in the precursor-ion, product-ion, and neutral loss scan modes with triple quadrupole instruments. However, the application of LC–MS and LC–MS–MS as screening techniques for the identification of unknown compounds and metabolites has been limited due to the up to date, current low sensitivity, low mass resolution, and limited structural information obtained under these scan modes, and to the lack of standards and LC–MS libraries [10].

An approach for increasing the selectivity, and avoiding false positive findings is the use of TOF-MS. Oa-TOF-MS instruments became commercially available some 3–4 years ago and combine the ability to perform accurate mass determination with an excellent full-scan sensitivity [13]. Thus, for compounds with molecular masses (M_r) up to about 1000, this accuracy provides an excellent confirmation of identity based on calculated elemental compositions. On the other hand, if full use is made of the resolution power of the TOF-MS, and narrow mass range chromatograms (± 0.1) are extracted, limits of detection (LODs) can compete with those of SRM detection [10].

Another very powerful technique results from the combination of a quadrupole front-end and an oa-TOF backend for MS–MS, in the so-called Q-TOF, where accurate mass determination at excellent sensitivity can be achieved after conventional low-energy collision induced dissociation (CID) in a hexapole collision cell [13].

However, neither of these two advance techniques (LC–oa-TOF-MS and LC–Q-TOF-MS) have been routinely employed yet for the qualitative or quantitative determination of steroids, drugs, and alkylphenolic surfactants in environmental samples, probably due to their, at the moment, high price compared to ion-trap and triple quadrupole instruments.

In this article, the LC–MS- and LC–MS–MS-based methods so far applied to current environmental monitoring of steroid sex hormones, drugs and alkylphenolic surfactants, are reviewed.

2. Sample preparation

The analysis of micropollutants in the environment constitutes a difficult task, first, because of the complexity of the matrices, and second, because of the normally very low concentrations of the target compounds. In essentially all cases of interest, substantial analyte enrichment is necessary to isolate the target compounds from the matrix and to achieve the LODs required. A typical analytical procedure includes, therefore, various sample preparation steps, such as filtration, extraction, purification, and evaporation; and, if the final determination is performed by bioassays or GC–MS, hydrolysis and derivatization are also frequently necessary. In the following

section, the main sample pretreatment procedures applied in the analytical determination by LC–MS or LC–MS–MS of steroid sex hormones and related synthetic compounds, drugs, and alkylphenolic surfactants, in aquatic environmental samples, are briefly discussed.

2.1. Steroid sex hormones and related synthetic compounds

2.1.1. Aqueous samples

The analytical methods published in the literature for the analysis of estrogens and progestogens in wastewater have been recently reviewed by López de Alda and Barceló [14]. According to this review, the filtration step, which is performed in the case of samples with high suspended matter content, and the concentration (rotary evaporation, stream of nitrogen, etc.), which is often carried out several times throughout the complete analytical procedure, do not lead to significant losses of the analytes. Thus, of the various steps taking part in the sample preparation procedure, the extraction/purification is the most critical.

Extraction of both estrogens and progestogens from water has been usually carried out by off-line solid-phase extraction (SPE) with either disks or, most frequently, cartridges (see Table 2).

The advantages and disadvantages of some commercially available sorbents, cartridges and devices for the off-line and on-line SPE of estrogens and progestogens from environmental matrices have been discussed recently [15].

Octadecyl (C_{18})-bonded silica has been the adsorbent most widely employed, although polymeric sorbents and graphitized carbon black (GCB) have also been used. The GCB material is presented as a quite selective adsorbent that permits the removal of most of the co-extracted impurities, such as humic acids [11,12,16]. This material behaves both as a nonspecific adsorbent and as an anion exchanger, and this feature is exploited to achieve selective neutral/acid class fractionation by stepwise desorption.

The use of two different sorbents—LiChrolut EN (0.2 g) and C_{18} (0.5 g, Varian Bond Elut) —layered-bed in custom packed glass columns has been reported by Ferguson et al. for the nearly quantitative extraction of estrogens from wastewater effluents [17]. Interestingly, these authors used immunosorbent extraction as a purification step previous to the analysis by LC–ESI-MS. The application of immunosorbent extraction to environmental analysis has been hampered by the difficulty in obtaining antibodies to common environmental pollutants. In this work, the authors prepared a mixed immunosorbent material for selective isolation of estradiol and estrone from extracts of wastewater, by taking advantage of the availability of commercially developed monoclonal antibodies. The mixed immunosorbent was found to be highly selective and to remove most of the matrix-related interferences that would otherwise cause severe ionisation suppression (approaching 100%) of the target analytes in the electrospray interface. With this approach, method LODs were well below those obtained by GC–MS and previous LC–MS methods, and were very similar to values reported for recent LC–MS–MS methods developed for analysis of estrogens in sewage effluents.

Promising results in the field of immunoaffinity extraction have also been obtained by Tozzi et al. [18], in a work in which an estrogen-binding affinity solid-phase, containing tetrapeptides selected by a combinatorial-binding approach, was prepared and applied to the preconcentration of estrogens from different types of water, including buffered water, tap water and river water. In these experiments, estrogens were found to be efficiently and selectively retained in the solid-phase column, which works well in aqueous medium, and quantitatively recovered in a few millilitres of methanol mobile phase.

Attempts to develop fully automated methodologies for the on-line SPE and analysis of estrogens in water have also been made in a few occasions [15,19–21], but in only two of them, LC–MS has been used for final determination [15,19]. In the most recent of these works [15], 10×2 mm I.D. Hy-Sphere-Resin-GP cartridges (Spark Holland, The Netherlands), were selected out of three other sorbents—C_{18} Baker (J.T. Baker, Deventer, The Netherlands), PLRP-S (Polymer Laboratories, Church Stretton), and Oasis HLB (Waters, Milford, MA, USA)—for the on-line SPE of the most en-

Table 2
Survey of LC–MS and LC–MS–MS methods for quantitative determination of steroid sex hormones and related synthetic compounds in aquatic environmental samples

Compounds	Matrix	Extraction	Detection method	LC column	Mobile phase	MS system	LOD (ng/l, ng/g)	Ref.
E2, E3, E1, EE, DES, PROG, LEV, NOR	Drinking and surface water, STP effluent	SPE (C_{18} col.)	ESI-MS (SIM)	LiChrospher 100 RP-18 (250×4 mm, 5 µm)	ACN–water	HP 1100[a]	2–500	[58]
E2, E3, E1, EE, DES, PROG, LEV, NOR	Water	On-line SPE (HySphere-Resin -GP col.)	ESI-MS (SIM)	LiChrospher 100 RP-18 (250×4 mm, 5 µm)	ACN–water	HP 1100[a]	<1	[15]
E2, E1	STP effluent	SPE (LiChrolut EN+C_{18} col.)+ immunoaffinity extraction	ESI(NI)-MS	Betasil C_{18} (150×2.1 mm, 3 µm)	ACN–water	Platform LCZ[b]	0.07–0.18	[17]
E2, E3, E1	River water	SPE (SDB-SC disk)	ESI(NI)-MS	Zorbax C_{18} (150×2.1 mm)	ACN–water	HP 1100[a]	1–50	[92]
E2, E3, E1, EE, DES	River water	SPE (C_{18} col.)	IS(NI)-MS (SIM)	Purospher STAR RP-18 (55×2 mm, 3 µm)	ACN–water	M-8000 ion trap[c]	3.2–10.6	[57]
E2, E3, E1, EE, DES	Water (tank, river, STP effl.)	SPE (C_{18} col.)	ESI(NI)-MS (SIM)	Phenomenex Luna C_{18} (150×4.1 mm, 3 µm)	Water–MeOH–ACN (ammonium acetate 10 m*M*)	HP 1100[a]	200	[56]
E2, E3, E1, EE, DES	Water (tank, river, STP effl.)	SPE (C_{18} col.)	ESI(NI)-MS–MS	Phenomenex Luna C_{18} (150×4.1 mm, 3 µm)	Water–MeOH–ACN (ammonium acetate 10 m*M*)	Quattro LC triple quadrupole[b]	5	[56]
E2, E3, E1, EE	River water, STP influent and effluent	SPE (Carbograph-4 col.)	ESI(NI)-MS–MS	Alltima C_{18} (250×4.6 mm, 5 µm)	ACN–water (postcolumn addition of ammonia)	Sciex API 2000 triple quadrupole[d]	0.08–0.6*	[11]
E2, E3, E1, EE, E3-3G, E2-3G, E1-3G, E3-16G, E2-17G, E3-3S, E2-3S, E1-3S	Sewage and river water	SPE (Carbograph-4)	ESI(NI)-MS–MS	Alltima C_{18} (250×4.6 mm, 5 µm)	ACN–water (postcolumn addition of ammonia)	Sciex API 2000 triple quadrupole[d]	0.003–15	[16]
E2, EE	Aquaria water	SPE (Sep-Pak C_{18})	APCI(PI)-MS (SIM)	Prodigy ODS (150×2 mm, 5 µm)	Water–methanol (0.2% formic acid)	G1946A MSD[e]	0.6–1	[55]
E2, E3, E1, EE	STP influent and effluent	SPE (Envi-Carb col.)	APCI(PI)-MS–MS	Alltima C_{18} (250×4.6 mm, 5 µm)	ACN–water	Sciex API 365 triple quadrupole[d]	0.5–1*	[12]
E2, E3, E1, EE, MES, equilin, testosterone, dihydrotestosterone, cyproterone	River water	SPE (C_{18} col.)	APCI(PI)-MS–MS	Hypersil ODS (100×4.6 mm, 3 µm)	0.1% Acetic acid–methanol	Sciex API 365 triple quadrupole[d]	1–10	[54]
E2, E3, E1, EE, DES, PROG, LEV, NOR	River sediment	PLE (acetone–methanol, 1:1), +SPE (LiChrospher ADS C4)	ESI-MS (SIM)	LiChrospher 100 RP-18 (250×4 mm, 5 µm)	ACN–water (NI) MeOH– water (PI)	HP 1100[a]	0.5–5	[24]
E2, E3, E1, EE, DES, PROG, LEV, NOR	River sediment	Ultrasonication (acetone–methanol, 1:1) +SPE (C_{18} col.)	ESI-MS (SIM)	LiChrospher 100 RP-18 (250×4 mm, 5 µm)	ACN–water	HP 1100[a]	0.04–1	[25]

