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# 16 Pharmaceutical Compounds in Estuarine and Coastal Waters

*John L. Zhou and Zulin Zhang*

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## 16.1 INTRODUCTION

Pharmaceuticals and personal care products (PPCPs) are a group of emerging contaminants of environmental concern that have remained largely unrecognized as such until recent advances in trace-level analytical measurements (Cha et al., 2006; Erickson, 2002; Gros et al., 2006; Lindsey et al., 2001). There is growing concern over the occurrence and fate of PPCPs in the environment, with evidence of adverse effects in terrestrial and aquatic organisms, and also the potential of some antibiotics to induce resistance in naturally occurring bacterial strains (Hirsch et al., 1998).

Over three thousand chemical substances are used in human and veterinary medicine (Ternes et al., 2004). Such pharmaceuticals include antiphlogistics/anti-inflammatory drugs, contraceptives,  $\beta$ -blockers, lipid regulators, tranquilizers, antiepileptics, and antibiotics (Ternes et al., 2004; Petrovic et al., 2005). Some typical pharmaceuticals classified by groups according to therapeutical effect and physicochemical properties are listed in Table 16.1. During and after treatment, humans and animals excrete a combination of intact and metabolized pharmaceuticals, many of which are generally soluble in water and have been discharged to the aquatic environment with little evaluation of possible risks or consequences to humans and the environment. In addition, chemicals that are components of personal care products number in the thousands, and are contained in skin care products, dental care products, soaps, sunscreen agents, and hair care products. Annual production exceeds  $1 \times 10^6$  tonnes worldwide (e.g., >553,000 tonnes was produced in Germany alone in 1993; Daughton and Ternes, 1999). Included in this category are fragrances (e.g., nitro and polycyclic musks), UV blockers (e.g., methylbenzylidene camphor), and preservatives (e.g., parabens). Unlike pharmaceuticals, personal care products enter wastewater and the aquatic environment after regular use during showering or bathing. The environmental fates and effects of many cosmetic ingredients are poorly known, although considerable persistence and bioaccumulation in aquatic organisms have been reported (Daughton and Ternes, 1999; Kallenborn et al., 2001).

In many aquatic environments, particularly in North America and Europe, pharmaceuticals, hormones, metabolites, biocides, musks, and flame retardants have been measured (Ternes, 1998;

