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The Current State-of-the-Art in the Determination of Pharmaceutical Residues in Environmental Matrices Using Hyphenated Techniques

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Several thousand tons of pharmaceuticals and their transformation products (metabolites and degradation products) are introduced into the environment each year. They affect both human health and the environment, therefore, analytical procedures enabling the determination of a wide range of pharmaceuticals at trace levels with minimal effort, time, and energy are required. Nowadays, hyphenated techniques are commonly applied in pharmaceutical analysis. This article provides a brief overview of the state-of-the-art and future trends in the determination of pharmaceutical residues and their transformation products with a focus on the most popular techniques in the field, such as ultra or high-performance liquid chromatography (UHPLC or HPLC) coupled with tandem mass spectrometry (MS/MS), quadrupole time-of-flight (QTOF), and a hybrid quadrupole/linear ion trap (QqLIT) in different environmental matrices (water, soil/sediment). Attention is paid to different aspects, benefits, and limitations of the application of UPLC and mass spectrometry–based techniques.

Keywords Environmental analysis, mass spectrometry, pharmaceuticals, UHPLC

INTRODUCTION

Pharmaceutical residues, so-called "emerging" or "new" unregulated contaminants, have raised a great deal of interest in recent years due to their potential to cause negative effects in the environment and later in living organisms. There is a high possibility that these compounds may be the reason for the increase in bacterial resistance to antibiotics (Díaz-Cruz and Barceló, 2006; Kim and Carlson, 2005) or disruption of the endocrine system (Vanderford et al., 2003) causing infertility (Liu et al., 2008) and accelerated maturation in females (Coetsier et al., 2006). Furthermore, some pharmaceuticals (especially antidepressants and antibiotics) may be subjected to bioaccumulation processes in aquatic organisms (mostly fish) (Chafer-Perica et al., 2010; Jo et al., 2011). Consumption of pharmaceuticals continuously increases and, furthermore, the introduction rate of such compounds to the environment may exceed the degradation rate, thus in such a case they would be detected worldwide. The exact risks associated with decades of persistent exposure to random combinations of low levels of pharmaceuticals is not yet well recognized (Gracia-Lor et al., 2011). Pharmaceuticals are mainly excreted in unchanged form; however, certain compounds may be at least partially metabolized or degraded in natural conditions to more harmful chemicals (Gros et al., 2006b; Hanyšová et al., 2005) (e.g., transformation of paracetamol; see Figure 1), thus both parent compounds and transformation products should be of concern during analytical research.

The environment is exposed to pharmaceuticals derived from the veterinary field, medicine, and agriculture and pharmaceuticals excreted from animals and humans (Fatta-Kassinos et al., 2011). Moreover, additional chemicals are delivered into the environment during the disposal of expired medications (Al-Odainia et al., 2010). Many pharmaceuticals cannot be totally removed during the treatment of drinking water (Zwiener, 2007) or during sewage treatment, thus they can be found in the effluents (Kosjek et al., 2007) and later in the environment. In addition, livestock excrement used as fertilizer is sprayed on farm fields, introducing pharmaceutical residues to food and water and later to humans and animals (Jelić et al., 2009; Kasprzyk-Hordern et al., 2007).

The first review on pharmaceutical residues, their environmental fate, and analysis was published more than 10 years ago (Halling-Sørensen et al., 1998). Since then, a number of procedures have been developed for the determination of

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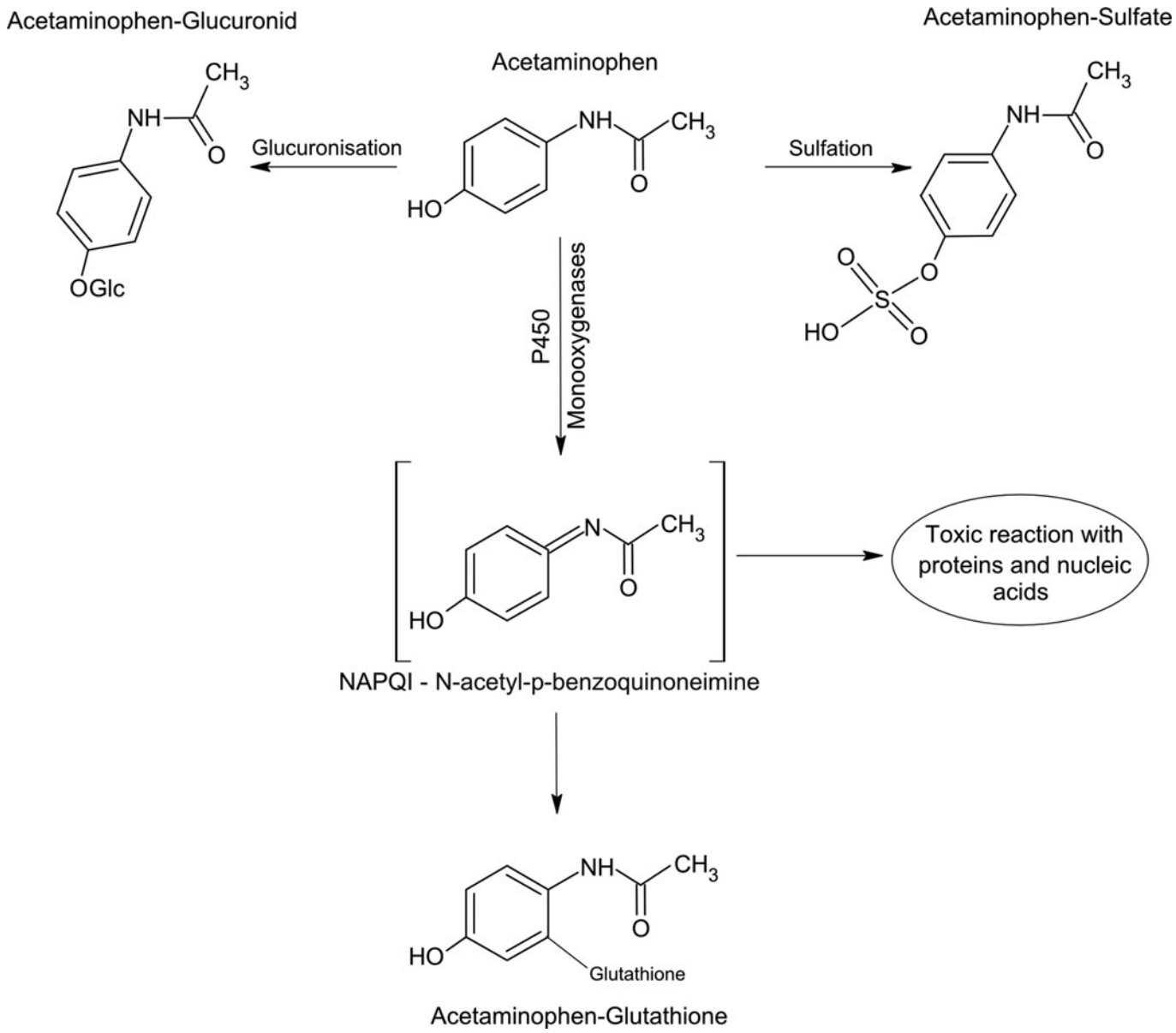

FIG. 1. Formation of metabolites as a result of various transformations of pharmaceuticals, with the example of acetaminophen (paracetamol) (Huber et al., 2009).

individual pharmaceuticals or a particular group of pharmaceuticals (Białk-Bielińska et al., 2009; Hernando et al., 2006; Lavén et al., 2009; Lee et al., 2007; Magnér et al., 2010; Piram et al., 2008; Seitz et al., 2006; Tölgyesi et al., 2010; Wu et al., 2010) in various matrices such as water (Farré et al., 2008; Ferrer et al., 2010) and solid samples (Silva et al., 2011; Vazquez-Roig et al., 2010). Hignite and Azarnoff (1977) were the first scientists to announce the problem of pharmaceutical residues in the environment, in 1977. As a result of this research, clofibric acid was determined in wastewater. The problem of pharmaceuticals started to be more widely recognized in the 1990s (Ternes, 1998). Nowadays, analytical procedures are often multi-residue, allowing for the simultaneous determination of pharmaceuticals from different classes (Babić et al., 2010; Bueno et al., 2007; Gómez et al., 2007; Gros et al., 2012; Pailler et al., 2009), since many different therapeutical compounds are present in the environment. This issue is a major challenge for analytical chemists due to the presence of a wide spectrum of pharmaceuticals in the environment, the necessity of identifying not only primary compounds, but also their transformation products, the diversity of matrices and different levels of pollutants, and a lack of specific standard and certified reference materials (Kot-Wasik et al., 2007). Pharmaceutically active compounds from different classes differ in polarity, solubility, pKa value and partition coefficient (log P), and in the stability in acidic or alkaline conditions (Kümmerer, 2009), as presented in Figure 2. All these