Abbreviations (not included in the text): Ref., reference; SFE, supercritical fluid extraction; E2, estradiol; E3, estriol; E1, estrone; EE, ethynyl estradiol; DES, diethylstilbestrol; PROG, progesterone; LEV, levonorgestrel; NOR, norethindrone; STP, sewage treatment plant; col., column or cartridge; ACN, acetonitrile; IS, ionspray or pneumatically assisted electrospray E3-3G, estriol 3-(β-D-glucuronide); E2-3G, 17β-estradiol 3-(β-D-glucuronide); E1-3G, estrone 3-(β-D-glucuronide); E3-16G, estriol 16α-(β-D-glucuronide); E2-17G, β-estradiol 17-(β-D-glucuronide); E3-3S, estriol 3-sulfate; E2-3S, estradiol 3-sulfate; E1-3S, estrone 3-sulfate; MES, mestranol.

[a] Hewlett-Packard. [b] Micromass. [c] Merck–Hitachi. [d] Perkin-Elmer. [e] Agilent.

vironmentally relevant estrogens from water using the automated sample preparation system Prospekt (Spark Holland). With the described methodology, up to 16 samples can be analysed in a fully automated, unattended way, and the no requirement for sample manipulation, other than the filtration carried out only in the case of samples with high turbidity, results in improved repeatability and accuracy. Further advantages of this technique are speed, cost, and improved sensitivity as the complete sample rather than a (minor) aliquot is subjected to separation and detection. With this procedure, LODs below 1 ng/l could be achieved for estrogens analysed by using an ESI interface in the negative mode of ionisation.

2.1.2. Solid samples

The analytical methods described so far in the literature for the determination of estrogens and progestogens in fresh water sediments [22] and in solid environmental samples in general [23] have been reviewed in two recently published articles. These reviews also cover the determination of other classes of EDCs (alkylphenols, polychlorinated compounds (dioxins, furans, and biphenyls), polybrominated diphenyl ethers, and phthalates) [22] and drugs [23], but they do not specifically discuss the use of LC–MS methods. As indicated in these articles, most of the environmental programs carried out to assess the presence and impact of natural and synthetic estrogens and progestogens in the aquatic environment have focused on the investigation of environmental waters and, to a lesser extent, of sewage sludge. On the contrary, soils and sediments have seldom been analysed and in only a few occasions final determination has been carried out by LC–MS [24–26].

Extraction of both natural and synthetic estrogens from river sediments has always been performed with acetone–methanol (1:1), using either sonication [25] or pressurized liquid extraction (PLE) [24], and clean-up of the extracts has been carried out by SPE with C_{18} columns (see Table 2). An interesting, novel methodology based on column switching LC–MS using restricted access materials (RAM) has been applied by Petrovic et al. for further, integrated clean-up and analysis. RAMs are bifunctional sorbents that combine size-exclusion and reversed-phase retention mechanisms tailored for the separation of macromolecular matrix components and the adsorption of low molecular target analytes, all in one step. These materials are, therefore, particularly well-suited for bioanalysis although they have found application as well in the analysis of environmental pollutants, such as pesticides [27–29]. According to Petrovic et al., the use of RAM precolumns, after SPE clean-up with C_{18} columns, enables the LC–MS determination of steroid sex hormones and other pollutants, such as alkylphenolic compounds and bisphenol A, in sediments at very low levels (LODs = 0.5–5 ng/g) due to the efficient removal of co-extracted matrix components and the consequent reduction of ion suppression effects. In this work, different LiChrospher ADS RAM precolumns (Merck, Germany), with C_4, C_8 and C_{18} modification of inner pore surface were tested. The ADS C_4 precolumn was found to be the most convenient in terms of recovery, selectivity and sensitivity. With the methodology described, a complete analysis, including efficient PLE of target compounds, on-line clean-up, chromatographic separation and MS detection takes approximately 2 h, which is a significant improvement in comparison to methods previously reported.

2.2. Drugs

2.2.1. Aqueous samples

Previous to extraction of target analytes from water matrices, the sample is filtered to subtract the suspended matter, and pH adjusted from values ranging from acid to alkaline pH (2, 3, 5, 7 and 9) depending on the acid (tetracyclines) or alkaline (phenazone-type) nature of the drugs under study. To avoid photodegradation, which affects specially to some compounds, such as fluoroquinolones and tetracyclines (TCs), samples are stored at low temperatures (~4 °C) in amber glass bottles until extraction. Due to the predicted low concentration levels of pharmaceuticals in the aquatic environment, enrichment of the target analytes prior to the detection, is essential. In most instances, extraction and preconcentration has been performed by SPE, with some exceptions which use lyophilisation [30,31] because it is fast and consumption of organic solvents is low (see Table 3), and because of its easy automatisation and on line attachment. For SPE,

Table 3
Survey of LC–MS methods used for quantitative analysis of human and veterinary drugs

Compounds	Matrix	Extraction	Detection method	LC column	Mobile phase	LC–MS system	LOD (ng/l)	Ref.
TCs, SAs, Trimethoprim, Tylosin, Ibuprofen, Ciprofloxacin, Enrofloxacine,	Stream waters	Tandem SPE Oasis HLB/MCX cartridges	ESI(PI)-MS	Luna C_8 (100×4.6 mm, 3 μm)	Binary gradient: Phase A: 10 m*M* ammonium formate in water –methanol (90:10) with 0.3% formic acid Phase B: 10 m*M* ammonium formate with 0.5% formic acid in methanol	1946B MS[a]	NR	[3]
Fluoxetine Gemfibrozil		SPE Oasis HLB	ESI(PI)-MS–MS	Metasil Basic C_8 (150×2.0 mm, 3 μm)	Binary gradient: Phase A: aqueous $NH_4H_2O_2$–CH_2O_2 buffer, 10 m*M*, pH 3.7 Phase B: acetonitrile			
Analgesics (phenazone), β-Blockers (propanolol), broncholitics (clenbuterol), antineoplastics (ifosfamide) lipid lowering agents (simvastatin)	Tap and surface waters	SPE PPL Bond-Elut	ESI(PI)-MS–MS	Nucleosil 120-3C_{18} (250×2.0 mm, 3 μm)	Binary gradient: Phase A: 20 m*M* ammonium acetate in water, pH 6.8 Phase B: 20 m*M* ammonium acetate in acetonitrile–methanol (2:1, v/v)	Sciex API 2000[b]	8–44	[61]
X-ray contrast media (iopamidol)		SPE LiChrolut EN	ESI(PI)-MS–MS		Binary gradient: Phase A: 2 m*M* ammonium formate in water, pH 7.0 Phase B: 2 m*M* ammonium formate in acetonitrile–methanol (2:1, v/v)		8–16	
Antibiotics SAs (sulfadiazine)		SPE Isolut ENV+	ESI(PI)-MS–MS		Binary gradient: Phase A: 20 m*M* ammonium acetate in water, pH 6.8 Phase B: 20 m*M* ammonium acetate in acetonitrile–methanol (2:1, v/v)		3.7–11	
Macrolides (erythromycin)		SPE Isolut ENV+	ESI(PI)-MS–MS		Binary gradient: Phase A: 20 m*M* ammonium acetate in water, pH 6.8 Phase B: 20 m*M* ammonium acetate in acetonitrile–methanol (2:1, v/v)		6.4–15	
PENs (amoxicillin)		SPE Isolut ENV +	ESI(PI)-MS–MS		Binary gradient: Phase A: 2 m*M* ammonium formiate in water, pH 7.0 Phase B: 2 m*M* ammonium formiate in acetonitrile–methanol (2:1, v/v)		15–21	
Caffeine Aminoantipyrine Propyphenazone Diazepam Nifedipine Glibenclamide Omeprazole Oxyphenbutazone Phenylbutazone Dihydrocarbamazepine	Groundwater and wastewaters	SPE Isolute C_{18}	ESI(PI)-MS–MS	LiChrospher 100RP C_{18}, (125×3.0 mm, 5 μm)	Binary gradient: Phase A: 20 m*M* ammonium acetate in water, pH 5.7–acetonitrile (9:1, v/v) Phase B: phase A–acetonitrile (4:6, v/v)	Sciex API 365[b]	10–50	[35]
TCs	Surface waters	SPE (except TCs) LiChrolut EN LiChrolut C_{18} Lyophiliz	ESI(PI)-MS–MS Except for chloramphenicol (ESI(NI))	LiChrospher 100RP C_8 (ec) (125×3.0 mm, 5 μm)	Binary gradient: Phase A: 10 m*M* oxalic acid –acetonitrile (9:1 v/v) Phase B: 10 m*M* oxalic acid –acetonitrile (4:6 v/v)	Sciex API III Plus[b]	50	[31]