**TABLE 16.1**  
**Pharmaceuticals and Their Physicochemical Properties**

Compound	Therapeutic Class	logK <sub>ow</sub>	pK <sub>a</sub>	MW	Formula
Ketoprofen	Analgesic/anti-inflammatories	3.12	4.45	254	C <sub>16</sub> H <sub>14</sub> O <sub>3</sub>
Naproxen		3.18	4.15	230	C <sub>14</sub> H <sub>14</sub> O <sub>3</sub>
Ibuprofen		3.97	4.91	206	C <sub>13</sub> H <sub>18</sub> O <sub>2</sub>
Indomethacine		4.27	4.5	358	C <sub>19</sub> H <sub>16</sub> ClNO <sub>2</sub>
Diclofenac		4.51	4.14	296	C <sub>14</sub> H <sub>10</sub> Cl <sub>2</sub> NO <sub>2</sub>
Meclofenamic acid		5.12	4.2	241	C <sub>15</sub> H <sub>15</sub> NO <sub>2</sub>
Acetaminophen		0.46	9.38	151	C <sub>8</sub> H <sub>9</sub> NO <sub>2</sub>
Propyphenazone	Lipid regulators/cholesterol-lowering statin drugs	1.94	n/a	230	C <sub>14</sub> H <sub>18</sub> N <sub>2</sub> O
Clofibric acid		n/a	n/a	214	C <sub>10</sub> H <sub>11</sub> O <sub>3</sub> Cl
Gemfibrozil		4.77	n/a	250	C <sub>15</sub> H <sub>22</sub> O <sub>3</sub>
Bezafibrate		4.25	n/a	362	C <sub>19</sub> H <sub>20</sub> ClNO <sub>4</sub>
Pravastatin		3.1	n/a	446	C <sub>23</sub> H <sub>36</sub> O <sub>7</sub>
Mevastatin		3.95	n/a	391	C <sub>25</sub> H <sub>38</sub> O <sub>5</sub>
Carbamazepine	Psychiatric drugs	2.47	7	236	C <sub>15</sub> H <sub>12</sub> NO
Fluoxetine		3.82	8.7	309	C <sub>17</sub> H <sub>18</sub> F <sub>3</sub> NO
Paroxetine		3.95	n/a	329	C <sub>19</sub> H <sub>20</sub> FNO <sub>3</sub>
Lansoprazole	Antiulcer agent	2.58	8.73	369	C <sub>16</sub> H <sub>14</sub> F <sub>3</sub> N <sub>3</sub> O <sub>2</sub> S
Loratadine	Histamine H <sub>1</sub> and H <sub>2</sub> receptor antagonists	5.20	n/a	383	C <sub>22</sub> H <sub>23</sub> ClN <sub>2</sub> O <sub>2</sub>
Famotidine		-0.64	n/a	337	C <sub>8</sub> H <sub>15</sub> N <sub>7</sub> O <sub>2</sub> S <sub>3</sub>
Ranitidine		0.27	n/a	314	C <sub>13</sub> H <sub>22</sub> N <sub>4</sub> O <sub>3</sub> S
Erythromycin	Antibiotics	3.06	8.8	734	C <sub>37</sub> H <sub>67</sub> N <sub>13</sub> O <sub>13</sub>
Azythromycin		4.02	8.74	749	C <sub>38</sub> H <sub>72</sub> N <sub>2</sub> O <sub>12</sub>
Sulfamethoxazole		0.89	6.0	253	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S
Trimethoprim		0.91	7.12	290	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O <sub>3</sub>
Ofloxacin		n/a	n/a	361	C <sub>18</sub> H <sub>20</sub> FN <sub>3</sub> O <sub>4</sub>
Atenolol	β-Blockers	0.16	9.6	266	C <sub>14</sub> H <sub>22</sub> N <sub>2</sub> O <sub>3</sub>
Sotalol		0.24	n/a	272	C <sub>12</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> S
Metoprolol		1.88	9.68	267	C <sub>15</sub> H <sub>25</sub> N <sub>3</sub> O <sub>3</sub>
Propranolol		1.2–3.48	9.5	260	C <sub>16</sub> H <sub>21</sub> NO <sub>2</sub>
Mebeverine	Gastrointestinal	n/a	n/a	429	C <sub>25</sub> H <sub>35</sub> O <sub>5</sub>
Thioridazine	Antidepressant	n/a	n/a	371	C <sub>21</sub> H <sub>26</sub> N <sub>2</sub> S <sub>2</sub>
Tamoxifen	Anticancer	n/a	n/a	372	C <sub>26</sub> H <sub>29</sub> NO
Monensin	Growth promoters	2.75–3.89	6.65	692	C <sub>36</sub> H <sub>61</sub> NaO <sub>11</sub>

*Note:* logK<sub>ow</sub>, log of the octanol-water partition coefficient; pK<sub>a</sub>, log of the ionization constant; MW, molecular weight; n/a, not available.

Kolpin et al., 2002; Hirsch et al., 1999; Hilton and Thomas, 2003). One of the principal sources is through the release of municipal wastewater. Some pharmaceuticals do not readily biodegrade in a marine environment, and have been detected in seawater (Weigel et al., 2002; Thomas and Hilton, 2004) and sediments (Samuelsen et al., 1992). Weigel et al. (2002) reported a wide distribution of clorfibric acid, caffeine, and N,N-diethyl-3-toluamide (DEET, an insect repellent) in concentrations up to 19, 16, and 1.1 ng L<sup>-1</sup>, respectively, throughout the North Sea, off Scotland, the outer and inner German Bight, as well as the Danish and Norwegian coasts. Samples collected from UK

estuaries had clorfibric acid concentrations of approximately  $100 \text{ ng L}^{-1}$  in two samples (Thomas and Hilton, 2004). Other frequently measured pharmaceutical compounds found in UK estuaries included clotrimazole (a typical antifungal agent, in 59% of samples, up to  $22 \text{ ng L}^{-1}$ ), ibuprofen (an analgesic, in 50% of samples, up to  $569 \text{ ng L}^{-1}$ ), and propranolol (an antihypertensive drug, in 41% of samples, up to  $56 \text{ ng L}^{-1}$ ), with several other drugs appearing in approximately one-third of the samples at lower concentrations (Thomas and Hilton, 2004).