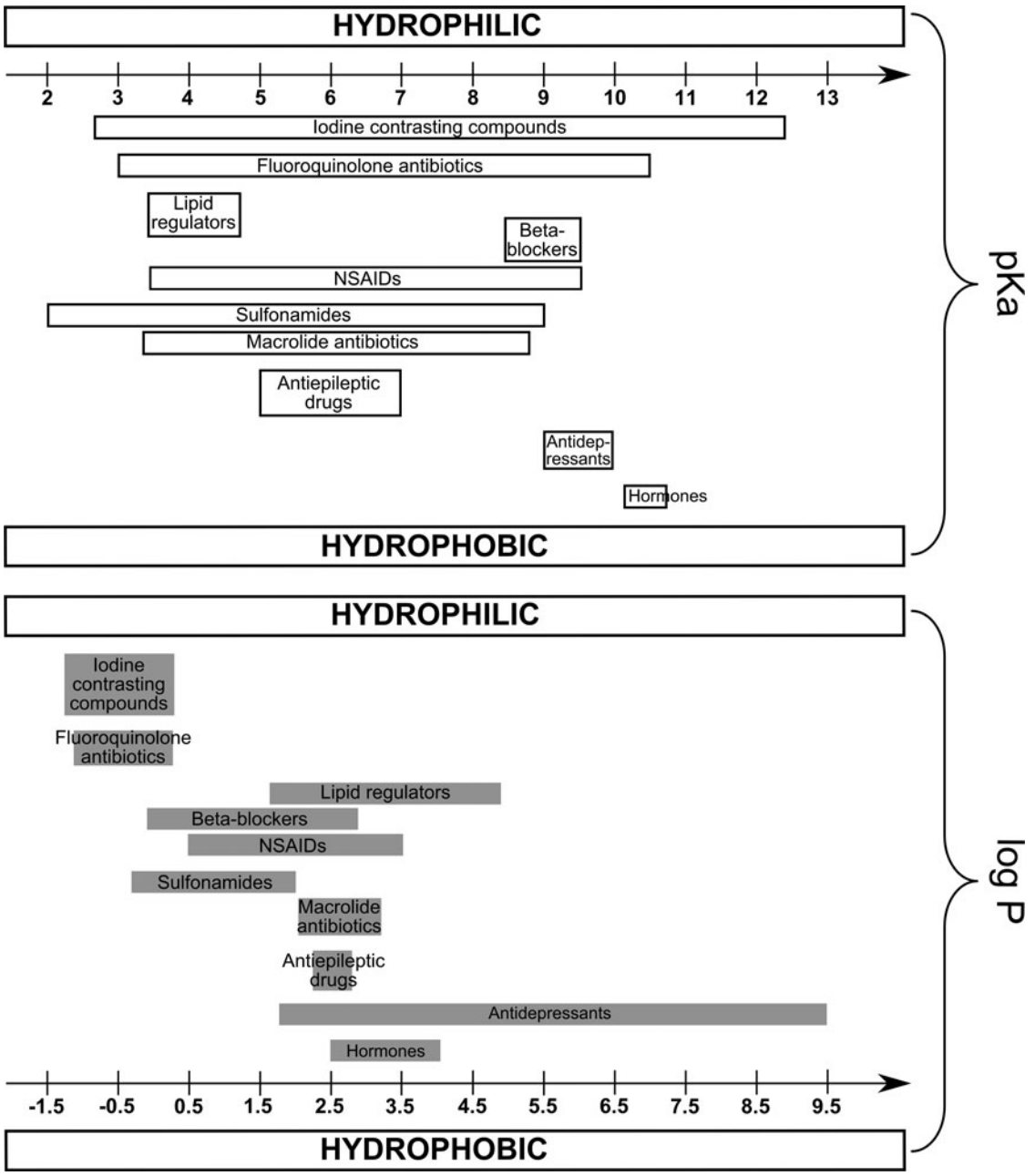

FIG. 2. Hydrophilicity/hydrophobicity, pKa, and log P values of the different classes of pharmaceuticals.

factors have a significant impact on the sample preparation step, as well as on the effects of qualitative and quantitative analysis, recoveries, and validation parameters.

Interest in the presence of pharmaceuticals and their impact on the environment has become great. Therefore, the aim of this article is a brief up-to-date review of the literature (from the past five years) concerning current practice in the determination and identification of a variety of pharmaceutical residues and their transformation products in environmental matrices using hyphenated techniques, especially liquid chromatography coupled with different types of mass spectrometers.

SAMPLE PREPARATION IN THE DETERMINATION OF PHARMACEUTICAL RESIDUES

Sample preparation for analysis is often a challenge for analysts (Fu et al., 2005). Pharmaceuticals are particularly problematic; the challenge lies mainly in the extraction and analysis of compounds that belong to various classes, thus differing in physico-chemical properties. Furthermore, environmental samples are often characterized by complex matrices and contain many interfering chemicals that may cause problems during analysis (e.g., matrix effect). Additionally, pharmaceuticals are found in the environment at very low concentration

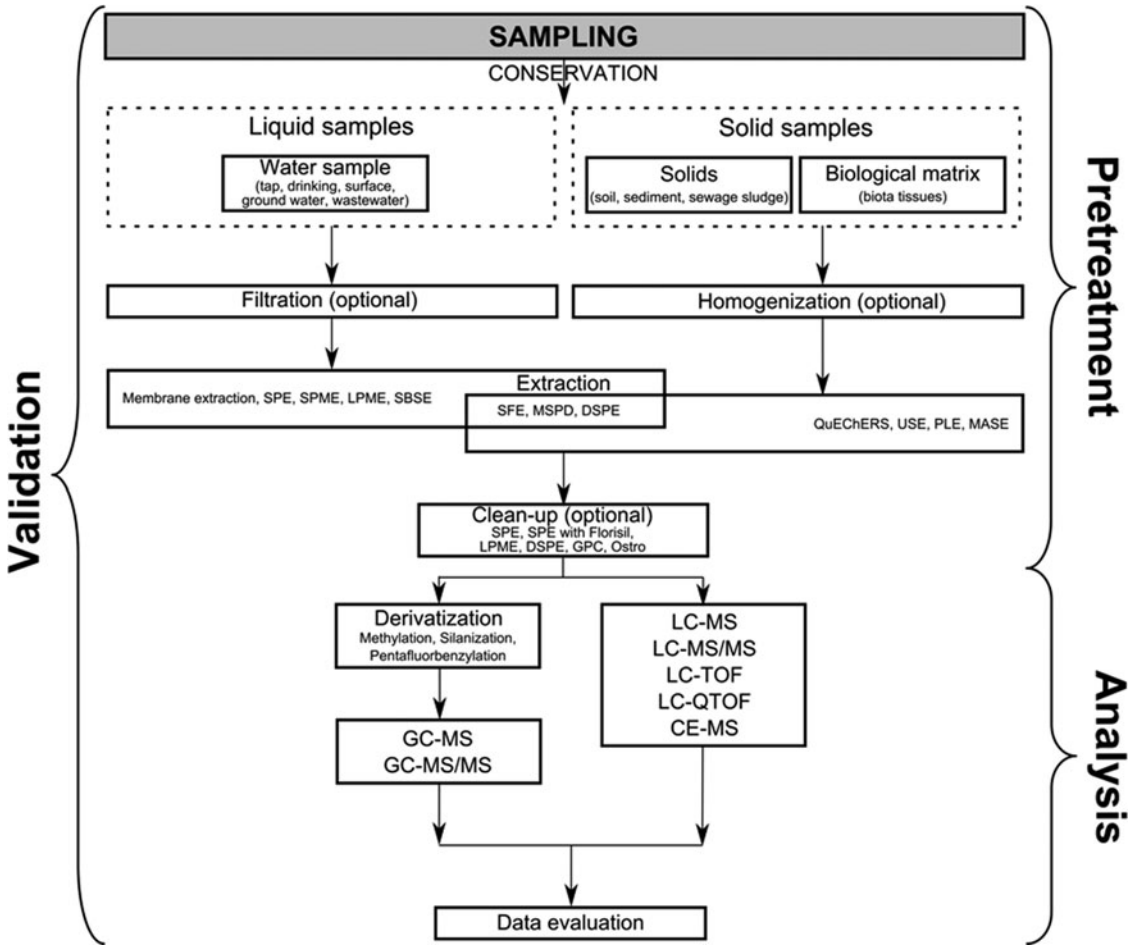

FIG. 3. General scheme of analytical procedures for determination of pharmaceuticals in samples with complex matrix.

levels (ng/L to μg/L), and therefore sample preparation, which must include concentration and purification, seems to be a crucial step in the analytical procedure. A general outline of analytical procedures (Fatta et al., 2007) for the determination of pharmaceuticals in environmental samples is presented in Figure 3.

A number of analytical procedures that use different types of extraction for sample preparation have been proposed. Pavlović et al. (2007) presented a comprehensive overview of sample preparation techniques currently used in pharmaceutical analysis, such as solid-phase extraction (SPE), solid-phase micro-extraction (SPME), stir-bar sorptive extraction (SBSE), membrane extraction, liquid-phase micro-extraction (LPME), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), matrix solid-phase dispersion (MSPD), dispersive solid-phase extraction (DSPE), ultrasonic extraction (USE), and microwave-assisted solvent extraction (MASE).

According to the number of procedures published by 2013, solid-phase extraction is the most applied in pharmaceutical analysis (Fatta et al., 2007; Pailler et al., 2009; Sun et al., 2009), commonly for the extraction of liquid matrices such as water, while PLE, MSPD, and MASE are good alternatives for solid samples (Pavlović et al., 2007). In the case of solid samples, the number of analytes determined in one analytical cycle is much lower, since there is a problem with developing an optimal sample pretreatment. These matrices are very complex due to the presence of many interferences, thus effective extraction of pharmaceuticals differing in physico-chemical properties is quite problematic. For instance, on one hand, when determining pharmaceuticals in biota or in solids, it is important to take an optimal amount of sample for analysis, on the other hand, such a sample size might be either too small, causing nondetection of target compounds, or too large, resulting in ion suppression/enhancement. Therefore, introducing a cleanup step seems to be the key for effective pharmaceutical extraction. Even in this case, the possibility of analysis of a wider spectrum of compounds is still limited. Most of the procedures include SPE (using reversed-phase sorbents) for cleanup (Baker and Kasprzyk-Hordern, 2011; Ding et al., 2011; Kaufmann et al., 2012; Radjenović et al., 2009); however, it eliminates only specific interferences, which may be only partially responsible for observed matrix effects.