Table 3. Continued

Compounds	Matrix	Extraction	Detection method	LC column	Mobile phase	LC–MS system	LOD (ng/l)	Ref.
PENs					Binary gradient: Phase A: acetate buffer, pH 5.7 –acetonitrile (9:1, v/v) Phase B: phase A –acetonitrile (4:6 v/v)		20	
SAs, macrolide antibiotics, trimethoprim chloramphenicol					Binary gradient: Phase A: acetate buffer, pH 5.7 –acetonitrile (9:1, v/v) Phase B: phase A –acetonitrile (2:8 v/v)		20	
Paracetamol[+] Clofibric acid Penicillin V Naproxen Benzafibrate Carbamazepine Diclofenac sodium Ibuprofen Mefenamic acid	River waters	SPE Bondesil ODS 40 μm	ESI–MS APCI-MS	YMC ODS-AM (250×2 mm)	Ternary gradient: Phase A: acetate buffer, pH 5.5 Phase B: water Phase C: methanol	HP 5989B[a]	0.04–1.1*	[59]
Ibuprofen Ketoprofen Naproxen Diclofenac Salicylic acid Gemfibrozil	River waters, wastewaters	SPE LiChrolut EN Oasis HLB	ESI(NI)-MS	LiChrospher 100RP C_{18} (125×3.0 mm, 5 μm)	Binary gradient: Phase A: acetonitrile Phase B: aqueous formic acid, pH 2	HP 1100[a]	NR	[65]
TCs Tylosin	Fertilized soil Dried liquid manure Soil water Ground water	LLE SPE Baker SDB1	ESI(PI)-MS–MS	Puresil C_{18} (150×4.6 mm)	Binary gradient: Phase A: formic acid (0.5%)+ ammonium acetate (1 m*M*)+ water (pH 2)	LCQ ion trap MS[c]	5[d] 50[d] 100 100	[62]
SAs TCs	Ground and surface water	SPE HLB	ESI(NI)-MS	Luna C_8 (100×4.6 mm, 3 μm)	Binary gradient: Phase A: 10 m*M* ammonium formate in water–methanol (90:10) with 0.3% formic acid Phase B: 10 m*M* ammonium formate with 0.5% formic acid in methanol	HP 1946B[a]	NR	[36]
TCs	Groundwater confined animal feeding wastewater	SPE HLB	ESI(PI)-MS–MS	BetaBasic C_{18} (250×2 mm, 5 μm)	Isocratic Water–5% formic acid –acetonitrile–methanol (23:40:25:12)	LCQ ion trap MS[c]	200–380	[63]

* Corresponding to the MS detection mode offering the best LOD. [+] Paracetamol could not be extracted by the described SPE method; NR, not reported.
Abbreviations (not included in the text): FAB, fast atom bombardment.
[a] Agilent; [b] Perkin–Elmer; [c] Finnigan; [d] Expressed in ng/g.

several adsorbent materials have been employed (see Table 3) being the reverse phase supports those used the most. Kolpin et al. [32] have designed a tandem SPE extraction system, attaching an hydrophilic–lipofilic balance (HLB) cartridge to the top of a mixed mode, HLB-cation exchange (MCX) cartridge in order to extract at once 21 antibiotics. Other authors [33], however, used mixed-phase cation-exchange (MPC) disk cartridges for a similar purpose, probing to be the most specific material and offering

the best recovery efficiencies for fluoroquinolone antibacterials. For some drugs, for instance for TCs, the use of SPE cartridges or disks, without any additional treatment, is not adequate. In addition, cartridge materials must not contain silanol groups, since they have been found to bind irreversibly to TCs. A precaution leading to a notorious improvement of extraction efficiencies is the silanisation, for instance with dimethyldichlorosilane [34], of all glassware getting in contact with either the water sample or the extract, or the use of other container materials, such as PTFE. These preventative measures are essential in the analysis of polar compounds, such as β-blockers, and of compounds with probed chelating capabilities, such as TCs [35]. Additional approaches to prevent chelation of metals by these type of compounds are washing off the cartridge using a diluted HCL solution and adding a strong quelator to the sample, for instance Na_2EDTA, which presents optimum solubility in water and, unlike oxalic acid, does not accumulate in the capillary interface when mass spectrometric detection follows [36].

Solid-phase microextraction (SPME) has also been tested for TCs extraction from water [37]. In this study, the optimisation of the on-line SPME–LC–MS method is described including choice of extracting fibre and desorption method (heating or salting out the analytes). Despite SPME eliminates the need for lengthy sample clean-up and is economic, the poor variety of fibres available compatible with LC–MS limits the widespread development of this technique.

2.2.2. Solid samples

The presence of pharmaceutical products in soil, sediment and sludge has scarcely being investigated as compared to aquatic media. To date, only one review devoted to the analysis of solid environmental matrices is available [23]. This work presents recent advances on the analysis of drugs in order to light up further research in this area.

Extraction of drugs from solid matrices has normally been performed by sonication or by simple blending or stirring of the sample with polar organic solvents or mixtures of them, or with aqueous solutions. The use of more advanced extraction techniques, such as PLE, has been reported by Reddersen et at. [38] for the analysis of phenazone in sludge obtained by filtration of filter flushing water from a drinking water treatment plant. The PLE was performed at 150 °C and 10.34 MPa pressure twice with ethyl acetate.

Clean-up of the extracts, when performed, has been carried out by SPE, liquid–liquid extraction (LLE), gel-permeation chromatography (GPC) and semipreparative LC. SPE has been preferred in most instances because it is fast, requires low volume of organic solvent, presents low contamination risk and can be used online. With the exception of Brambilla et al. [39] who used strong cation-exchange (SCX) cartridges, SPE cleanup of the extracts has always been performed with reversed-phase adsorbents. The use of other advanced clean-up techniques, such as immunoaffinity extraction or molecular imprinted materials (MIPs) for this kind of applications has not been reported in the literature.

2.3. Alkylphenolic compounds

The widespread use and questionable environmental acceptability of alkylphenol ethoxylates (APEOs) make them a focus of interest in environmental analytical chemistry. Although often present in concentrations several orders of magnitude higher than concentration of steroid hormones, the analysis of APEOs and their neutral and acidic degradation products still requires multistep sample pretreatment aimed at the reduction of the matrix content and the enrichment of the target compounds.

2.3.1. Aqueous samples

For preconcentration of alkylphenolic surfactants from aqueous samples, SPE is considered the most appropriate technique, in terms of its speed, selectivity and percentage of recovery, and it is preferred over conventional methods (e.g. LLE extraction). Octadecyl (C_{18}) bonded silica has been the SPE material most widely employed for extraction of both neutral and acidic alkylphenolic compounds [17,40–44] with the efficiency of extraction from wastewater and surface water being higher than 80% for all compounds investigated. Strong anionic exchange (SAX) [45] and GCB have also been employed [46] (see Table 4). Desorption of trapped analytes is

Table 4
Survey of LC–MS and LC–MS–MS methods for quantitative determination of alkylphenol ethoxylates and their metabolites in aquatic environmental samples

Compounds	Matrix	Extraction	Clean-up	Separation and detection method	LC column	Mobile phase	MS system	LOD (ng/l, ng/g)	Ref.
NPEO (n_{EO} = 1–19)	Marine sediment	Soxhlet (hexane–IPA, 70:30)	SPE-CN	NP-LC–ESI-MS	Spherisorb CN (250×3 mm, 5 μm)	A: toluene B: 0.5 m*M* NaOAc in toluene–MeOH–water (10:88:2, v/v)	VG Quattro tandem MS[a]	2–10	[66]
APEO (n_{EO} = 1–3), APs, XNPs	Estuarine sediment	High-temperature continuous flow sonication (MeOH)	1. SPE-NH_2 2. RP-LC fractionation	RP-LC–ESI-MS	Keystone C_8 (150×2.1 mm, 5 μm)	MeOH–water, (80:20, isocratic)	Platform LCZ[c]	0.04–0.92 ng/l	[17,93]
			RP-LC fractionation (two columns in series)	Mixed mode LC–ESI-MS				21.5 NP	[94]
				0.78–37.3 NPEO					
APEO, APEC, AP, halogenated derivatives	River water sediment, sludge,	Sonication (DCM–MeOH, 3:7)	SPE-C_{18}	RP-LC–ESI-MS	LiChrospher 100 C_{18} (250×4, 5 μm)	MeOH–water (PI) ACN–water (NI)	HP 1100[b]	20–50 ng/l 5–25 ng/g (sludge) 2–10 ng/g (sediment)	[71]
APEOs, APECs, APs, halogenated derivatives	River sediment, sludge	PLE	C_{18}	RP-LC–ESI-MS	LiChrospher 100 C_{18}	MeOH–water (PI)	HP 1100[b]	1–5 ng/g	[95]
			RAM (ADS C4)		(250×4, 5 mm)	ACN–water (NI)			
								0.5–5 ng/g	[24]
NPEO, OPEO (n_{EO} = 6–15)	Wastewater, sludge	LLE (DCM)	–	RP-LC–ESI-MS	Phenomenex Luna C_{18} (250×2 mm, 5 μm)	MeOH–water (both containing 5 m*M* NH_4-acetate + 0.5 m*M* trichloroacetic acid)	Esquire[c]	100 ng/g	[96]
OP	Fish tissue, water	MAE (DCM–MeOH, 2:1) SPE (Sep-Pak C_{18})	SPE–NH_2	RP-APCI-ESI–MS	Zorbax Eclipse XDB C_{18} (150×2.1 mm)	MeOH–water	HP 1100[b]	100 ng/l 10 ng/g (muscle) 50 ng/g (liver)	[87]
NPEO, NPEC, CAPEC	Wastewater	SPE–GCB	–	RP-LC–ESI-MS	Alltima C_{18} (250×4.6 mm, 5 μm)	ACN–water (both containing 1 mmol formic acid)	Finnigan AQA[d]	Not reported	[83]
NPEO	STP samples	SPE-C_{18}	Sequential elution in 4 fractions	FIA-APCI-MS	Bypassing analytical column	MeOH–water (3:7) containing 0.05 *M* NH_4OAc	TSQ 700 triple quadrupole[e]	Not reported	[49,50]
NPEO, NPEC, NP	Waste water, river water, sludge	SPE-C_{18} Sonication (DCM–MeOH, 3:7)	SPE-C_{18}	RP-LC–APCI-MS	Hypersil Green ENV (150×4.6 mm, 5 μm)	A: MeOH–CAN (1:1) B: water, both containing 0.5% HOAc	VG Platform[a]	80–200 ng/l	[47,48]
NPEC, NP, halogenated derivatives	River water, drinking water, sludge	SPE-C_{18} PLE (MeOH–acetone, 1:1)	SPE-C_{18}	RP-LC–ESI-MS–MS	Purospher STAR RP-18 (55×2 mm, 3 μm)	ACN–water	Quattro LC[a]	1–2 ng/l 0.5–1.5 ng/g	[85]

[a] Micromass; [b] Hewlett-Packard; [c] Bruker Daltonics; [d] Thermoquest; [e] Finnigan.

generally performed with methanol, acetone, dichloromethane or with their mixtures.