The concentrations of pharmaceuticals present in the aquatic environment are generally in the sub  $\text{ng L}^{-1}$  range and do not necessarily represent a major threat to drinking water quality. The consequence of a continuous presence of low concentrations of pharmaceuticals for ecosystems is still not fully understood. A discussion of various aspects of ecotoxicology of pharmaceuticals in the environment can be found in recent reviews (Cunningham et al., 2006; Fent et al., 2006; Crane et al., 2006; Hernando et al., 2006). It is quite clear that environmental risk assessment must be based on reliable data about the actual concentrations of pharmaceuticals in aquatic systems. Therefore, efficient analytical methods are of major importance.

Fast progress in the development of analytical procedures for residue analysis of pharmaceutical drugs has been facilitated by the existence of considerable expertise in other micro-organic pollutants residue analysis. Strategies successfully used for routine analysis of traces of some polar organic contaminants have been modified and subsequently applied to residue analysis of pharmaceuticals. In many cases, the common procedures involve sampling, sample treatment (e.g., preconcentration, cleanup step) by solid-phase extraction (SPE) or related techniques, followed by analysis using chromatography in combination with mass spectrometry (MS) as detector. When residue analysis of pharmaceuticals became an important issue in the 1990s, gas chromatography (GC) was the preferred chromatographic technique together with various derivatization procedures for the analytes. Nowadays, GC-MS may still be the perfect technique for certain classes of pharmaceuticals (Togola and Budzinski, 2008), although high-performance liquid chromatography (HPLC) hyphenated with atmospheric pressure ionization-MS has established itself as a better choice for simultaneous determination of pharmaceuticals of widely differing structures (Buchberger, 2007). In this chapter, the analysis of pharmaceuticals in estuarine and coastal waters will be discussed, including the sampling, preconcentration, and instrumental measurement.

## 16.2 SAMPLING

The first task in any analysis is sampling. The sample being taken should be representative, the composition of which is as close as possible to the whole mass of whatever (e.g., estuarine water) is being analyzed. Obtaining a good sample is a crucial first step in the chemical analysis process. Prior to sampling, a sampling strategy should be drawn concerning the locations of sampling, the number of samples to be taken, where to conduct replicate sampling, size of samples, and storage and transport of samples (see Chapter 1). Preparation should also ensure that all the *in situ* measurement equipment is calibrated, and necessary sampling tools and containers are cleaned appropriately before sampling.

There are two sampling methods, spot and passive sampling, which are complementary to each other. Currently, the most widely used technique for performing monitoring of organic contaminants is spot sampling followed by laboratory-based extraction and analysis. In general, spot sampling uses a glass sampler or stainless steel sampler such as buckets (Gulkowska et al., 2007), some of which can be opened underwater to prevent the sampling of the surface microlayer (Zhou et al., 1996). The sample volume is typically 1–2 L (Gulkowska et al., 2007; Roberts and Thomas, 2006), although for seawater samples this may be increased to 10–100 L. General biocides such as sodium azide (final concentration of 0.02 M) are added to each sample on site to inactivate bacteria and prevent sample degradation during storage and processing. The samples are stored in a refrigerator below  $4^{\circ}\text{C}$  until filtration and extraction. Prior to use, all glassware is thoroughly soaked with

detergents (e.g., Decon-90) and cleaned with ultrapure water, before further treatment (e.g., rinsed with distilled solvents such as dichloromethane and methanol, or baked in a furnace). The procedure should be adjusted for the compounds to be analyzed, based on their physicochemical properties (e.g., solubility, polarity).