Nowadays, current trends in sample preparation specifically within the context of global pharmaceutical analysis are the use of fast, simple, one-step, and efficient methods in order to provide readily prepared samples. These features are met by, among others, TOXI TUBES, a quick version of LLE (Fourel et al., 2010; Lam et al., 2008); QuEChERS, quick extraction of analytes from solid matrices (Stubbings and Bigwood, 2009); DPX, a novel dispersive solid-phase extraction (Foster et al., 2009), and MIPs (molecularly imprinted polymers) (Hoshina et al., 2011). Each of these extraction techniques is highly specific and provides excellent recoveries for most of the concerned compounds (polar and nonpolar) from various matrices (water: surface water, wastewater, drinking water; solid samples: soils, sediments).

UHPLC Analysis

There exists an ever-increasing need for sensitive, fast, and multicompound analytical procedures that can be used in laboratories conducting simultaneous monitoring of the environment for the presence of dozens of pharmaceuticals belonging to different therapeutical classes. There is a current trend of applying ultrahigh-performance (or pressure) liquid chromatography (UHPLC). UHPLC became a powerful approach mainly because of the possibility of direct application of high-performance liquid chromatography (HPLC) conditions previously established. UHPLC uses short, narrow bore columns with sub-2 μm particles, which result in ultrahigh back-pressures, and mobile phases at high linear velocities (Hsieh et al., 2007; Kalovidouris et al.; 2006, Petrović et al., 2006; Yu et al., 2006). This provides much faster elution of a large number of analytes in higher peaks with smaller width below 10 s at base (Figure 4), resulting in improved chromatographic resolution, increased peak capacity (López-Serna et al., 2011), and reduced co-elution of interferences (consequently decreasing matrix effects during ionization) (Wu et al., 2010). Shortening time of analysis even a little in comparison to conventional HPLC (Petrović et al., 2006) is a great opportunity for routine analysis where the high sample throughput is required (Gros et al., 2012). Nevertheless,

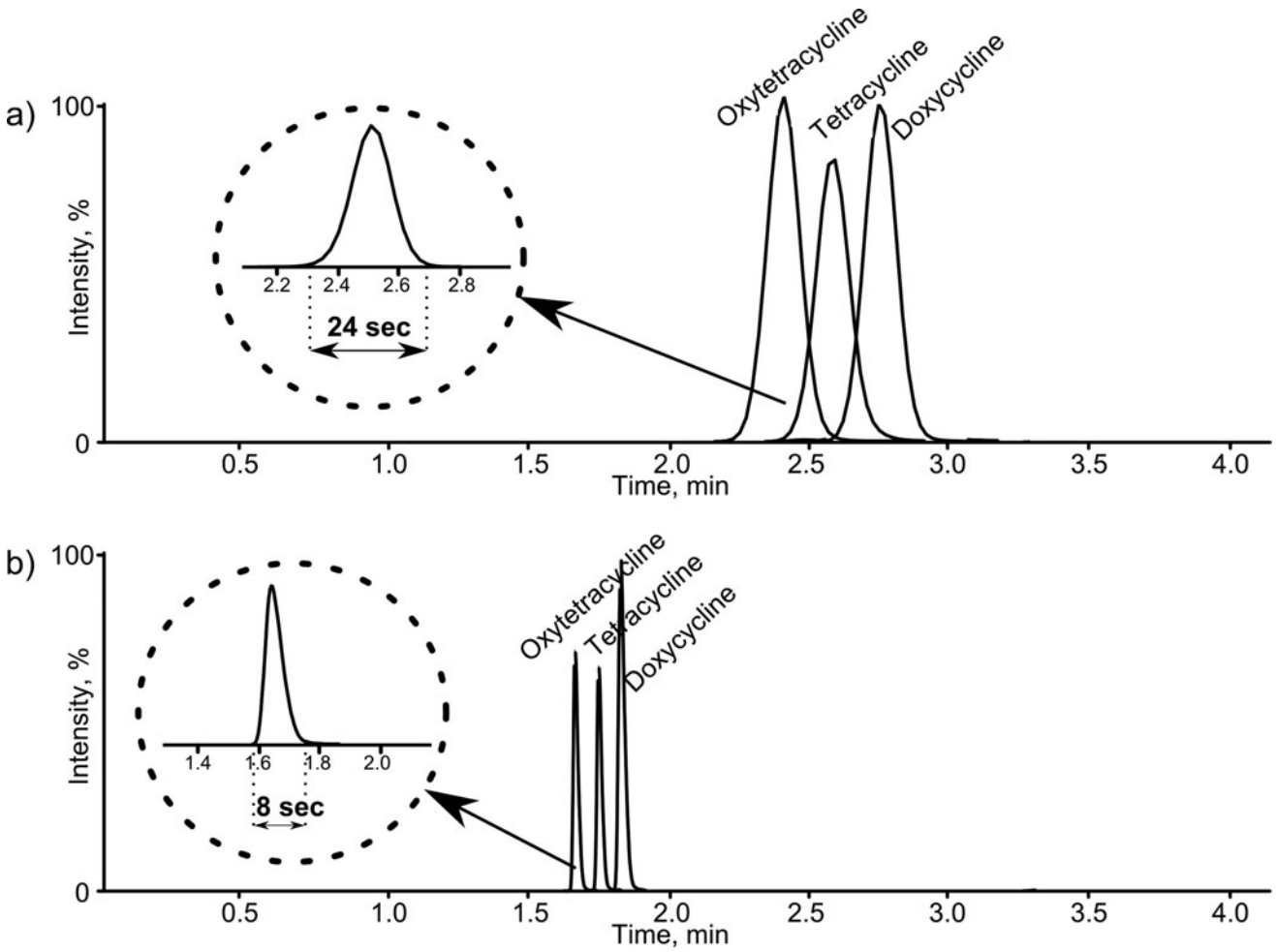

FIG. 4. Comparison of HPLC and UHPLC chromatographic separation of 3 antibiotics, a) HPLC separation - column: Agilent Eclipse C18 (4.6 $\times$ 100 mm, 3.5 μm); b) UHPLC separation - column: Acquity BEH C18 (50 $\times$ 2.1 mm, 1.7 μm).

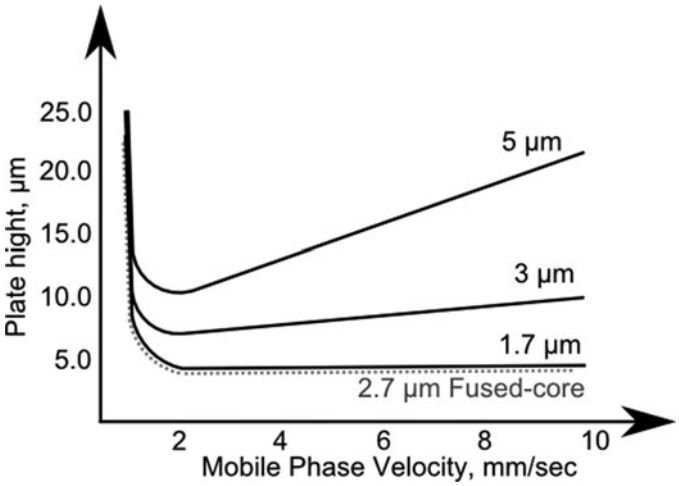

FIG. 5. Van Deemter plot for various particle sizes.

fast chromatographic separation may be provided by application of a few approaches: by increasing the flow rate of the mobile phase, by reducing the column particle diameter or its length.

Comparing HPLC columns, where particle size is 3 or 5 μm, the efficiency is inversely proportional to the mobile phase flow rate. According to the van Deemter plot (Figure 5) when the particle size decreases, the curve becomes flatter and is not greatly affected by high flow rates. Therefore, smaller particle size provides much better efficiencies in a wider flow rate range. This flat region allows combining flow rate increase with column length reduction to obtain higher separation without a significant decrease in resolution. However, pressure is inversely proportional to the square of particle size diameter (Darcy's law) and using columns with sub-2 μm particles will cause extremely high back-pressures, thus specially designed instruments are necessary. Although the UHPLC system must be resistant to high pressures, that is not the only requirement for such instruments. They must have the ability to work with columns with reduced diameter (mostly 2.1 mm) in (ultra) fast modes, thus limiting frictional heating and/or solvent compressibility. This and the other pros and cons of using UHPLC systems have been well described by Nguyen et al. (2006). Although UHPLC instrument-based procedures are widely described in the published literature, it must be emphasized that most of them use conventional columns with 3 or 5 μm particles, and thus these procedures are not in fact (ultra) fast UHPLC.

Several procedures dedicated to pharmaceutical analysis in environmental matrices were developed with the application of UHPLC, and they are summarized in Table 1. As can be seen, in the past few years application of UHPLC and columns with sub-2 μm particles has become especially popular in analysis of environmental water. All of the procedures are based on reversed-phase chromatography using predominately Acquity BEH C18 columns packed with 1.7 μm particle size. These columns are based on hybrid particle technology, which was first introduced in 2005 by Waters. BEH columns, dedicated to UHPLC, are based specifically on ethylene bridged hybrid (BEH) technology (Figure 6) and enable its application in a much wider pH range (1–12), and also provide a versatile and robust separation due to intrinsic chemical stability in comparison to typical silica-based HPLC columns. We should mention here the procedure developed by Gros et al. (2012) for determination of 81 pharmaceutical compounds and their metabolites in surface water and wastewater using an Acquity BEH C18 column (particle size 1.7 μm) and an Acquity HSS T3 column (particle sieze 1.8 μm). A fast chromatographic separation in less than 7 minutes in positive ion mode and 4 minutes in negative ion mode was achieved by working with high flow rate of mobile phase of about 0.5 and 0.6 mL/min. Quantitation and confirmation were performed by using quadrupole-linear ion trap tandem mass spectrometry in selected reaction monitoring (SRM) mode. Although two transitions were selected for this purpose, an additional tool—an information-dependent acquisition (IDA) experiment—for confirmation of positive findings was used. The great advantage of the procedure was speeding up the sample preparation step by decreasing the sample volume in the case of wastewater to 25 mL for influent and 50 mL for effluent. The procedure was sensitive (method quantitation limits (MQLs) in the range 1–50 ng/L) and reliable (a significant number of isotopically labeled internal standards).