A sequential SPE procedure using two cartridges of different SPE material (C_{18} and polymeric) coupled in series was developed to extract and fractionate APEOs and their acidic and neutral degradation products [47,48]. A similar fractionation scheme using selective elution with solvents of different polarity and selective desorption potential (hexane–diethyl ether, 8:2, v/v; diethyl ether; methanol–water, 2:8, v/v and methanol) was applied for successful analysis of alkylphenolic compounds by flow-injection analysis (FIA)-MS [49,50].

2.3.2. Solid samples

A review of the sample preparation methods for the determination of APEOs and their degradation products in solid environmental matrices has been recently published by Petrovic and Barceló [51]. The common approach for extracting alkyphenolic compounds from solid samples includes either traditional exhaustive extraction techniques, such as Soxhlet extraction or sonication, or advanced extraction techniques based on elevated temperatures and pressures, such as PLE or microwave-assisted extraction (MAE) (Table 4). Typical solvents for the extraction of APEOs and alkylphenols (APs) have been methanol, dichloromethane, dichloromethane–hexane, hexane–acetone or hexane–i-propanol mixtures [51]. All these solvents yielded satisfactory extraction efficiency and maintained the integrity of the oligomeric distribution while allowing the preconcentration of APEOs.

A modification of PLE, extraction with pressurized (supercritical) hot water, as well as extraction with hot water under subcritical conditions, have also been used for the analysis of polar alkylphenolic compounds. Field and Reed [52] evaluated subcritical (hot) water extraction of the nonylphenol ethoxycarboxilates (NPECs) NPE_1C–NPE_4C over a range of temperatures from 25 to 100 °C at a pressure of 350 bars using ethanol-modified hot water (30% ethanol), which yielded quantitative recovery of native NPECs from sludge.

The extract obtained by exhaustive extraction techniques typically contains a large number of matrix components, which may coelute with the analytes and disturb the quantitative analysis and cause suppression of the analyte signal in LC–MS analysis. Therefore, subsequent cleanup or fractionation of extracts is indispensable. The common approach for extract clean-up in analysis of alkylphenolic compounds is based on SPE using different types of commercially packed cartridges (C_{18}, CN or NH_2). Recently, a column-switching system using precolumns packed with RAM was successfully employed for the simultaneous analysis of alkyphenolic compounds and steroid sex hormones in sediments [24].

3. Analysis

3.1. Steroid sex hormones and related synthetic compounds

To date, the techniques most commonly employed for the environmental analysis of steroid sex hormones and related synthetic compounds have been immunoassays and, to a greater extent, GC–MS [14]. However, LC–MS has gained in popularity in the last years and nowadays is considered to be the most promising analytical method for the determination of steroids [53]. The main advantage of using LC is that the enzymatic hydrolysis, required for the immunoassay analysis of both conjugated (glucuronides, sulfates, etc.) and unconjugated estrogens and progestogens, and the derivatization that normally precedes a subsequent GC–MS analysis, can be avoided.

Prior to MS detection, the LC separation of both conjugated and unconjugated estrogens and progestogens, has always been performed on octadecyl silica stationary phases (see Table 2).

As mobile phases, mixtures of water–methanol and, more frequently, water–acetonitrile, with gradient elution from 10 to 50% to 100% organic solvent have normally been used.

Modification of the mobile phase, when performed in attempt to improve the sensitivity of MS detection, has been accomplished with acetic acid 0.1% [54], formic acid 0.2% [55], ammonium acetate 10 m*M* [56], or by postcolumn addition of ammonia [11,16] (see Table 2).

According to Benijts et al. [57], who studied in detail the influence of different mobile phase com-

positions on the ionisation efficiency of an ionspray interface, a mixture of water and acetonitrile, without addition of bases or buffer systems is the best choice for optimal ionisation of estrogens.

In a first series of experiments, Benijts et al. investigated the effect of acetonitrile and methanol as organic modifiers on the ionisation of estradiol. It was observed that an increasing amount of organic modifier gradually increases the ionisation efficiency of the ion source for estradiol, this effect being more apparent with acetonitrile than with methanol. However, on reaching nonaqueous conditions (100% organic) in a gradient system, the electrolytic dissociation of analytes and solvation of the resulting ions seem to be reduced, especially in acetonitrile, and, therefore, the use of 100% organic mobile phases should be avoided.

The use of mobile phase additives, such as ammonium hydroxide, isopropylamine and triethylamine (TEA), commonly employed to both improve LC separation and ionisation efficiency in LC–(NI)MS, was also investigated by Benijts et al. [57] and it was found that none of these volatile bases improved the estradiol signal. On the contrary, TEA even had a negative impact on ionisation of the analyte.

Buffers are also usually added to the LC eluent for chromatographic purposes. In LC–MS, volatile buffers, such as formic acid–ammonium formate or acetic acid–ammonium acetate, are primarily used at concentrations of around 10 m*M* (ESI) and 50 m*M* (APCI). However, according to the studies carried out by Benijts et al. even low concentrations of these buffers in the LC mobile phase result in an extreme ion suppression [57].

The effects of mobile-phase additives on the ionisation efficiency of estrogens and progestogens were also evaluated by López de Alda et al. [58]. As Benijts et al., these authors found that modification of the acetonitrile–water mobile phase with methanol in various proportions, acetic acid 0.5%, or triethylamine 5 m*M*, did not significantly improve the MS signals.

These findings are, however, in contrast with those expressed by Baronti et al. [11] and Gentili et al. [16]. According to these authors the postcolumn addition of a basic agent, such as methanolic ammonia, serves to promote deprotonation of the weakly acidic estrogens, thus resulting in a drastic increase of the response of the ESI-MS system.

For LC–MS analysis of steroid sex hormones and related synthetic compounds, both APCI, and to a greater extent, ESI have been used. As indicated in Table 2, the LC–MS analysis of estrogens has been carried out in most instances with an ESI interface operating in the negative ion mode of ionisation (NI). With this technique the sensitivity achieved in the analysis of the most relevant estrogens is considerably better than that of the ESI interface operating in the positive ion mode of ionisation (PI) and the APCI interface operating in the NI mode [11,58]. However, some recent studies [12,54,55] indicate that the APCI interface operating in the PI mode can furnish sensitivities comparable in many cases to that of the negative ion ESI.

Table 5 summarizes the quantitation and diagnostic ions used by the various authors whose methods are reviewed here for the determination of estrogens and progestogens in the SIM or the SRM mode. As can be seen, the base peak selected for quantitation of estrogens in the SIM mode, or as precursor for collisionally induced dissociation in the SRM mode, corresponds to the $[M+H-H_2O]^+$ ion ($[M+H]^+$ for estrone) when positive ion APCI is used as interface and to the deprotonated analyte molecule $[M-H]^-$ when the interface used is negative ion electrospray.

Figs. 2 and 3 show full-scan product-ion spectra obtained for estriol, estradiol and estrone by LC–APCI(PI)-MS–MS [12] and LC–ESI(NI)-MS–MS [16], respectively, and the fragmentation scheme purported in each case.

LC–APCI(PI)-MS–MS was used, among others, by Lagana et al. to investigate the presence of estradiol, estriol, estrone, and diethylstilbestrol in sewage effluents. Under the MS experimental conditions selected in this work, the ion at *m/z* 133, probably due to a vinylbenzene structure (see Fig. 2), was the most abundant product from the three estrogens estrone, estriol, and EE, whereas estradiol principally generated a dihydronaphthalenic structure at *m/z* 159.