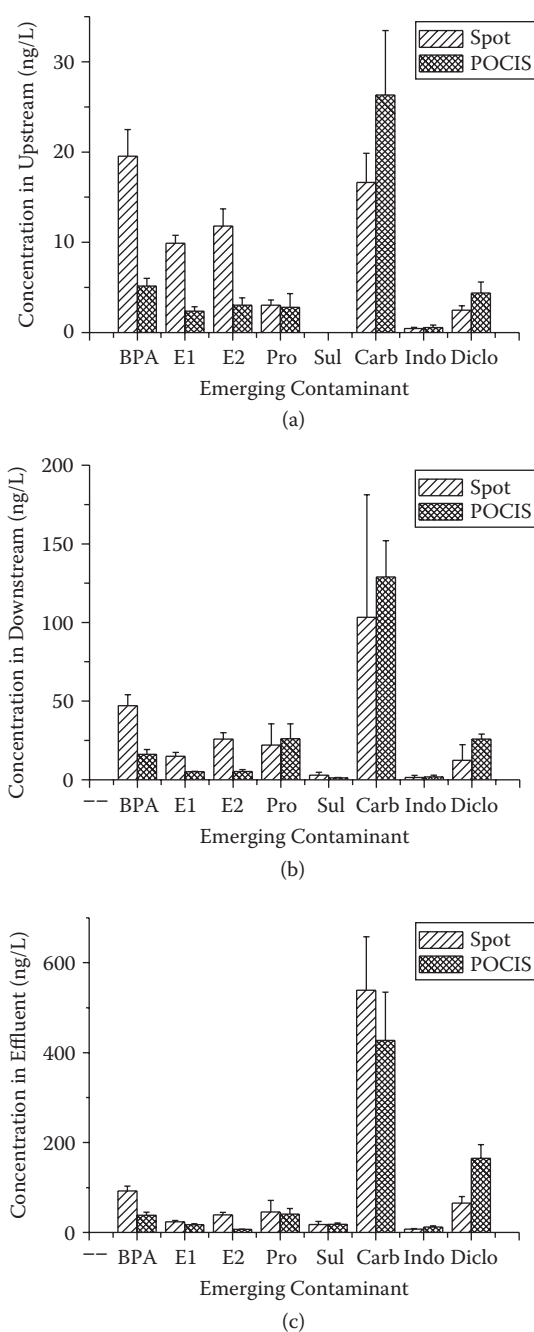
Spot sampling is a well-established technique that is easy to perform and inexpensive, and requires limited expertise. However, it only yields an instantaneous measurement of pollutant levels and suffers from the uncertainty of short- and long-term concentration variations, which occur in the aquatic environment. An increase in sampling frequency or the use of flow- and time-weighted automatic samplers may reduce such uncertainty; however, the associated increase in costs can be prohibitive. There has been rapid development in the use of passive sampling devices such as polar organic chemical integrative sampler (POCIS) (Alvarez et al., 2004), Chemcatcher (Mills et al., 2007), and silicon rod (Paschke et al., 2007) that allow continuous monitoring of aqueous pollutants, with the disadvantage of using organisms (passive sampler could mimic the bioconcentration of pollutants in aquatic organisms but not suffer from adverse effects as organisms). Of the various passive sampling devices, the most widely used is POCIS, which comprises a solid receiving phase (sorbent) sandwiched between two microporous polyethersulfone (PES) membranes (Figure 16.1a; Zhang et al., 2008). POCIS samples from water and thereby enables the chemical concentration to be estimated as follows (Alvarez et al., 2004; Vrana et al., 2005):

$$M_s = C_w R_s t \quad (16.1)$$

where  $M_s$  is the mass of analytes in the receiving phase at time  $t$ , and  $C_w$  represents time-weighted average concentration in water during the deployment period.  $R_s$  is the sampling rate of the system, which may be interpreted as the volume of water cleared of analyte per unit of exposure time by the device (Vrana et al., 2006; Zhang et al., 2008). Although little has been reported on the application of POCIS for pharmaceutical residue measurements in seawater, it has been successfully applied in surface and estuarine water (Jones-Lepp et al., 2004; Petty et al., 2004; Togola and Budzinski, 2007). Figure 16.1b and c shows the operating process for applying POCIS to pharmaceutical monitoring in river and estuarine waters (Zhang et al., 2008).