Fast separation in UHPLC also provides narrow peaks, which are obtained by using a small detection volume and fast acquisition rate while maintaining high efficiency. In the case of complex matrices (e.g., environmental water, solids), application of mass spectrometry became a conscious choice (Wang, 2009), mostly due to high sensitivity and possibility of confirmation of the presence of target compounds. However, hyphenated techniques are described in the next section.

Although most procedures developed recently are based on ballistic gradients (Nardi and Bonelli, 2006), there are procedures based on fast isocratic LC-MS runs (Badman et al., 2010; Heinig and Bucheli, 2003), high flow rates using shorter columns with smaller particles (Murphy et al., 2002), and monolith or fused-core columns (Hsieh et al., 2007; Mallett and Ramírez-Molina, 2009). The last-named, fused-core columns, have recently become very popular and competitive for conventional UHPLC columns and instruments. Fused-core or "superficially porous" columns, first commercialized by Advanced Materials Technology in 2007, consist of fused-core silica particles surrounded by porous silica coating (Table 2) (Núnez et al., 2012). These kind of columns exhibit efficiencies comparable to those of typical UHPLC columns with sub-2 μm particles (Figure 5); however, application of such columns does not cause high back-pressures (up to 50% of back-pressure of UHPLC column) (Cunliffe and Maloney, 2007; Gritti et al., 2010), thus it is possible to use them in conventional HPLC systems with a pressure limit of 400 bars (Figure 7). This advantage is obtained due to narrow particle size distribution and high density of such particles (DeStefano et al., 2008). There are a few recent studies comparing UHPLC and fused-core columns for

TABLE 1
Application of UHPLC-based procedures for determination of pharmaceuticals in various matrices

Compounds	Matrix	Sample preparation	Analytical technique	Column	Mobile phase flow rate	LOD/LOQ	Ref.
			Water samples				
20 pharmaceuticals	Urban wastewater	SPE (Oasis HLB)	UHPLC-MS/MS	Acquity BEH C18 (50 × 2.1 mm, 1.7 μm)	0.3 mL/min	LOQ 0.8–44 ng/L	Gracia-Lor et al., 2010
76 pharmaceuticals	Slaughterhouse wastewater	SPE (Oasis HLB)	UHPLC-MS/MS	Acquity BEH C18 (100 × 2.1 mm, 1.7 μm)	0.3 mL/min	MQL 0.2–30 ng/L	Shao et al., 2009
55 pharmaceuticals	Wastewater	SPE (Oasis HLB, MCX, MAX, WCX)	UHPLC-(QqLIT) MS/MS	Acquity BEH C18 (100 × 2.1 mm, 1.7 μm)	0.8 mL/min	LOQ 0.02–50 ng/L	Huerta-Fontela et al., 2010
47 pharmaceuticals	Wastewater, surface water	SPE (Oasis HLB)	UHPLC-MS/MS	Acquity HSS T3 (100 × 2.1mm, 1.8 μm)	0.3 mL/min	LOQ < 50 ng/L	Gracia-Lor et al., 2011
32 biologically active compounds	Tap water, wastewater	SPE (Oasis HLB)	UHPLC-QTOF-MS	Acquity C18 (50 × 2.1mm, 1.7 μm)	0.2 mL/min	MDL 0.1–300 ng/L	Farré et al., 2008
74 pharmaceuticals	Surface and ground water, wastewater	SPE (Oasis HLB)	UHPLC-MS/MS	BEH C18 (100 × 2.1 mm, 1.7 μm)	0.4 mL/min	LOD 0.01–50 ng/L	López-Serna et al., 2011
54 pharmaceuticals	Surface water, wastewater	SPE (Oasis MCX)	UHPLC-MS/MS	BEH C18 (100 × 1.0 mm, 1.7 μm)	0.1 mL/min	MDL 1–51 ng/L	Batt et al., 2008
17 compounds (pharmaceuticals, personal care products)	Surface water, wastewater	SPE (Oasis HLB)	UHPLC-MS/MS	Acquity BEH C18 (50 × 2.1 mm, 1.7 μm)	0.3 mL/min		Gracia-Lor et al., 2012
81 pharmaceuticals	Surface water, treated water	SPE (Oasis HLB)	UHPLC-(QqLIT) MS/MS	Acquity HSS T3 (50 × 2.1 mm, 1.8 μm) Acquity BEH C18 (50 × 2.1 mm, 1.7 μm)	0.5 mL/min 0.6 mL/min	MQL 1–50 ng/L	Gros et al., 2012

(Continued on next page)

TABLE 1
Application of UHPLC-based procedures for determination of pharmaceuticals in various matrices *(Continued)*

Compounds	Matrix	Sample preparation	Analytical technique	Column	Mobile phase flow rate	LOD/LOQ	Ref.
25 pharmaceuticals	Surface water	SPE (Oasis MCX)	UHPLC-MS/MS	Acquity BEH C18 (150 × 1 mm, 1.7 μm)	0.05 mL/min	MQL 0.3–30 ng/L	Kasprzyk-Hordern et al., 2008
15 steroidal contraceptives	River water	SPE (ENVI-18)	UHPLC-MS/MS	Acquity BEH C18 (50 × 2.1 mm, 1.7 μm)	0.1 mL/min	MDL 0.5–3.4 ng/L	Sun et al., 2009
28 pharmaceuticals, 10 estrogens	River water	SPE	UHPLC-QTOF-MS	Acquity C18 (50 × 2.1 mm, 1.7 μm)	0.4 mL/min	LOD 0.05–2.2 ng/L	López-Roldán et al., 2010
10 pharmaceuticals	Seawater	SPE (XAD-2)	UHPLC-QTOF-MS	Acquity HSS T3 (100 × 2.1 mm, 1.8 μm)	0.6 mL/min	1–13 ng/L	Magnér et al., 2010
4 pharamceuticals	Seawater	SPE (Oasis HLB)	UHPLC—ESI low-energy CID MS/MS	Acquity BEH C18 (50 × 2.1 mm, 1.7 μm)	0.5 mL/min	0.4–1 ng/L	Wu et al., 2010
			Solid samples				
20 compounds (pharmaceuticals, steroid hormones, and endocrine-disrupting personal care products)	Sewage sludge	UE + SPE (Oasis HLB) cleanup	UHPLC-MS/MS	Zorbax Eclipse XDB C18 (50 × 2.1 mm, 1.8 μm)	0.3 mL/min	MQL 0.1–3 ng/g	Yu et al., 2011
60 drugs of abuse	Wastewater suspended particulate matter	PLE + SPE (Oasis MCX) cleanup	UHPLC-MS/MS	Acquity BEH C18 (150 × 1 mm, 1.7 μm)	0.04 mL/min	MQL 0.05–20.33 ng/g	Baker and Kasprzyk-Hordern, 2011

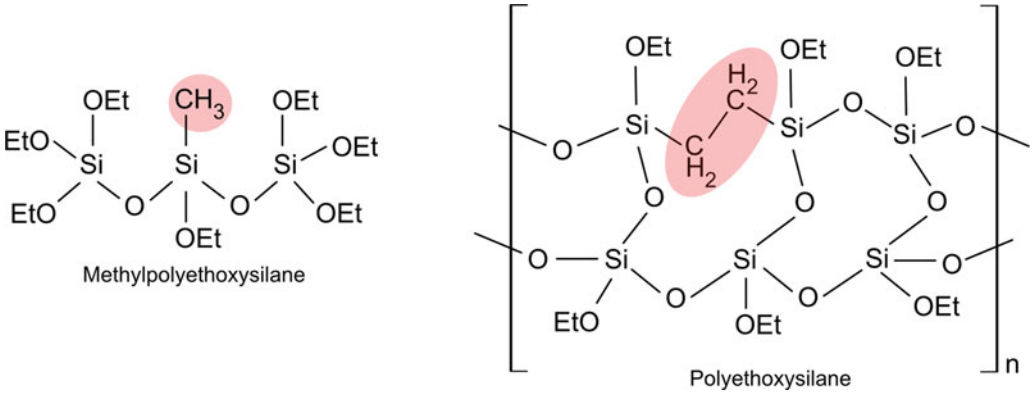

FIG. 6. UHPLC columns based on ethylene bridged hybrid (BEH) technology.

pharmaceutical analysis (Abrahim et al., 2010; Fanigliulo et al., 2010; Ruta et al., 2012). Also, a good overview of the application of fused-core columns was recently presented by Núnez et al. (2012). A very interesting method was presented by Badman et al. (2010), who developed UHPLC-MS/MS procedures using short columns packed with fused-core particles and high (1.0–3.0 mL/min) flow rates. These methods provided comparable or even better performance to slower assays for accuracy, precision, sensitivity, specificity, and ruggedness. Furthermore, run time was limited to less than 1 minute while maintaining the quality of the results. Gilart et al. (2012) proposed a methodology for determination of 15 pharmaceutical compounds in wastewater based on the fused-core column Ascentis Express C18 (100 × 4.6 mm, 2.7 μm). Despite the small particle size of 2.7 μm and high flow rate of 0.6 mL/min, the separation time was about 20 minutes. This may be due to a "large" internal column diameter of 4.6 mm and slow gradient increase (it takes 23 minutes to increase ACN content from 10% to 100%) as well. Nevertheless, the authors developed an efficient extraction procedure with the application of MIPs, which provided the selective extraction of target compounds and significantly reduced the matrix effect in comparison to the conventional SPE protocol.