In LC–ESI(NI)-MS–MS the fragmentation pathways followed by these estrogens are, as is shown in Fig. 3, different from those just indicated for APCI(PI). The full scan product-ion spectrum for

Table 5
Quantitation and diagnostic ions and transitions used for the LC–MS and LC–MS–MS analysis of the most environmentally relevant estrogens and progestogens in the aquatic environment

Compound	Detection technique	Ionisation mode	SIM (m/z and base peak)	MRM transition (m/z and base peak)	Ref.
E2	ESI-MS	NI	271 $[M-H]^-$		[17,24–26,57,58]
	ESI-MS MS	NI		271→183 $[M-C_5H_{12}O]^-$ 271→145 $[M-C_8H_{14}O]^-$ 271→143 $[M-C_8H_{16}O]^-$	[11,16,56]
	APCI-MS	PI	255 $[M+H-H_2O]^+$		[55]
	APCI-MS–MS	PI		255→159 255→133	[12,54]
E3	IS-MS	NI	287 $[M-H]^-$		[24–26,57,58]
	ESI-MS MS	NI		287→171 $[M-C_6H_{12}O_2]^-$ 287→145 $[M-C_8H_{14}O_2]^-$ 287→143 $[M-C_8H_{16}O_2]^-$	[11,16,56]
	APCI-MS–MS	PI	287 $[M+H-H_2O]^+$	271→133	[12]
E1	ESI-MS	NI	269 $[M-H]^-$		[17,24–26,57,58]
	ESI-MS MS	NI	269 $[M-H]^-$	269→145 $[M-C_8H_{12}O]^-$ 269→143	[11,16,56]
	APCI-MS–MS	PI	271 $[M+H]^+$	271→133	[12]
EE	ESI-MS	NI	295 $[M-H]^-$		[17,24–26,57,58]
	ESI-MS MS	NI		295→159 $[M-C_9H_{12}O]^-$ 295→145 $[M-C_{10}H_{14}O]^-$	[11,56]
	APCI-MS	PI	279 $[M+H-H_2O]^+$		[55]
	APCI-MS–MS	PI		279→133	[12]
DES	ESI-MS	NI	267 $[M-H]^-$		[24–26,57,58]
E3-3G	ESI-MS–MS	NI	463 $[M+H]^+$	463→287 463→113	[16]
E3-3S	ESI-MS–MS	NI	367 $[M+H]^+$	367→287 367→80	[16]
E3-16G	ESI-MS–MS	NI	463 $[M+H]^+$	463→287 463→85	[16]
E2-3G	ESI-MS–MS	NI	447 $[M+H]^+$	447→271 447→113	[16]
E2-3S	ESI-MS–MS	NI	351.1 $[M+H]^+$	351→271 351→80	[16]
E2-17G	ESI-MS–MS	NI	447.2 $[M+H]^+$	447.2→271.2 447→85	[16]
E1-3G	ESI-MS–MS	NI	445.2 $[M+H]^+$	445→269 445→113	[16]
E1-3S	ESI-MS–MS	NI	349.01 $[M+H]^+$	349→269 349→145	[16]
NOR	ESI-MS	PI	321 $[M+Na]^+$		[25,26,58]
	APCI-MS	PI	299 $[M+H]^+$		[58]
LEV	ESI-MS	PI	335 $[M+Na]^+$		[25,26,58]
	APCI-MS	PI	313 $[M+H]^+$		[58]
PROG	ESI-MS	PI	337 $[M+Na]^+$		[25,26,58]
	APCI-MS	PI	315 $[M+H]^+$		[58]

estradiol shows losses consistent with ring cleavages (i.e. losses of $C_5H_{12}O$ and $C_8H_{14}O$) to give major product ions at m/z 183 and 145, respectively. The $[M-H]^-$ ion from estriol at m/z 287 gives major product ions at m/z 171 and 145 upon excitation. These ions relate to losses of $C_6H_{12}O_2$ and $C_8H_{14}O_2$, respectively, from the steroidal ring system. The $[M-H]^-$ ion from estrone at m/z 269 gives major product ions at m/z 145 and 143 (loss of $C_8H_{12}O$ and $C_8H_{14}O$) and the $[M-H]^-$ ion from EE gives major fragment ions at m/z 159 and 145, from what are believed to be losses of $C_9H_{12}O$ and $C_{10}H_{14}O$, respectively [11,16,56].

In addition to the more frequently investigated free estrogens, Gentili et al. [16] have studied the presence of conjugated (glucuronides and sulfates) estrogens in sewage and river waters by LC–ESI(NI)-MS–MS. Conjugated estrogens, likewise the unconjugated ones, present singly charged molecular anions as the most abundant ions. After collision induced dissociation, both glucuronides and sulfates produce fragment ions corresponding to the free estrogen at m/z $[M-176]^-$ and m/z $[M-80]^-$, respectively, and to the glucuronide (stabilized by a double bond) and sulfate anions at m/z 175 and m/z 80, respectively. Fig. 4 shows the reconstructed ion

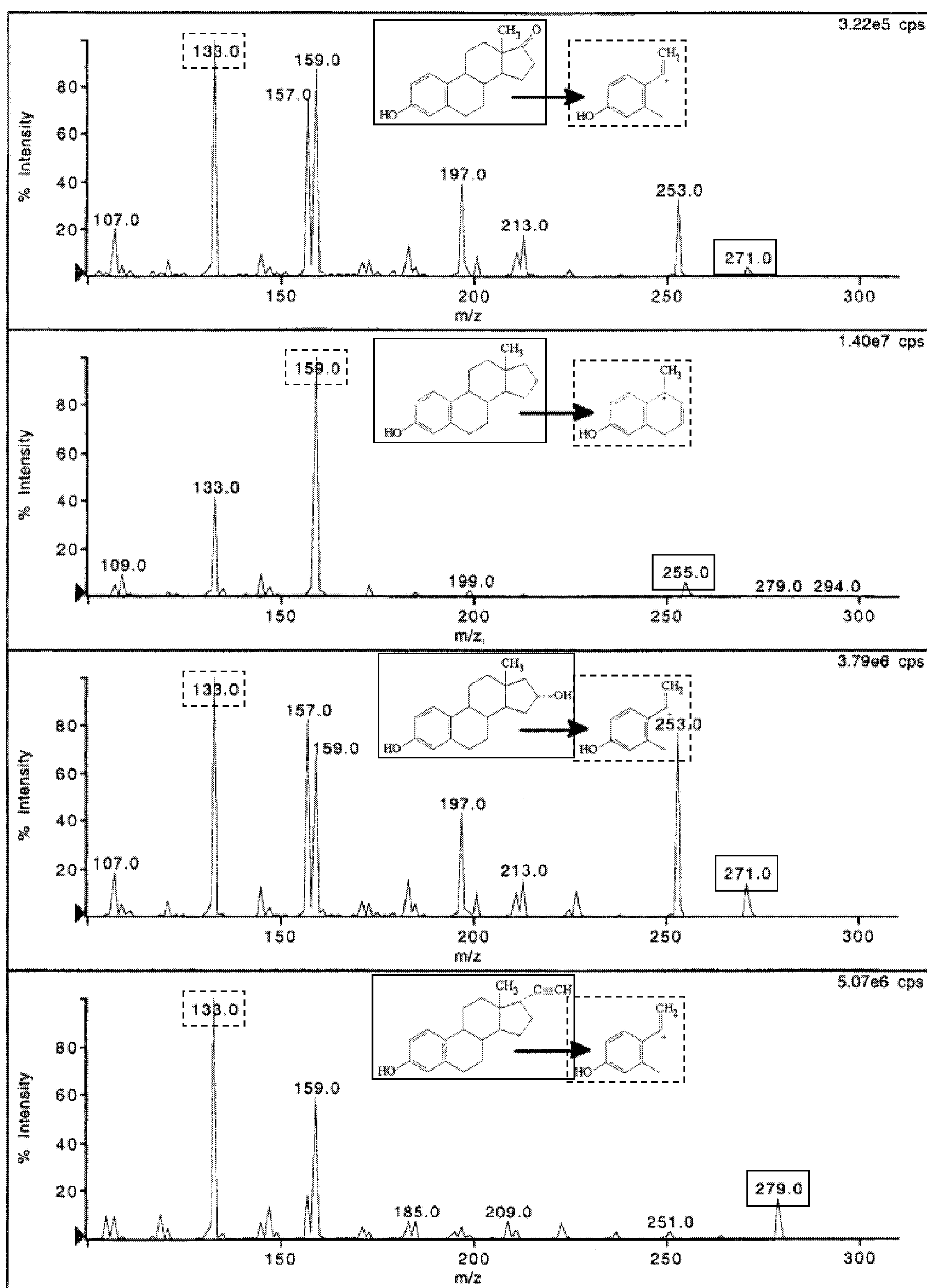

Fig. 2. Full-scan daughter ion spectra and hypothetical chemical structures of (□) precursor and (⁝⁝) product ions obtained from the flow injection analysis of (a) estrone, (b) estradiol, (c) estriol, and (d) ethynyl estradiol, by APCI(PI)-MS–MS. From Laganà et al. [12].

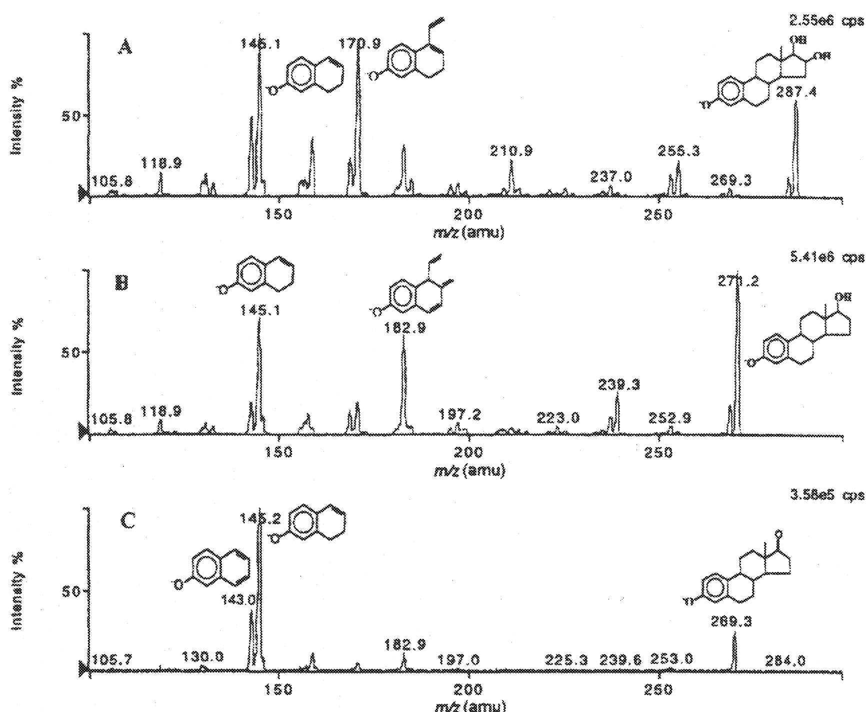

Fig. 3. Full scan product-ion spectra of (A) estriol; (B) estradiol; (C) estrone by LC–ESI(NI)-MS–MS. From Gentili et al. [16].

chromatograms obtained in the analysis of estrone-3-sulfate in a river water, and in a standard solution. As illustrated, two different SRM transitions, one corresponding to the formation of the free estrogen (349→269) and another one corresponding to the formation of a characteristic fragment of estrone (349→145), are recorded for both quantitation and confirmation, respectively.