The POCIS is versatile, and by changing the sequestering medium, specific chemicals or chemical classes can be targeted. It is common to have POCIS of several different configurations deployed together to maximize the data obtained. There are two configurations of POCIS that are typically used. One is a generic system that is useful for general hydrophilic organic contaminant purposes, and the other is for pharmaceutical sampling. The generic configuration contains the triphasic sorbent admixture of Isolute ENV<sup>+</sup> polystyrene divinylbenzene (Argonaut Technologies, Redwood City, California) and Ambersorb 1500 carbon (Rohm and Haas, Philadelphia, Pennsylvania) dispersed on S-X3 Biobeads (200-400 mesh, Bio-Rad, Hercules, California). This mixture exhibits excellent trapping and recovery of many pesticides, natural and synthetic hormones, and other wastewater-related contaminants (Alvarez et al., 2004, 2005). The pharmaceutical configuration uses the Oasis HLB sorbent (Waters, Milford, Massachusetts) for sequestering the chemicals of interest. This configuration is necessary, as many pharmaceuticals, with multiple functional groups, have a tendency to strongly bind to the carbonaceous component of the sorbent admixture. The membrane acts as a semipermeable barrier, allowing chemicals of interest to pass through to the sorbent, while excluding particulate matter, biogenic material, and other large, potentially interfering substances. The polyethersulfone membrane (Pall Gelman Sciences, Ann Arbor, Michigan) contains water-filled pores, 0.1  $\mu\text{m}$  in diameter, to facilitate transport of the hydrophilic chemicals. The POCIS was designed to mimic respiratory exposure of aquatic organisms to dissolved chemicals without the inherent problems of metabolism, depuration of chemicals, avoidance of contaminated areas, and mortalities of test organisms. Also, dietary uptake of polar organic compounds likely represents only a small fraction of residues accumulated in aquatic organism tissues (Huckins et al., 1997).





**FIGURE 16.2** Comparison of the mean contaminant concentrations between spot water sampling and those predicted by POCIS in (a) upstream, (b) downstream, and (c) effluent outfall, Ouse Estuary, West Sussex, UK. BPA, bisphenol A; E1, estrone; E2, 17 $\beta$ -estradiol; Pro, Propranolol, Sul, sulfamethoxazole; Carb, carbamazepine; Indo, indomethacine; Diclo: diclofenac.

Thus, the POCIS provides a worst-case exposure scenario for aquatic organisms, enables a concentration of sufficient amounts of bioavailable hydrophilic organic chemicals for some biomarker tests, and permits determination of the biologically relevant time-weighted average concentrations in water.

The POCIS devices can be deployed at the sampling site for a duration ranging from 1 week to 2 months (Alvarez et al., 2005; Zhang et al., 2008). Quality control is achieved using both fabrication and field blanks ( $n = 3$ ) for each analytical technique. Fabrication blanks account for any background contribution due to interferences from POCIS components and for contamination incurred during laboratory storage, processing, and analytical procedures. Field blank POCISs are used as quality control (QC) samples for transport, deployment, and retrieval procedures (note that these POCIS blanks are sealed again in the same shipping cans and stored frozen during the exposure period). The field blank POCISs are treated identically as the deployed devices, with the exception that they are not exposed to waters at the monitoring sites.

The procedures for the recovery of sequestered chemical residues from the deployed POCIS are as follows: Briefly, the POCISs are disassembled and the sorbent transferred into glass gravity-flow chromatography columns or glass beakers (Alvarez et al., 2005; Zhang et al., 2008). Chemical residues are recovered from the sorbent by organic solvent elution/extraction. Methanol is widely used to recover the pharmaceuticals. The extracts are reduced in volume by rotary evaporation and under a gentle stream of nitrogen, and then are ready for further instrumental analysis.

Zhang et al. (2008) has described the use of POCIS for analysis of emerging contaminants, including pharmaceuticals, and compared the predicted compound concentrations in water with those measured by spot sampling. As shown in Figure 16.2, for the pharmaceuticals propranolol, sulfamethoxazole, carbamazepine, indomethacin, and diclofenac, their mean aqueous concentrations measured by spot sampling varied: 3.0–45.6 ng L<sup>-1</sup>, <LOD–17.6 ng L<sup>-1</sup>, 16.6–539 ng L<sup>-1</sup>, 0.4–7.2 ng L<sup>-1</sup>, and 2.4–65.2 ng L<sup>-1</sup>, respectively. Their concentrations predicted by POCIS were 2.8–40.5 ng L<sup>-1</sup>, <LOD–18.2 ng L<sup>-1</sup>, 26.3–427 ng L<sup>-1</sup>, 0.5–11.9 ng L<sup>-1</sup>, and 4.4–165 ng L<sup>-1</sup>, respectively. It is apparent that for most samples, the predicted pharmaceutical concentrations by POCIS are similar to those by spot sampling. In addition, POCIS was validated and deployed for monitoring pharmaceuticals in estuarine systems (Togola and Budzinski, 2007), confirming the potential application of passive samplers for routine monitoring of seawater quality.