TABLE 2
Fused-core columns available on market

Brand name	Producer	Fused core diameter (μm)	Layer size (μm)	Total particle diameter (μm)
HALO	Advanced Materials Technology	1.7	0.5	2.7
Kinetex	Phenomenex	1.9	0.35	2.6
Accucore	Thermo Fisher Scientific	1.9	0.35	2.6
Ascentis	Sigma-Aldrich	1.7	0.5	2.7
Poroshell	Agilent	1.7	0.5	2.7

Recently, HPLC and UHPLC have become basic procedures in multi-residue detection for pharmaceutical analysis (Babić et al., 2010; Fukutsu et al., 2006; Piram et al., 2008). As can be seen in Tables 1 and 3, the vast majority of procedures apply to the analysis of water samples (especially wastewater and surface water). Some of these methodologies allow for the determination of a large number of compounds (e.g., Gros et al. (2012), 81 compounds; Shao et al. (2009), 76 compounds; Lopez-Serna et al. (2010), 74 compounds) in a very short time of analysis. An interesting application was presented by Nuijs et al. (2010), who proposed an HILIC-MS/MS method for the determination of 13 pharmaceuticals in wastewater. The application of hydrophilic interaction liquid chromatography provided good separation, especially for metformin, a highly polar compound that does not exhibit any retention in conventional reversed-phase chromatography. The procedure provided low lower limits of quantification (LLOQs) in the range 1–20 ng/L (except for metformin, 500 ng/L) and high recoveries for most of the compounds. Although recovery for metformin was quite low (∼20%), it was found in wastewater in the highest concentration, 94 μg/L. Recently, even chiral chromatography has been applied for analysis of pharmaceuticals and drugs of abuse (Bagnall et al., 2012). Bagnall et al. (2012) compared two chiral LC-QTOF-MS methodologies using CBH (cellobiohydrolase) and Chirobiotic V columns with cellobiohydrolase and vancomycin as chiral selectors. Application of the Chirobiotic V column provided lower values of method detection limits (MDLs) (0.2–22.8 ng/L) for most of the compounds; however, the CBH column gave a much better resolution of enantiomers. Recoveries were satisfactory for both procedures (> 80%).

APPLICATION OF HYPHENATED TECHNIQUES IN PHARMACEUTICAL ANALYSIS

Nowadays, hyphenated techniques, meaning the combination of LC with mass spectrometry, are those most often applied in the determination of pharmaceuticals and their transformation products, including highly polar, volatile, and thermally labile compounds (Hao et al., 2006) present at ultra-trace levels in the

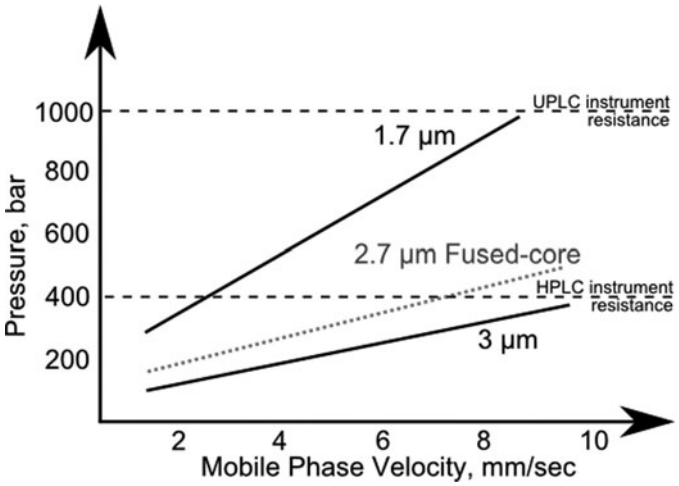

FIG. 7. Correlation between mobile phase velocity, pressure complied in the system, and particle size of stationary phase.

environment. In order to obtain sufficient amounts of data per peak while using UHPLC, only those MS instruments can be coupled that can work at low dwell times and low inter-channel and inter-scan delays. That is why tandem mass spectrometry in the SRM acquisition mode (Gracia-Lor et al., 2010; Kaufmann et al., 2012; Yu et al., 2011) is the most efficient tool for identification and quantification of trace levels of pharmaceuticals in different matrices (Hao et al., 2007), as can be seen in Table 1, due to its high sensitivity, selectivity, and robustness (Plumb et al., 2004). Other MS analyzers such as quadrupole linear ion trap MS (QqLIT-MS) (Gros et al., 2012) or quadrupole time-of-flight MS (QTOF-MS) (Farré et al., 2008; Min et al., 2009) have been used for UHPLC applications as well. In the case of MS/MS the importance of good chromatographic separation is often neglected due to the virtual separation (obtained in SRM mode), which in consequence can lead to ion suppression and isobaric interferences. This is a great problem, especially in trace analysis where matrix components may cause nondetection of target compounds (Petrović et al., 2006). Combining tandem MS with UHPLC can significantly reduce the problem of co-elution and signal suppression, which may occur between interferences and the ionization process (Wu et al., 2010), and mass spectral overlap due to obtaining much narrower peaks. On the other hand, in the case of narrow peaks, mass spectrometers must gain spectra more rapidly without losing accuracy. That is why lately scientists also combine UHPLC with quadrupole time-of-flight mass spectrometry (QTOF-MS) (Gracia-Lor et al., 2011), which can manage the problem of rapidity and meet present expectations. Additionally, superior chromatographic efficiency and separation significantly increase the potential for the identification of nontarget compounds in various samples (Terzic & Ahel, 2011).

UHPLC-MS/MS Analysis

There is growing interest in the application of UHPLC (Sun et al., 2009; Yu et al., 2011) (Table 1), since the combination of UHPLC and tandem MS significantly increases the sensitivity of the developed methods. This is due to the fact that MS/MS itself provides high sensitivity in the SRM mode, and application of UHPLC results in much narrower peaks with higher intensity, which enables lowering the limits of detection. Gracia-Lor et al. (2010) presented a UHPLC-MS/MS multi-residue method for the determination of 20 pharmaceuticals in urban wastewater. The LOQs ranged from 3.6 to 427 ng/L and 8.6 to 974 ng/L for effluent and influent wastewater, respectively. Application of UHPLC provided not only greater resolution and increased sensitivity, but also, which is highly important, the higher flow rate shortened the time of analysis. Furthermore, using a fast acquisition triple quadrupole analyzer makes feasible the selection of short dwell times (10 ms) and the simultaneous monitoring of up to three transitions per compound in multiple reaction monitoring (MRM) mode, assuring reliable identification for all target analytes. The general focus is on aqueous samples, due to the lack of a proper analytical procedure for the determination of pharmaceuticals sorbed on soil, sediments, or biosolids (Wu et al., 2008). It seems necessary to analyze such matrices to understand the distribution, fate, and behavior of pharmaceuticals in the environment (Barron et al., 2010) and in wastewater treatment plants (WWTP) (Radjenović et al., 2009). Furthermore, sewage sludge from WWTP is used as fertilizer or soil amendments, introducing extra amounts of pharmaceuticals to the environment (Jones-Lepp and Stevens, 2007). It seems very important to investigate the presence of compounds that do not biodegrade and pharmaceuticals of a hydrophobic nature that significantly influence interactions with solids (Radjenović et al., 2009). Baker and Kasprzyk-Hordern (2011) developed the first multi-residue method for the determination of important illicit drugs and pharmaceuticals in wastewater suspended particulate matter using PLE-SPE-UHPLC-MS/MS. Excellent quantification levels were provided for nearly all compounds at < 2.6 ng/g. In the case of analytes that exhibit a poor fragmentation, where it is not possible to confirm the compound using a second transition in SRM mode, different solutions for the screening and confirmation of pharmaceuticals based on UHPLC-QTOF-MS have been proposed by Petrović et al. (2006). Similarly, López-Roldán et al. (2010) used LC-MS/MS for the quantification and confirmation of contamination peaks of target analytes in surface water; however, additional analysis was done by LC-QTOF-MS in order to obtain an extra point of confirmation when needed and for obtaining reliable results. This approach was used to confirm the presence of ibuprofen and gemfibrozil in all samples tested. Despite the extra cost of performing two analyses, other scientists applied this solution as well. Furthermore, the presence of the compounds (e.g., tetracycline, oxytetracycline), whose second transition is very weak, cannot be confirmed when the analyte is at a very

TABLE 3
Multi-residue procedures for pharmaceutical analysis with application of hyphenated techniques