Progestogens, likewise conjugated estrogens, have received little attention to date, and very few works have investigated their environmental occurrence [25,26,58]. According to the work published by López de Alda and Barceló [58], progestogens can be detected in the positive ion mode of operation with both APCI and ESI. The base peak used for quantitation of progestogens corresponds to the protonated analyte molecule when APCI is used as interface and to adducts of the analyte molecule with one sodium atom when the interface employed is ESI. However, the sensitivity reported for the analysis of progestogens with the ESI interface is about 10-fold better than that of the APCI interface.

The use of triple-quadrupole mass spectrometers in LC–MS–MS has substantially increased the selectivity and sensitivity of the determination, resulting in LODs far better than those achieved by use of single-quadrupole LC–MS. Croley et al. [56] compared the performance of three optimized mass spectrometric protocols —LC–ESI(NI)-MS (SIM), LC–ESI (NI)-MS–MS (MRM) and GC–MS–MS on an ion trap instrument— for the determination and quantitation of steroid sex hormones in environmental matrices. The LODs achieved with the LC–MS–MS method (5 ng/l) were comparable to those obtained with the GC–MS–MS method (2–20 ng/l) and about 40 times better than those of the LC–MS (200 ng/l).

3.2. Drugs

The detection and analysis of drugs and their metabolites within biological fluids in pharmacokinetic studies was one of the first applications of LC–MS in the 1980s and it is still one of the major areas of application. Now that drugs and their environmental fate have attracted the attention of

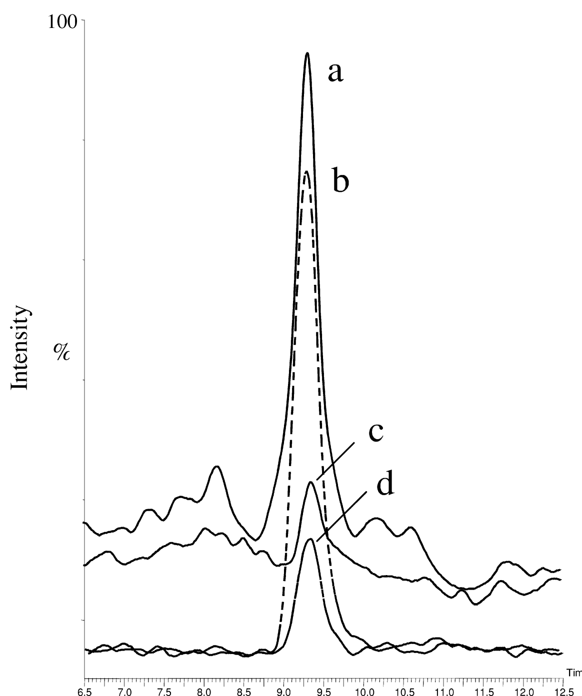

Fig. 4. Reconstructed ion chromatograms obtained from the LC–ESI(NI)-MS–MS analysis of estrone-3-sulfate in a standard mixture (1 ng/ml) of estrogens and progestogens (a and c), and in a river water containing estrone-3-sulfate at a concentration of 1.3 ng/l (concentration factor 1000) (b and d). Chromatograms (a) and (b) correspond to the SRM transition m/z 349→269, and chromatograms (c) and (d) to the SRM transition m/z 349→145.

aquatic chemists and the public, methods are being adapted or developed for detecting these compounds in water [34].

MS is becoming more popular in the field of pharmaceuticals determination as compared to UV or fluorescence detection. Radioimmunoassay has also been reported as a screening method for antibiotics detection, however, its low selectivity only allows semiquantitative results. Capillary electrophoresis is another analytical technique available that has scarcely been applied by environmental researchers [59,60].

For LC–MS and LC–MS–MS analysis of pharmaceuticals, ESI has been the ionisation technique of choice. Ahrer et al. [59] compared the performance of APCI and ESI in the analysis of diverse drugs (paracetamol, clofibric acid, penicillin V, carbamazepine, etc.) and found ESI to provide the best LODs (0.05–1.1 μg/l). The same interfaces, in both the positive and the negative ionization modes, were evaluated by Lindsey et al. [36] for the analysis of eleven sulfonamide and TCs antimicrobials. Under optimised conditions both ESI and APCI in the positive ion mode worked well. However, ESI was finally selected, because it provided the best sensitivity towards chlortetracycline.

MS and MS–MS determinations have been carried out in SIM and SRM mode, respectively, for improved sensitivity as compared to scan modes. Mostly, the precursor ion corresponds to the proton adduct of the molecular ion, $[M+H]^+$, of the respective analyte as is presented in Table 6 for some of the currently monitored pharmaceuticals. Using tandem MS it is possible to distinguish individual compounds having the same molecular mass by the different fragments obtained after the induced collision with an inert gas (argon). So, whenever possible, it is preferable MS–MS detection for a better selectivity and sensibility, in particular in complex matrices.

LC of target drugs in extracts obtained from environmental waters has been carried out with different columns, mostly C_{18}. As mobile phases, mixtures of water–methanol and acetonitrile at different pH, have normally been used. Modification of the mobile phase is usually performed in attempt to improve the sensitivity of MS detection, and has been accomplished with acetate [35,59,61,62], formate [3,61,62], or formic acid [63] (see Table 3).

Limits of detection are in the low ng/l range for all the pharmaceuticals under investigation, underlining the good performance data of the developed methods here reported.

Very recently, Kolpin et al. from the US Geological Survey has conducted the first nationwide assessment of the occurrence of organic wastewater contaminants, including pharmaceuticals and hormones, in streams susceptible to contamination, i.e. downstream of intense population and livestock production, across the US, during 1999 and 2000 [32]. Five methods were selected to accomplish this study, three of them being based on LC–MS and the other two on GC–MS. All the investigated pharmaceutical and related compounds, with the exception of steroids and hormones, were determined using LC–ESI(PI)-MS. A brief overview of the five methods

Table 6
The *m/z* values and base peaks of precursor and product ions used in the LC–ESI(PI)-MS–MS analysis of several β-blockers, β_2-simpathomimetics, antibiotics and other neutral pharmaceuticals

Compound	Precursor ion	Product ion 1	Product ion 2	Product ion 3
Salbutamol[a]	240 $[M+H]^+$	222 $[M-H_2O+H]^+$	148 $[166-H_2O]^+$	166 $[M-H_2O-tert.butyl^+ +2H]^+$
Terbutalin[a]	226 $[M+H]^+$	152 $[M-H_2O-tert.butyl^+ +2H]^+$	125	107 [methylenphenol]$^+$
Fenoterol[a]	304 $[M+H]^+$	135 [propylphenol]$^+$	107 [methylenphenol]$^+$	286 $[M-H_2O+H]^+$
Timolol[a]	317 $[M+H]^+$	261 $[M-tert.butyl^+ +2H]^+$	244 $[M-tert.butylamine+H]^+$	188 (cleavage of side chain)
Clenbuterol[a]	277 $[M-H]^+$	203 $[M-H_2O-tert.butyl^+ +2H]^+$	259 $[M-H_2O+H]^+$	168 $[203-Cl]^+$
Celiprolol[a]	380 $[M+H]^+$	251 (cleavage of side chain)	307 $[M-tert.butylamine+H]^+$	324 $[M-tert.butyl^+ +2H]^+$
Clarithromycin[b]	750 $[M+H]^+$	116 $[cladinose-OCH_3+H]^+$	592 $[M-desosamine+H]^+$	158 $[desosamine+H]^+$
Roxitromycin[b]	838 $[M+H]^+$	158 $[desosamine+H]^+$	680 $[M-desosamine+H]^+$	116 $[cladinose-OCH_3+H]^+$
Sulfamethazine[b]	279 $[M+H]^+$	124 $[aminodimethylpyridine+H]^+$	186 [aminophenyl]$^+$	–
Trimethoprim[b]	293 $[M+H]^+$	123 $[M-trimetoxyphenyl]^+$	231 $[M-2CH_3O+H]^+$	–
Chloramphenicol[b]	323 $[M-H]^+$	152 [nitrobenzylalcohol carbanion]$^-$	176 $[194-H_2O]^-$	194 $[M-dichloroacetamide-H]^-$
Chlortetracycline[b]	479 $[M+H]^+$	444 $[M-H_2O-NH_3+H]^+$	462 $[M-NH_3+H]^+$	461 $[M-H_2O\ +H]^+$
Doxycycline[b]	445 $[M+H]^+$	428 $[M-NH_3+H]^+$	410 $[M-H_2O-NH_3+H]^+$	–
Oxytetracycline[b]	461 $[M+H]^+$	426 $[M-H_2O-NH_3+H]^+$	443 $[M-H_2O+H]^+$	201
Tetracycline[b]	445 $[M+H]^+$	410 $[M-H_2O-NH_3+H]^+$	427 $[M-H_2O+H]^+$	154
Cloxacillin[b]	453 $[M+NH_4]^+$	160 [cleavage in β-lactam+H]$^+$	277 [cleavage in β-lactam+H]$^+$	178
Dicloxacillin[b]	487 $[M+NH_4]^+$	160 [cleavage in β-lactam+H]$^+$	311 [cleavage in β-lactam+H]$^+$	212
Methicillin[b]	381 $[M+H]^+$	165 [dimethoxybenzaldehyd]$^+$	222 [cleavage in β-lactam+H]$^+$	150 [165-methyl]$^+$
Nafcillin[b]	432 $[M+NH_4]^+$	171 [ethoxynaphthyl]$^+$	199 [ethoxynaphthylcarbonyl]$^+$	181
Oxacillin[b]	419 $[M+NH_4]^+$	144 [phenylisoxazolyl+H]$^+$	243 [M−methylphenylisoxazolyl]$^+$	172
Caffeine[c]	195 $[M+H]^+$	138 $[M-CH_3-N-CO+H]^+$	110 $[M-CO-N(CH_3)-CO+H]^+$	–
Omeprazole[c]	346 $[M+H]^+$	136 $[M-COH_3-(C_7N_2H_4)-SO-CH_2]^+$	198 $[M-COH_3-C_7N_2H_4]^+$	–
Erythromycin[b]	716$[M-H_2O+H]^+$	522 $[M-desosamine-2H_2O+H]^+$	558 $[M-desosamine-H_2O+H]^+$	158 $[desosamine+H]^+$