### 16.3 SAMPLE PREPARATION

The sample preparation is an important step in analysis, particularly since the concentration levels of pharmaceuticals found in environmental water samples are generally too low to allow a direct injection into a chromatographic system. Therefore, efficient preconcentration steps are necessary that should also result in some sample cleanup. Several techniques have been developed and optimized, with solid-phase extraction (SPE) being the most frequent. Also, solid-phase microextraction (SPME), liquid-phase microextraction (LPME), and lyophilization have been applied (Fatta et al., 2007). In a review of thirty-two pharmaceutical studies, Fatta et al. (2007) found that most (twenty-eight studies) used SPE for extraction from water samples. This extraction procedure can be based on multiple equilibria between the liquid phase and the sorbent in SPE cartridges.

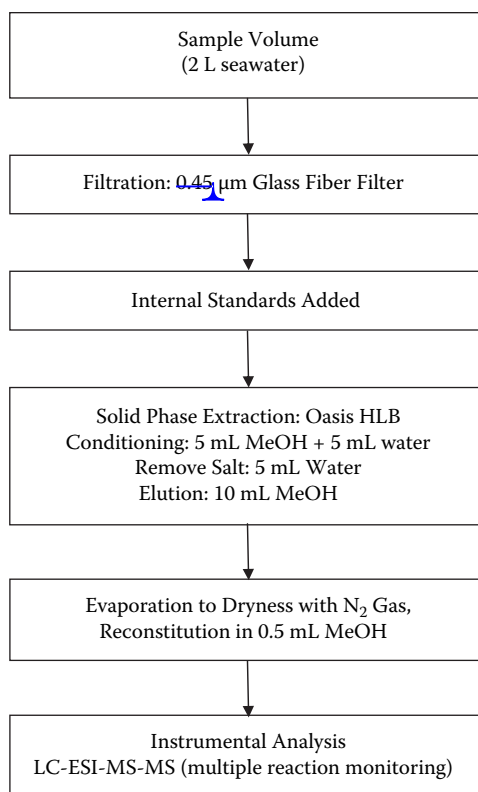
Pharmaceuticals of adequate hydrophobicity can easily be preconcentrated using any reversed-phase material such as alkyl-modified silica or polymer-based materials (Buchberger, 2007). Deprotonation of acidic compounds and protonation of basic compounds should be suppressed to ensure sufficient hydrophobicity of the analytes. Therefore, acidic pharmaceuticals should be preconcentrated under acidic conditions, whereas basic analytes should be preconcentrated at an alkaline pH range. Alternatively, mixed-mode SPE materials can be used that exhibit both reversed-phase and cation exchange properties due to the presence of sulfonic acid groups on the hydrophobic surface of the particles. Using acidified sample solutions, acidic and neutral analytes would be extracted by hydrophobic interactions, whereas protonated basic analytes would interact

via ion exchange mechanisms. Such an approach has been used by, among others, Stolker et al. (2004) for SPE of a set of thirteen pharmaceuticals of different classes. Mixed-mode materials with reversed-phase and anion exchange properties have been used under slightly basic conditions for antibiotics containing carboxylic acid functionality (Benito-Pena et al., 2006).

From the practical point of view, it might be desirable to extract pharmaceuticals from water samples without any pH adjustment (Buchberger, 2007). Furthermore, various (neutral) pharmaceuticals may exhibit significant hydrophilic properties, which make it difficult to enrich them on conventional alkyl-modified silica materials. SPE procedures for extraction of polar compounds from aqueous samples are still a big challenge in analytical chemistry. A recent review has summarized new SPE materials that can improve the recoveries for polar analytes (Fontanals et al., 2005). These SPE cartridge materials are mainly polymeric sorbents that improve the retention of polar compounds either by novel functional groups in the polymeric structure (resulting in a hydrophilic-hydrophobic balance material) or by considerably increased surface area. Some of these new materials have turned out to be well suited for multiclass analysis of pharmaceuticals in water samples. A number of different SPE stationary phases (Table 16.2) have been evaluated for the extraction of the selected pharmaceutical compounds (Hilton and Thomas, 2003; Weigel et al., 2004; Zhang and Zhou, 2007). Nowadays, one of the most widely used sorbents is a copolymer of divinylbenzene and vinylpyrrolidone, which has been commercialized under the trade name Oasis-HLB by Waters. Weigel et al. (2004) demonstrated that this sorbent can simultaneously extract acidic, neutral, and basic pharmaceuticals at neutral pH. Multiresidue methods for different classes of pharmaceutical using Oasis-HLB at neutral pH have also been reported recently by Barceló and co-workers (Gomez et al., 2006; Gros et al., 2006). Trenholm et al. (2006) developed a comprehensive method for the analysis of fifty-eight potential endocrine-disrupting compounds and pharmaceuticals using a single SPE step based on Oasis-HLB. Various other studies can be found describing the successful use of Oasis-HLB for pharmaceuticals in water (Petrovic et al., 2006; Zhang and Zhou, 2007). A typical multiresidue analysis would include filtration of the seawater, conditioning of the Oasis HLB material (between 60 and 500 mg packed into a suitable cartridge) by several milliliters of methanol and ultrapure water, application of up to 2 L of sample at a flow rate of approximately 10 ml min<sup>-1</sup>,