Compounds	Year published	Matrix	Sample preparation	Analytical technique	Column	LOD/LOQ	Ref.
				Water samples			
8 pharmaceuticals and drugs of abuse	2012	Surface water, wastewater	SPE (Oasis HLB)	HPLC-QTOF-MS	Chirobiotic V (250 × 4.6 mm, 5 μm) Chiral-CBH (100 × 2 mm, 5μm)	MDL 0.2–22.8 ng/L MDL 2.1–10.7 ng/L	Bagnall et al., 2012
15 pharmaceuticals	2012	Wastewater	MIPs	HPLC-MS/MS	Fused-Core Ascentis Express C18 (100 × 4.6 mm, 2.7 μm)	MDL 1.5–7 ng/L	Gilart et al., 2012
16 pharmaceuticals	2011	Surface water, drinking water	SPE (Oasis HLB)	HPLC-MS/MS	Supelco C-18 (150 × 2.1 mm, 5 μm)	MDL 0.1–9.9 ng/L	Wang et al., 2011
15 pharmaceuticals and their metabolites	2011	Wastewater	SPE (Oasis MCX)	HPLC-MS/MS	Kinetex C18 (100 × 2.1 mm, 2.6 μm)	LLOQ 0.5–25 ng/L	Tarcomnicu et al., 2011
13 pharmaceuticals	2010	Wastewater	SPE (Oasis HLB, MCX, MAX)	HPLC-MS/MS	Phenomenex Luna HILIC (150 × 3 mm, 5 μm)	LLOQ 1–500 ng/L	Nuijs et al., 2010
12 pharmaceuticals	2010	Wastewater	SPE (Strata X)	HPLC-MS/MS	Phenomenex - Synergy Fusion C18 (150 × 2 mm, 4 μm)	MDL 0.1–5 ng/L	Babić et al., 2010
8 pharmaceuticals	2010	Surface water, wastewater	SPE (Speeddisk - phenyl)	HPLC-MS/MS	Pursuit Pentafluorphenyl (100 × 4.6 mm, 5 μm)	LOD < 0.05–5 ng/L	Steene et al., 2010
23 pharmaceuticals	2010	Wastewater, river water	SPE (Oasis MCX)	HPLC-MS/MS	Agilent Zorbax SB-C18 (100 × 2.1 mm, 1.8 μm) Agilent Zorbax Extend-C18 (100 × 2.1 mm, 1.8 μm)	MDL 1–281 ng/L	Al-Odainia et al., 2010
11 steroid compounds	2010	River water	SPE (Oasis HLB and MCX)	HPLC-MS/MS	Supelco Supelcosil ABZ + PLUS (150 × 3mm, 3 μm)	LOD 0.06–0.92 ng/L	Tölgyesi et al., 2010
46 pharmaceuticals	2010	Surface water, seawater, wastewater	SPE (Oasis HLB)	HPLC-MS/MS	Polaris C18-Ether (150 × 2 mm, 3μm)	MQL 1.6–160 ng/L	Nödler et al., 2010
70 pharmaceuticals	2010	Drinking water	SPE (Oasis HLB)	HPLC-MS/MS	Agilent Zorbac Eclipse Plus (100 × 2.1 mm, 3.5 μm) Agilent Zorbax HILIC Plus (100 × 2.1 mm, 3.5 μm)	LOD 0.02–20 μg/L	Ferrer et al., 2010
15 pharmaceuticals	2009	Wastewater	SPE (Oasis MCX and MAC)	HPLC-QTOF-MS	Waters Acquity HSS T3 (100 × 2.1 mm, 1.8 μm)	MDL 2.1–285 ng/L	Lavén et al., 2009

(Continued on next page)

TABLE 2
Multi-residue procedures for pharmaceutical analysis with application of hyphenated techniques *(Continued)*

Compounds	Year published	Matrix	Sample preparation	Analytical technique	Column	LOD/LOQ	Ref.
11 pharmaceuticals	2009	Wastewater, surface water	SPE (Oasis HLB, Chromabond C-18EC, Chromabond EASY, and Bond Elut PLEXA)	HPLC-MS/MS	NUCLEODUR C-18 ISIS (125 × 2 mm, 3 μm)	LOD 0.3–2 ng/L	Pailler et al., 2009
21 pharmaceuticals	2008	Industrial and hospital wastewater	SPE (Oasis MCX)	HPLC-MS/MS	C18 X-Bridge (150 × 2.1 mm, 3.5 μm) for corticosteroids Hibar Purospher Star (250 × 4.6mm, 5 μm) for β-blockers	IDL 0.1–77.3 pg	Piram et al., 2008
38 pharmaceuticals and 10 metabolites	2007	Wastewater	SPE (Oasis HLB)	HPLC-QTRAP-MS/MS	Agilent Zorbax SB C18 (250 × 3 mm, 5 μm)	MDL 0.04–50 ng/L	Bueno et al., 2007
29 pharmaceuticals	2006	Surface water, wastewater	Filtration, SPE (Oasis HLB, Isolute ENV+, Isolute C18, Strong cation Exchange Oasis MCX)	HPLC-MS/MS	Purospher Star RP-18 endcapped (125 × 2.0 mm, 5 μm)	MDL 1–160 ng/L	Gros et al., 2006a
5 pharmaceuticals	2006	Natural water and treated water	SPE (Isolute SPE C18)	HPLC-MS/MS	Purospher Star RP-18 endcapped (125 × 2.0 mm, 5 μm)	MDL 7–26 ng/mL	Hernando et al., 2006
				Solid samples			
12 pharmaceuticals	2012	Sediment	MSPD	HPLC-MS/MS	Synergy Fusion C18 (150 × 2.0 mm, 4 μm)	LOD 0.125–500 ng/g	Pavlović et al., 2012
15 pharmaceuticals	2011	Biosolids	ASE + SPE (Oasis HLB) cleanup	HPLC-MS/MS	Luna C18 (150 × 4.6 mm, 3 μm)	LOQ < 488 μg/kg	Ding et al., 2011
17 pharmaceuticals	2010	Soil, sediments	PLE + SPE (SAX and Oasis HLB) cleanup	HPLC-MS/MS	Luna C18 (150 × 2 mm, 3 μm)	MQL 0.25–23 ng/g	Vazquez-Roig et al., 2010
32 pharmaceuticals	2010	Soil, sediments	PLE + SPE (Oasis MAX and HLB) cleanup	HPLC-MS/MS	XTerra MS-C18 Column (2.1 × 100 mm, 3.5 μm) Atlantis HILIC (2.1 × 100 mm, 2 μm)	LOQ 0.3–7.1 ng/g	Pérez-Carrera et al., 2010
31 pharmaceuticals	2009	Sewage sludge	PLE + SPE (Oasis HLB cleanup)	HPLC-MS/MS	Purospher Star RP-18 (125 × 2.0 mm, 5 μm)	MQL < 258.5 ng/g	Radjenović et al., 2009

low concentration in the sample or matrix effects are very high, because the second transition cannot be observed. In such a case second analysis (when the concentration of the analyte is high enough) using QTOF-MS for analyte confirmation may be a good choice. Although MS/MS is a very efficient tool in target analysis, it fails in the analysis of nontarget pharmaceuticals not initially included in the multi-residue procedure (Bueno et al., 2007).

UHPLC-QqLIT-MS Analysis

A hybrid instrument designed to increase versatility, such as a quadrupole linear ion trap (QqLIT), is the latest trend in the determination of pharmaceuticals. This kind of system combines triple quadrupole scanning functionality with sensitive linear ion trap scans due to the closure of the third quadrupole by repulsive direct current voltages (Bueno et al., 2007). Due to the powerful scan combination of SRM mode and IDA (information-dependent acquisition) software in the same run, it is possible to obtain accurate quantification and structural information simultaneously (Jelić et al., 2009). Furthermore, the LIT mode provides enhanced sensitivity in full scan and enhanced product ion (EPI) scan (Gros et al., 2009). Quadrupole ion trap is suitable for both quantitation and confirmation due to its specific feature of running the third quadrupole in two different modes at the same time. This allows the instrument to work as a typical triple quadrupole (SRM, product ion, precursor ion, neutral loss) and as a sensitive ion trap at the same time (Gros et al., 2009; Hopfgartner et al., 2004). Application of ion trap mass spectrometry is still scarce; however, it has already been applied to water samples such as wastewater (Huerta-Fontela et al., 2010; Unceta et al., 2010), river water (Chen et al., 2008; Madureira et al., 2009), and surface water (Gros et al., 2012). The first combination of UHPLC and QqLIT for the analysis of pharmaceuticals in the environment was published by Huerta-Fontela et al. (2010). A fast method for the analysis of 55 pharmaceuticals in wastewater was developed. The application of UHPLC technology made possible fast separation in less than 9 minutes with good sensitivity and reduced matrix effect. Moreover, the QqLIT instrument provided additional confirmation by using an IDA experiment. Later, Gros et al. (2012), as mentioned above, developed an UHPLC-QqLIT-MS method with an IDA experiment combined with a library search for determination of 81 pharmaceutical compounds and their metabolites in wastewater.

LC-QTOF-MS Analysis

Liquid chromatography-quadrupole time-of-flight MS (LC-QTOF-MS) applications for the determination of pharmaceuticals in various samples are still scarce; however, several authors have reported on the application of LC-QTOF for screening, confirmation, and quantitative analysis due to its advantages (Petrović et al., 2006). QTOF is a powerful tool in pharmaceutical analysis compared to MS/MS in SRM mode (Magnér et al., 2010) due to accurate mass determination errors, below 5 ppm (Petrović et al., 2006), and full-scan analysis. Accurate mass measurements provide highly reliable identification of target and nontarget compounds and additionally allow obtaining the elemental composition of parent and fragment ions. Through full-scan information collected during analysis, further data evaluation and compound identification are possible; because no extra analysis is needed, additional cost and time are avoided (Bueno et al., 2007). Petrović et al. (2006) developed the UHPLC-QTOF-MS method for screening and confirmation of 29 pharmaceutical compounds belonging to different therapeutical classes in wastewater. Unequivocal identification of target compounds was based on accurate mass measurements of (pseudo)molecular ions in TOF mode and product ions in QTOF mode. Quantitation was performed in TOF mode using the narrow window extracted ion chromatograms (nwXICs) of each analyte. Despite the quantitative possibility of QTOF-MS in pharmaceutical analysis, sensitivity was one order of magnitude lower than with an MS/MS instrument working in SRM mode. Magnér et al. (2010) presented a multi-residue method based on UHPLC-QTOF-MS for the determination of 10 compounds in surface seawater. Even though triple quadrupoles in SRM mode provide necessary sensitivity during the analysis of environmental samples, only a certain number of selected analytes can be monitored; furthermore, due to the low mass resolution in full-scan mode, MS/MS does not provide the needed sensitivity (Ibáñez et al., 2008; Petrović et al., 2006). QTOF-MS detector is an alternative to MS/MS for full-scan analysis, due to its high mass accuracy and possibility of identifying target and nontarget analytes. However, the LODs achieved by Magnér et al.'s (2010) method ranged from 1 to 13 ng/L, indicating sensitivity comparable to that in MRM mode. Terzic and Ahel (2011) developed a comprehensive procedure for the identification of nontarget polar contaminants in aquatic sediments using UHPLC-QTOF-MS. The complexity of the matrix in the identification of unknowns requires high efficiency and resolution of the chromatographic system coupled with mass spectrometric detection. The application of UHPLC and QTOF-MS provides sharper peaks, significantly reducing the problem of co-elution and mass spectra overlap (Plumb et al., 2004). The use of fast UHPLC and QTOF-MS provides high sensitivity in full-scan mode, allowing the analysis of both target and nontarget analytes, so that untargeted retrospective analysis is possible in order to extend the test for new compounds. That is why QTOF-MS detection is probably irreplaceable for investigation of metabolites (Liu et al., 2010), isomers (Bartók et al., 2010), and degradation products (Prakash et al., 2007; Radjenović et al., 2007) in different pharmaceuticals. Obtaining and verifying the structure of metabolites are done by comparing changes in exact molecular mass and comparing the masses of fragment ions that are produced from precursor ions of an adequate drug. Furthermore, TOF-MS is suitable for tracking the metabolic routes of drugs in organisms (Castro-Perez et al., 2005; Liu et al., 2008; Rousu et al., 2009; Strano-Rossi et al., 2010).