[a] Data from Ref. [97].
[b] Data from Ref. [31].
[c] Data from Ref. [35].

used, as well as a comparison between them is included. A similar study was conducted in Baden-Württemberg, Germany, during the year 2000 [61]. In this study, Sacher et al. monitored a large number of pharmaceuticals and endocrine disrupting chemicals in ground waters across the city in order to set up a data base on the occurrence of the aforementioned compounds. From the total of the 60 target drugs, 45 of them were determined following quite similar LC–ESI(PI)-MS–MS methods. As well, Ternes et al. [35,64] indicates LC–ESI-MS–MS as the technique of choice to assay polar, unstable and high-molecular-mass compounds such as most pharmaceuticals and their metabolites, however points out the difficulty in the enrichment step for highly polar compounds, as well as the low resolution and the suppression of signals in the electrospray interface due to matrix impurities.

The occurrence of 18 antibiotics corresponding to the classes of macrolides, sulfonamides (SAs), penicillins (PENs) and tetracyclines in several distinct water samples, were quantified by LC–ESI-MS–MS [30,31]. However, in these works, the samples preconcentration were conducted by means of lyophilisation after the filtration and the addition of Na_2EDTA to the sample in order to avoid the complexation of the analytes (especially tetracyclines) with the metals present in the water samples.

With the aim of improving LODs, Ahrer et al. [59] and Ahrer and Buchberger [60] have developed diverse methods based on the combination of LC or capillary electrophoresis (CE) with MS. For LC–MS two types of interfaces, ESI and APCI, were employed in the analysis of several river waters. Sample pretreatment was performed by SPE for LC–MS and with a combination of LLE and SPE for CE–MS. Because of rather high standard deviations of the recoveries due to the three extractions steps needed in CE–MS (two LLE prior to one SPE), a quantita-

tion method based on standard addition is recommended. Fragmentation patterns can be quite different in APCI compared to ESI, as was found for naproxen. Generally, ESI interface was more efficient for the drugs investigated in this study compared to APCI, resulting in a higher sensitivity. LODs for CE–MS (between 4.9 and 19 ng/l) were, as expected, poorer than those obtained by LC–MS (0.05–1 ng/l) [59]. Farré et al. [65] have applied a new LC–ESI-MS method in the monitoring of some acidic and very polar analgesics in surface waters and wastewater. Results were compared with those obtained in parallel by means of a previously established high resolution GC–MS method, obtaining a good agreement.

In the analysis of drugs residues in environmental solid matrices MS detection has not been as widespread used as UV or fluorescence, and, when applied, ESI has been the interface chosen. The latest and most advanced work on drug analysis on soils, has been conducted by Hamscher et al. [62], which reports the development of a new method to determine persistent TCs residues in soil fertilised with manure by LC–MS–MS and confirmation by MS–MS–MS. In Fig. 5 the LC chromatograms and the tandem mass spectra of an amended soil sample containing the antibiotics tetracycline and chlortetracycline are represented.

With regards to the accuracy of the methods described, relatively low recoveries were occasionally reported for some drugs. These low recoveries could be explained, apart from the aforementioned losses by adsorption onto glass surfaces, in terms of the formation of complexes between the drug and some matrix components, such as divalent cations (Ca^{2+} or Mg^{2+}),or bonding to suspended natural organic matter, such as humic acids [30]. In addition, the interaction of these compounds with residual silanol groups and metal ions in the LC column, often results in notorious peak tailing and variable analyte recoveries [63].

3.3. Alkylphenolic compounds

The inherent low volatility of the alkylphenolic surfactants hampers the application of GC for their analysis. Separation without derivatization is limited to the lower molecular mass APEO oligomers and to some degradation products (e.g. alkylphenols). On the other hand, LC allows determination of a whole range of oligomers and is nowadays a routinely used method.

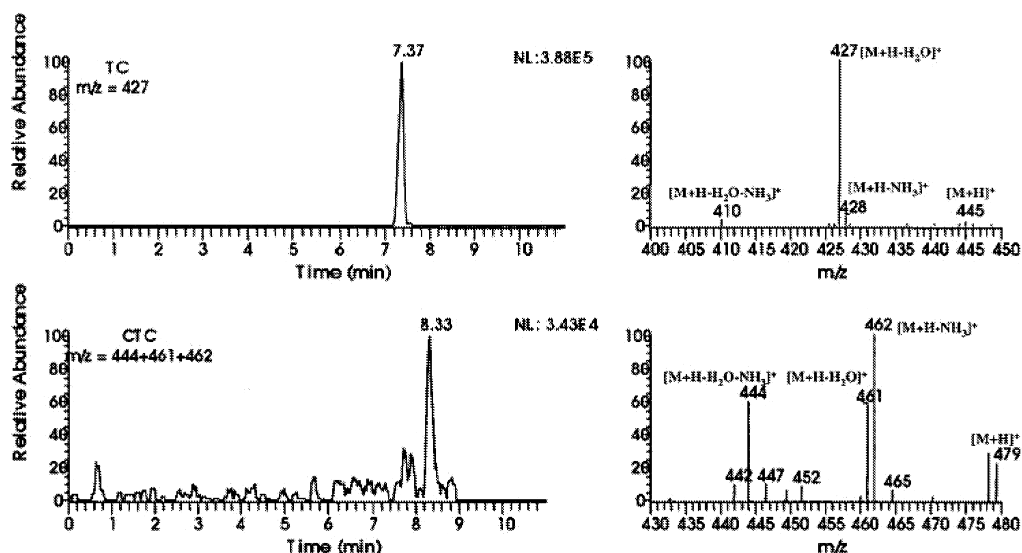

Fig. 5. Ion chromatograms and the corresponding tandem mass spectra of tetracycline and chlortetracycline obtained for a sample of an agricultural soil amended with manure. From Hamscher et al. [62].

The LC separation of APEOs and their metabolites may be carried out either by normal-phase (NP)-LC or by reversed-phase (RP)-LC (see Table 4). In normal-phase systems, the APEOs are separated according to the increasing number of ethylene oxide units, while corresponding homologues with the same number of ethoxy units but different alkyl substituents co-elute—i.e. octylphenol ethoxylates (OPEO) and nonlyphenol ethoxylates (NPEO). RP-LC that allows separation according to the character of the hydrophobic moiety is particularly well suited to separate alkyl-homologues, while the various oligomers containing the same hydrophobic moiety elute in one peak. Eluting all the oligomers into one peak has the advantages of increasing the peak intensity and therefore, increasing the sensitivity of determination. However, in RP-LC–ESI-MS, the interference of isobaric doubly charged ions $[M+2Na]^{2+}$ of highly ethoxylated APEOs, that interfere with singly charged ions of less ethoxylated APEOs (e.g. odd numbered pairs $NPE_{15}O$–NPE_5O), is reported to cause an error up to 40% in the quanitification of less ethoxylated NPEOs [66]. Intermediate resolution can be obtained by using C1-RP columns which also provide separation according to the number of ethoxy groups. Recently, Ferguson et al. [67] reported on the application of a mixed-mode LC separation, which operates with both size-exclusion and reversed-phase mechanisms, for the comprehensive analysis of NPEOs and NP in sediment and sewage samples.

With regards to detection, nonionic surfactants APEOs were early analysed by LC–thermospray-MS. However, nowadays, ESI and APCI are the ionisation methods of choice.

The trace analysis of APEOs and their metabolites by LC–MS or LC–MS–MS using API has been recently reviewed by Petrovic et al. [68] and the performances of two ionisation methods, APCI and ESI, in terms of selectivity and sensitivity toward oligomeric mixtures of APEOs has been discussed. ESI-MS methods, combined with NP-LC [66,69,70] or RP-LC [17,24,46,71,72] have been frequently used for the quantitative analysis of ethoxylates in environmental and wastewater samples. Several authors have also reported the identification and determination of APEOs in industrial blends, wastewaters and environmental samples by APCI-MS using FIA [49,50] or preceded by LC, using both RP [47,48,73,74] and NP [75,76] separation.

Generally, ESI interface is more often used for the analysis of alkylphenolic compounds due to the higher sensitivity, especially for alkylphenols [77]. Using an ESI interface APEOs show a great affinity for alkali metal ions, yielding almost exclusively evenly spaced sodium adducts $[M+Na]^+$, due to the ubiquity of sodium in the solvents and surfaces, while the formation of protonated molecules is not a dominant process. However, formation of distinct adducts with ions originating from the buffer, the sample and/or the introduction system (e.g. H^+, Na^+, K^+), water clusters (especially when using APCI interface), dimeric complexes and doubly charged ions, such as disodium adducts [17,46,68] are also reported. The use of mobile phase additives (e.g. ammonium acetate or formate, formic, acetic or trifluoroacetic acid, ammonium hydroxide) assures the reproducible adduct formation. For example, when an ammonium buffer was used, sodium adducts and protonated molecules are suppressed, and the spectrum become less complicated with primarily $[M+NH_4]^+$ adduct ion visible.