**TABLE 16.2**  
**Different Types of SPE Cartridges Being Used for Pharmaceutical Extraction**

Cartridge	Description	Manufacturer
DSC-C <sub>18</sub>	Polymerically bonded, octadecyl	Supelco
DSC-Si	Unbonded acid-washed silica sorbent	Supelco
DSC-SCX	Aliphatic sulfonic acid, Na <sup>+</sup> counterion	Supelco
DSC-SAX	Quaternary amine, Cl <sup>-</sup> counterion	Supelco
Strata-X-CW	Polymeric weak cation	Phenomenex
Strata SDB-L	Styrene-divinylbenzene polymeric	Phenomenex
Chromabond-Easy	Bifunctionally modified polystyrene-divinylbenzene adsorbent resin	Macherey-Nagel
Chromabond-C <sub>18</sub> Hydra	Octadecyl-modified silica	Macherey-Nagel
Chromabond-Drug	Modified silica	Macherey-Nagel
Isolute C <sub>18</sub>	Octadecyl	International Sorbent Technology
Isolute C <sub>18</sub> /ENV <sup>+</sup>	C <sub>18</sub> hydroxylated polystyrene-divinylbenzene	International Sorbent Technology
Isolute C <sub>8</sub>	Octadecyl functionalized silica	International Sorbent Technology
Oasis HLB	Poly(divinylbenzene- <i>co</i> - <i>N</i> -vinyl-pyrrolidone)	Waters
Oasis MCX	Poly(divinylbenzene- <i>co</i> - <i>N</i> -vinyl-pyrrolidone, -SO <sub>3</sub> H)	Waters
Varian Bond Elut C <sub>18</sub>	Irregularly based acid-washed silica	Varian



**FIGURE 16.3** The procedures involved in the extraction and analysis of pharmaceuticals in estuarine and seawater samples.

rinsing the cartridge with several milliliters of ultrapure water to remove salt, drying the SPE material by applying a vacuum to remove excess water, elution with approximately 10 ml of methanol (it may be necessary to repeat the elution step), evaporation of the extracts under a gentle stream of nitrogen (see Section 15.3.4), and reconstitution in 0.5 ml of methanol or a mixture of methanol and ultrapure water containing internal standards that are then ready for instrumental analysis. The whole analytical procedure is illustrated in Figure 16.3 (Zhang and Zhou, 2007). All the glassware used for the extraction is baked at 400°C for 4 hours to eliminate any organic contaminants. All the solvents are of distilled-in-glass grade.

Hilton and Thomas (2003) have shown that Strata-X is useful for extracting selected pharmaceuticals, after comparing seven types of SPE cartridges. So in a few cases Strata-X (a polydivinylbenzene resin containing piperidone groups manufactured by Phenomenex) has been employed for generic SPE procedures (Hilton and Thomas, 2003; Roberts and Bersuder, 2006). Nebot et al. (2007) also used Strata-X successfully to determine the concentrations of a range of human pharmaceuticals in surface and wastewater, but none of the target compounds were above limit of detection (LOD) in seawater samples collected offshore Scotland. Strata-X may have properties similar to those of Oasis-HLB, but at present there is not enough data in the literature to allow this comparison to be made.