TABLE 4
Comparison of MS-based instruments (Krauss et al., 2010)

Parameter	Mass spectrometer		
	MS/MS	QqLIT-MS	QTOF-MS
Resolution (FWHM)	5,000	10,000	20 000
Mass accuracy (ppm)	50	50	3
Maximum sensitivity	fg (SRM)	fg (SRM, full-scan)	pg (full-scan)
Dynamic range	10^4	10^3	10^2–10^3

COMPARISON OF MS-BASED TECHNIQUES

Recently, researchers have shown a great interest in the application of hyphenated techniques in analysis of pharmaceutical compounds at low concentrations in complex matrices. As can be seen in Table 1, UHPLC-MS/MS is the most often applied technique in the determination of pharmaceutical residues; however, UHPLC-QTOF-MS has recently emerged as a powerful tool (Feng et al., 2009; Ojanperä et al., 2006; Pelander et al., 2009) due to high mass accuracy measurements. A brief comparison, based on Krauss et al.'s (2010) review of MS-based instruments, is presented in Table 4. Tandem MS and QqLIT-MS are still the leading instruments in target analysis due to their high sensitivity and selectivity in SRM mode. However, MS/MS fails in the analysis of unknown compounds, where QTOF-MS is the option of choice. Although, QTOF-MS has a high resolving power of about 20,000 FWHM (full width at half maximum), sensitivity is more limited but still enough to investigate compounds at trace levels in complex matrices due to accurate mass measurements of both precursor and product ions (Petrović and Barcelo, 2006). It must be remembered that even the best analytical instruments are not able to detect a compound if the sample is not well pretreated, causing non-ionization of the analyte, ion suppression, or high background interferences.

The selected reaction monitoring (SRM) mode allows monitoring the precursor to product ion transition by using either MS/MS or QqLIT-MS instruments. Its high sensitivity, selectivity, repeatability, and dynamic range (Pfeifer et al., 2002; Stolker et al., 2004) make these the most commonly used instruments in quantitative analysis of target compounds. However, monitoring only one SRM transition might be insufficient and cause false positive findings, thus at least two transitions per analyte are needed. In some cases such as poor fragmentation of a compound or nonspecific transition (e.g., loss of H_2O or CO_2), this approach is not adequate. There are several solutions, e.g., additional analysis by an LC-QTOF-MS instrument for the confirmation of existing analytical tools (López-Roldán et al., 2010; Petrović et al., 2006), as proposed by Petrović et al. (2006), as mentioned above. It is a suitable approach when compounds of interest are present in the sample at sufficiently high concentration levels since the sensitivity and dynamic range of QTOF-MS (Magnér et al., 2010) is much lower than in MS/MS instruments operated in SRM mode (Petrović and Barcelo, 2006). However, according to Ibanez et al. (2009), in the new generation of QTOFs produced after 2005, instrumental detection limits (IDLs) were lowered and comparable to those obtained with MS/MS (Farré et al., 2008). Technical solutions such as attenuation of the ion beam in order to avoid detector saturation were introduced to increase the dynamic range (Magnér et al., 2010). Also, a good option seems to be the application of IDA, offered by hybrid instruments such as QqLIT-MS. The IDA function allows for targeted screening, monitoring of one SRM transition per analyte, and an enhanced product ion scan (EPI) at the same time, thus obtaining MS/MS spectra that can be compared with library spectra afterwards (Gros et al., 2012). However, these solutions may fail when co-eluting or isobaric compounds appear, which can be differentiated only by separation performed on a column with different polarity. Therefore, proper separation cannot be neglected.

Despite many advantages of target analysis in SRM mode performed by LC-MS/MS, no other data are recorded at the same time, causing a loss of information for structural elucidation or identification of unknowns (Petrović et al., 2006). This kind of information can be obtained in scan mode; however, the sensitivity is relatively poor due to low mass resolution (Ibáñez et al., 2008; Magnér et al., 2010) and limited availability of comprehensive mass spectral libraries (Terzic and Ahel, 2011). Consequently, LC-MS/MS is not suitable for trace analysis in scan mode. In contrast, LC-QTOF-MS (in either TOF-MS or TOF-MS/MS mode) is a great alternative for the identification of known-unknown compounds. Although, the MS libraries are still unsuitable for nontarget analysis of pharmaceutical compounds, there are some approaches that may help in identification and structural elucidation (Zedda and Zwiener, 2012). The application of full-scan or targeted MS/MS mode provides sufficient sensitivity for the identification and quantification of selected analytes and allows for further screening of nontarget compounds (Ibáñez et al., 2009; Magnér et al., 2010), such as transformation products (metabolites, degradation products).

Advantages that make LC-QTOF-MS such a powerful tool include the possibility of accurate mass measurements (to four decimal places with mass error < 3 ppm) (Williamson and Bartlett, 2007), due to its high resolving power of around 20,000 FWHM (Figure 8(A), 8(C)), elemental composition and/or isotopic ratio (Figure 8(B)). For identification, a comparison is made between the precise value of the mass of the neutral and mono-isotopic compound with the obtained mass taken from ionic m/z value of determined typical adducts with a mass error usually in the range of 1 to 3 ppm (Figure 8(D)). Nevertheless, identification may be uncertain, due to the existence of many structural and steric isomers of a given molecular formula. False positives of such analyses are an ongoing concern and inhibit further screening (Sun et al., 2009).

Procedures developed based on mass spectrometry must fulfill the criterion of identification points (IPs). According to European Commission Decision 2002/657/EC (European

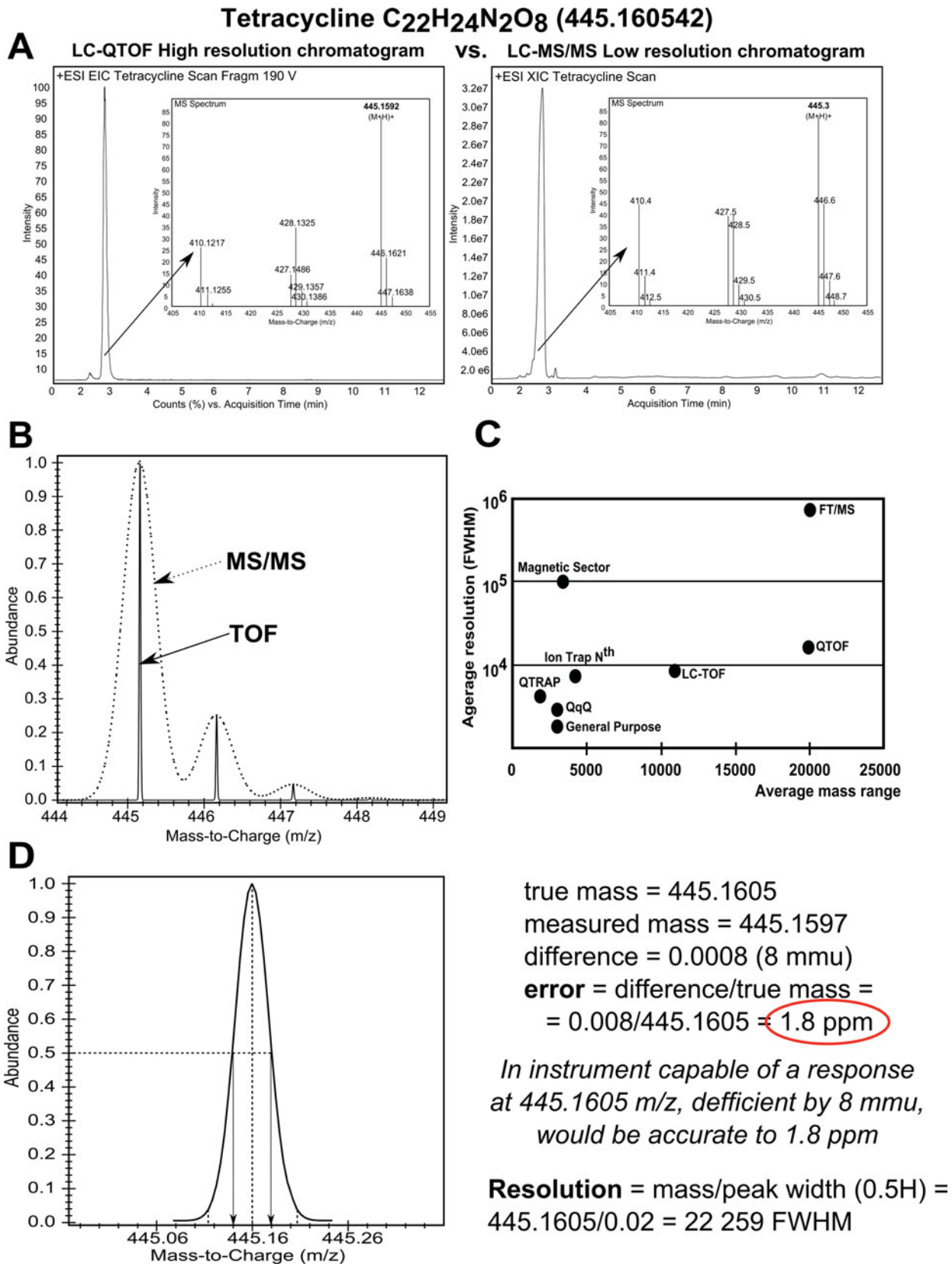

FIG. 8. High resolution QTOF-MS technique vs. low resolution MS/MS technique (description in the text).