Halogenated APEOs, formed during chlorination at wastewater and drinking water treatment plants, were analysed by LC–ESI-MS [71]. Like their nonhalogenated precursors they show a great affinity for alkali metal ions yielding exclusively evenly-spaced (Δ44) sodium adduct peaks $[M+Na]^+$ with no further structurally significant fragmentation. For this group of compounds the characteristic doublet signal of brominated and chlorinated compounds, respectively, due to different contribution of their isotopes (^{79}Br:$^{81}Br=100{:}98$ and ^{35}Cl:$^{37}Cl=100{:}33$, respectively) allows identification of isobaric oligomers (e.g. $ClAP_nEO$ and $BrAP_{n-1}EO$).

LC–MS–MS has been seldom used in the analysis of APEOs. The precursor ion scanning of m/z 121 and 133 and multiple reaction monitoring (MRM) applying FIA-APCI(PI)-MS–MS were used for a rapid screening of NPEOs in wastewaters and industrial blends [49,50]. The precursor scan of m/z 121 is characteristic for ethoxylates with 1–4 chain units, while m/z 133 is characteristic for NPE_5O–$NPE_{16}O$ [78,79]. APCI-MS–MS showed ethoxy

chain fragments at *m*/*z* 89, 133, 177 and the diagnostic fragment of OPEOs at *m*/*z* 277 and at *m*/*z* 291 for NPEOs [80,81].

Persistent acidic metabolic products of APEOs, alkylphenoxy carboxylates (AP_nEC) and dicarboxylates (CAPEC) were detected, in both, the NI mode [68,71,82,83] and the PI mode [84]. In the NI mode, using ESI, APECs give two types of ions, one corresponding to the deprotonated molecule $[M-H]^-$ and the other to $[M-CH_2COOH]^-$ in the case of APE_1Cs and $[M-CH_2CH_2OCH_2COOH]^-$ for the APE_2Cs. The relative abundance of these two ions depends on the extraction voltage. Neutral losses of the carboxylated ethoxy chain and carboxylated alkyl chain, respectively, and methanol loss followed by formation of acylium ions, were found to be typical fragmentation patterns for methylated CAPECs detected by ESI in the PI mode [84].

MS–MS spectra of APECs [82,85,86] shows intense signal at *m*/*z* 219 (for NPEC) and *m*/*z* 205 (for OPEC) that is produced after the loss of the carboxylated (ethoxy) chain, and sequential fragmentation of the alkyl chain resulting in ions *m*/*z* 133 and 147.

Using an ESI in the negative ion mode, alkylphenols (OP and NP) give exclusively deprotonated molecules (*m*/*z* 205 for OP and *m*/*z* 219 for NP), whereas using an APCI, at higher voltages, using so-called in-source CID, the spectra show fragmentation that closely resembles that obtained by the MS–MS technique [87]. Alkylphenols give, in addition to the $[M–H]^-$ ion, fragment *m*/*z* 133, resulting from the loss of a C_5H_{12} (OP) and C_6H_{14} (NP) group. However, the sensitivity of detection, using an APCI source was reported to be approximately 40–50 times lower than that obtained with an ESI source [77]. An example of LC–MS–MS analysis of alkylphenolic compounds in sewage sludge is shown in Fig. 6.

Product ion scan of deprotonated molecules of brominated NPECs and NP [85] yields intense signals at *m*/*z* 79 and *m*/*z* 81 corresponding to the $[Br]^-$ (ratio of isotopes 1.02), while the fragmentation of the side chain was suppressed and resulted

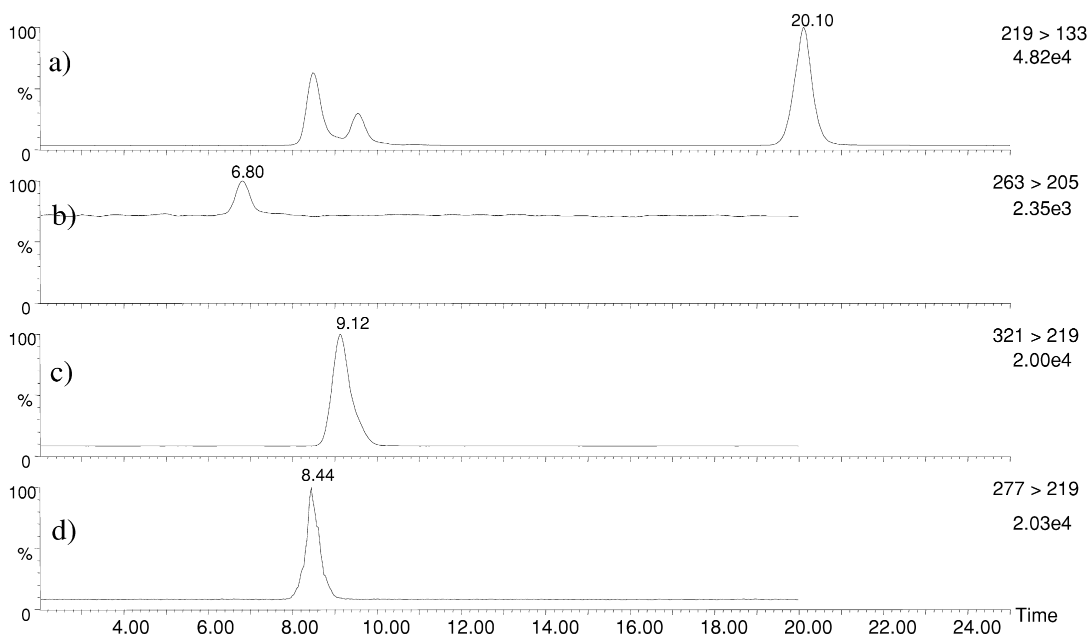

Fig. 6. LC–MS–MS chromatogram of a sewage sludge sample. Traces: (a) MRM channel *m*/*z* 219→133-NP ($t_R = 20.10$); b) MRM channel *m*/*z* 263→205-OPE_1C ($t_R = 6.80$ min); (c) MRM channel *m*/*z* 321→219 NPE_2C ($t_R = 9.12$); (d) MRM channel *m*/*z* 277→219−NPE_1C ($t_R = 8.44$ min).

just in a low-intensity fragment at *m/z* 211/213 produced after the loss of C_6H_{14}. For chlorinated NPs and NPECs the predominant fragmentation occurred primarily on the alkyl moiety leading to a sequential loss of *m/z* 14 (CH_2 group), with the most abundant fragments at *m/z* 167 for ^{35}Cl and *m/z* 169 for ^{37}Cl with the relative ratio of intensities of 3.03. Fragment corresponding to $[Cl]^-$ was produced only when sufficient collision energy was applied. Based on these fragmentation pathways a reliable and sensitive quantification method with LODs down to 1–5 ng/l was developed and applied to study occurrence of halogenated alkylphenolic compounds derived from chlorination treatment in a drinking water treatment plant [85].

4. Conclusions and future perspectives

The application of advanced LC–MS technologies to environmental analysis has allowed the determination of a great number of compounds, especially polar compounds, that were previously difficult or even impossible to analyze. In particular, the introduction of API interfaces and triple quadrupole analyzers has greatly improved the sensitivity and selectivity of detection and today, the analysis of steroids, many pharmaceuticals, and alkylphenolic surfactants in the environment is possible at the ng/l and ng/g level, and even at the pg/l and pg/g level, in the routine bases.

However, to date the most important value and application of current LC–MS techniques in the environmental field is the determination of known, target compounds, since the capacity of these techniques for screening and identification of unknowns is relatively low. Thus, 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 some few groups of compounds, such APEOs.

The recent introduction of oa-TOF-MS and Q-TOF instruments, which yield accurate mass determination at sensitivities comparable to those of a triple quadrupole instrument operating in the MRM mode, is expected to change this picture. However, the currently high price of these instruments has precluded a more extended use for noncommercial purposes, and so, their application in the environmental field is expected only in the long term.

Other technology that holds promise for future environmental research, although at present exhibits poor sensitivity, is on-line LC–nuclear magnetic resonance (NMR)-MS [88–90]. With the fabrication of nanoliter-volume NMR probes to be coupled to microseparation strategies, the measurement of analytes in complex matrices is becoming viable. The complementary structural information that NMR provides can be used to solve the structures of unknown pollutants and to uncover many important environmental processes. However, before its application to trace-level analysis, as encountered in environmental studies, can be seriously considered, the highly unequal sensitivity of NMR and MS will have to be addressed.

On the other hand, the combined sensitivity and selectivity of modern MS instrumentation has caused analytical chemists to reconsider the early steps in their procedures. Because of the improved sensitivity and selectivity of the detection systems, sample preparation is becoming easier, and probe of it is the current trend towards a more extensive application of automated on-line methodologies with simple sample pretreatment and high sample throughput.

However, despite the high selectivity of LC–MS-based methodologies, and in particular of LC–MS–MS, false negative findings can still occur due to the often high complexity of environmental matrices. Therefore, the application of stringent confirmation and identification criteria [91], in terms of retention time, base peak and diagnostic ions, relative abundances, etc., is essential.

In the area of sample pretreatment, important progress has been made also with regards to the preparation of selective supports, especially immunosorbents, for the SPE and purification of environmental samples.

The application of the above mentioned advanced sample preparation and detection techniques will no doubt expand our knowledge about the presence, fate, and persistence of known and newly identified environmental pollutants and their degradation products, the efficiency of their removal in sewage treatment plants and waterworks, and the degree of human and wildlife exposition, all of which will help

environmentalists (i) assess potential risks, (ii) define priority pollutants, (iii) propose quality criteria, and (iv) suggest remediation actions.

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