TABLE 5
Amount of IPs for analyte achieved by MS/MS and QTOF-MS (Grimalt et al., 2007; Ruyck and Ridder, 2007)

Detection	IP per precursor ion	IP per product ion	IP earned	
QqQ-MS/MS	1	1.5	1 MRM (1 precursor ion > 1 product ion)	2.5
			2 MRM (1 precursor ion > 2 product ions)	4
QTOF-MS	2	2.5	MS/MS (1 precursor ion > 1 product ion)	4.5
			MS/MS (1 precursor ion > 2 product ions)	7

Commision, 2002), compounds are divided into two groups: Group A, prohibited compounds, and Group B, legal compounds. For the identification and confirmation of a compound from Group B, it is necessary to collect three IPs and from Group A four IPs (Stolker et al., 2000). Table 5 presents the amount of IPs achieved for MS/MS and QTOF-MS.

When using only LC-MS/MS, it may be possible that two product ions cannot be achieved for a specific compound due to only one product ion being obtained during fragmentation. In such cases, three or four IPs are not obtained for those analytes. An innovative solution requires additional analysis of LC-QTOF-MS for confirmation of the compound (López-Roldán et al., 2010; Petrović et al., 2006), as mentioned in the previous section. QTOF-MS measures the accurate mass of the target analyte, providing that extra IP for the confirmation needed to obtain reliable results.

A significant number of analytical procedures based on UHPLC-MS/MS have been developed for the determination of various pharmaceuticals (Table 1) in different matrices; however, LC-QTOF-MS procedures for environmental applications are still scarce. LC-QTOF-MS is still more often used for screening and confirmation of pharmaceuticals (Stolker et al., 2004), for elucidation of unknown contaminants (Ibáñez et al., 2005), and for identification of analytes and their transformation products (Wren, 2005). Krauss et al. (2010) presented an excellent overview and future trends of the application of high resolution mass spectrometry in environmental analysis of polar compounds. Furthermore, it is worth mentioning reviews recently published by Richardson and coworker (Richardson, 2012; Richardson and Ternes, 2011), who summarize achievements in analysis of emerging pollutants in environmental matrices.

LEGISLATION AND ANALYTICAL METHODOLOGY

For many years there was no regulation that specified the maximum allowable level of pharmaceuticals in the environment. In 2010, the European Union published a list of pharmacologically active compounds with maximum residue limits in foodstuffs of animal origin (Commission Regulation (EU) No 37/2010).

There are no regulations on the level of pharmaceuticals in environmental waters. The U.S. Environmental Protection Agency (EPA) created a program entitled "Contaminant Candidate List" (CCL) to identify pharmaceutical pollution in drinking water. The most recent CCL3 was finalized in 2009 and for the first time included 10 pharmaceutical compounds (erythromycin, 17 alpha-estradiol, 17 beta-estradiol, equilenin, equilin, estriol, estrone, ethinylestradiol, mestranol, norethindrone, and nitroglycerin). Fortunately, the public's awareness is now higher than before, and per the request from several member states, the World Health Organization (WHO) published a rapid review in 2011 of the state of pharmaceuticals in drinking water (WHO, 2011).

A significant number of articles have been published reporting the occurrence of pharmaceutical compounds in various matrices. However, it seems that all of the published procedures so far are insufficiently effective in order to be legislated. In 2007 the EPA published a procedure for the determination of such compounds, however, the procedure included only water, soil, sediments, and biosolids.

The procedure, developed in 2007 by the EPA (U.S. EPA, 2007) and described in the standard EPA 1694, allows for the determination of 75 pharmaceuticals using 20 isotope internal standards. This EPA protocol can be used for samples of soil, sediments, biosediments, and water. It uses solid-phase extraction Oasis HLB columns for the preparation of liquid samples, solvent extraction (ACN) for isolation of compounds from solid samples, and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) at the final determination step.

Pharmaceuticals are divided into four specific groups. Compounds belonging to the first three are extracted in acidic environments (pH$\sim$2), and pharmaceuticals belonging to the last are extracted in an alkaline environment (pH$\sim$10). Pharmaceutical analysis is carried out in SRM mode with monitoring of one typical transition for the individual compounds.

Compounds from the first two groups and the fourth are analyzed in the positive mode, while the third group is analyzed in the negative mode. The limit of detection and quantification depends more on the composition of the sample matrix and on the presence of interference compounds in it than on the limitations imposed by the apparatus.

The quantitation of pharmaceuticals defined by the EPA 1694 standard, depending on the presence/absence of isotopic analogues of the compounds, is carried out in three ways:

- Isotopic dilution techniques (if there are available isotopic analogs of the compounds) and multipoint calibration of all analytes.
- Internal standard method (if the compound does not have an isotopic analogue) and multipoint calibration of all analytes.

- Isotopic dilution technique using isotopic compounds that are not analogues of any compound of interest (though they should belong to the group of analyzed compounds).

CONCLUSION AND FUTURE PERSPECTIVES

There is a clear need for analytical methods that will be selective and reliable and provide multi-residue analysis of a wide spectrum of pharmaceuticals. Moreover, there is a demand for procedures that can ensure identification and confirmation of the presence of analytes at ultra-trace levels. Future research should provide quantitation of pharmaceuticals from different groups (characterizing different physico-chemical properties) in a very short time with good separation results and high throughput. There is a current trend of applying ultra performance (pressure) liquid chromatography (UHPLC). UHPLC uses short, narrow bore columns with sub-2 μm particles, which result in ultrahigh back-pressures and mobile phases at high linear velocities. This provides much faster elution of a large number of analytes in higher peaks with smaller width below 10 s at base, resulting in improved chromatographic resolution, increased peak capacity, and reduced co-elution of interferences. However, a great competitor for pharmaceutical analysis is fused-core columns, which exhibit efficiencies comparable to those of typical UHPLC columns with sub-2 μm particles and do not cause high back-pressures; thus it is possible to use them on conventional HPLC systems.

Since there are only a few works presenting the chemical fate of drugs in the environment, it is necessary to expand our knowledge about pharmaceutical residues in the environment. Today most attention is paid directly to parent compounds, while the analysis of transformation products of pharmaceuticals is still secondary. In wastewater treatment plants, not all of the polar pharmaceuticals are eliminated and particular attention is needed to find the routes of decomposition and the environmental fate of pharmaceuticals.

LC-MS/MS in the past few years has become a superior analytical technique for the determination of pharmaceutical residues and the identification of known-unknown transformation products. However, developed procedures must be both timesaving and cost-effective, thus providing as many results as possible in one analytical cycle. LC-MS/MS operated in SRM mode is an effective tool due to its high sensitivity as well as selectivity. The slightly less selectivity of QTOF is offset by the higher resolution of UHPLC chromatography. Even though a time-of-flight mass spectrometer has a smaller linear range even with the use of the extended dynamic range mode, with the use of quadratic regression, there is a very good correlation of quantitative results with both QTOF and MS/MS. Moreover, full-scan acquisition enables data processing at any time. Data storage and processing require a high-performance computer and software when generating a few GBs of data each day (raw file: $<$ 1 MB for MS/MS, 90–900 MB for QTOF).

With QTOF-MS, known-unknowns like metabolites or substances not commercially available could be identified by extract mass chromatograms based on theoretical mass. Accurate mass measurements and isotopic profiling studies enable suggesting a possible formula of the unknown with the elemental composition tool. QTOF analyzers have recently emerged with enhanced sensitivity and dynamic range.

Nevertheless, it is crucial to develop new analytical procedures for determining either parent compounds or their transformation products. It is a priority to use fast—meaning UHPLC—and accurate—meaning QTOF—techniques. Therefore, it seems that despite the higher costs than those of LC-MS/MS instruments, LC-QTOF-MS will be gradually introduced into routine analysis. The major advantage of QTOF-MS–based instruments is the possibility of accurate mass measurements and the possibility of retrospective analysis of full-scan data, which enables searching for unknown contaminants. Smart approaches combine target analysis and nontarget screening in the same analytical run. Furthermore, the latest QTOF-MS instruments are now comparable to MS/MS in SRM mode in terms of sensitivity, which makes them competitive in theanalytical field.

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ABBREVIATIONS

ACN	acetonitrile
BEH	ethylene bridged hybrid
DPX	disposable pipette extraction
DSPE	dispersive solid-phase extraction
EPI	enhanced product ion
FWHM	full width at half maximum
HILIC	hydrophilic interaction chromatography
HPLC	high-performance liquid chromatography
IDA	information-dependent acquisition
IDL	instrument detection limit
LLOQ	lower limit of quantification
LOD	limit of detection
LOQ	limit of quantification
LPME	liquid-phase micro-extraction
MASE	microwave-assisted solvent extraction
MDL	method detection limit
MQL	method quantification limit
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MSPD	matrix solid-phase dispersion

PLE	pressurized liquid extraction
QqLIT	quadrupole linear ion trap
QTOF	quadrupole time of flight
QuEChERS	quick, easy, cheap, effective, rugged, and safe
SBSE	stir bar sorptive extraction
SFE	supercritical fluid extraction
SPE	solid-phase extraction
SPME	stationary-phase micro-extraction
SRM	selected reaction monitoring
UHPLC	ultrahigh performance (pressure) liquid chromatography
US EPA	United States Environmental Protection Agency
USE	ultrasonic extraction
WHO	World Health Organization
WWTP	wastewater treatment plant

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