SPE of pharmaceuticals is often done offline, and is useful for on-site sampling (performing the preconcentration step in the field, followed by the elution step in the lab). It is a technique that is also well-suited for online procedures and automation in the laboratory. The SPE cartridge can be installed in the injection valve instead of the injection loop, and the preconcentrated analytes directly

eluted onto the analytical column. An example of this approach is the work of Pozo et al. (2006), who determined sixteen antibiotics in surface and groundwater samples. In such a setup, the SPE cartridge is generally reused for a series of samples. Contrary to this configuration, fully automated SPE procedures with single-use cartridges can be realized by commercially available instrumentation, such as the Symbiosis<sup>TM</sup> Environ manufactured by Spark ~~Ltd~~. This robotic system includes an automated cartridge exchange module that transfers the cartridge after the preconcentration step into the flow of mobile phase of HPLC (Rodriguez-Mozaz et al., 2007). This approach, which has primarily been used with pharmaceutical residues analysis, could be applied in seawater monitoring. There are some significant advantages with the approach (Fatta et al., 2007):

1. Direct injection of untreated seawater samples
2. Automatic sample cleanup or analyte enrichment
3. Elimination of conventional manual sample pretreatment steps
4. Faster procedures
5. Methods are less prone to errors, resulting in better reproducibility
6. Reduction of health risks
7. Samples can be run unattended (e.g., overnight or over the weekend)

## 16.4 INSTRUMENTAL ANALYSIS

The majority of pharmaceuticals lack sufficient volatility and as such are not directly compatible with GC analysis (Buchberger, 2007). Various groups of pharmaceuticals can be derivatized to make them suited for GC analysis. Although such procedures may be time-consuming and can introduce errors due to side reactions during the derivatization, they are still widely in use and well established for routine work. The major advantage of GC-MS is the fact that the usual ionization modes such as electron impact (EI) or chemical ionization (CI) are generally less affected by the matrix of the sample than ionization modes used by, for example, HPLC-MS. Typical derivatization reagents for acidic pharmaceuticals include pentafluorobenzylbromide (Reddersen and Heberer, 2003), methyl chloromethanoate (Weigel et al., 2004), methanol/BF<sub>3</sub> (Verenitch et al., 2006), and tetrabutylammonium salts (for derivatization during injection) (Lin et al., 2005). Phenazone-type drugs have been derivatized by silylation using *N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) (Zuhlke et al., 2004). Silylation procedures are also commonly used for synthetic estrogens (Quintana et al., 2004; Fernandez et al., 2007), although careful selection of the reagent and the reaction conditions is necessary to avoid side reactions (Shareef et al., 2006). Derivatization reactions that are useful for sorptive extraction combine with thermal desorption GC.

Generally, the use of GC-MS seems to be a well-established approach for residue analysis of pharmaceuticals. Correctly, there is a trend toward tandem MS techniques as the MS component of choice for this type of analysis. The advantages of such instruments will be discussed in more detail in the context of HPLC-MS.

Despite the indisputable merits of GC procedures for residue analysis of certain classes of pharmaceuticals, HPLC shows much more universal applicability (Buchberger, 2007). In some cases, when just a few analytes of a certain class are to be analyzed, even a simple UV absorbance detection may be feasible. This has been demonstrated for residues of oxytetracycline in water, which can be detected at 360 nm (Himmelsbach and Buchberger, 2005). Fluorescence detection may also have some benefits, as shown for the determination of some other compounds, such as anthracycline cytostatics and fluoroquinolones (Mahnik et al., 2006; Golet et al., 2001, 2002). Nevertheless, MS detection involving atmospheric pressure ionization, such as electrospray ionization (ESI), is nowadays state of the art.

Although single-quadrupole instruments have been successfully used, when HPLC-MS procedures started to be developed for pharmaceutical residue analysis (Ahrer et al., 2001), more sophisticated mass analyzers are nowadays commonly employed that allow an unequivocal confirmation

of the identities of the analytes. Triple quadrupole (QqQ) MS instruments have become widely used with HPLC for environmental analysis. When using a QqQ instrument, false positive results can be avoided if the ions of at least two ion-ion transitions are used in combination with at least one ion intensity ratio. Several studies have dealt with HPLC-QqQ/MS for multiclass analysis of pharmaceuticals (Castiglioni et al., 2005; Rodriguez-Mozaz et al., 2004; Miao et al., 2004; Ternes et al., 2005; Gomez et al., 2006; Gros et al., 2006; Zhang and Zhou, 2007). Precursor ions and product ions used for quantification and confirmation purposes have been compiled for a wide range of pharmaceutical compounds (Petrovic et al., 2005).

An example of a typical multipharmaceutical residue analysis by HPLC-QqQ/MS is given in Figure 16.4 (Zhang and Zhou, 2007). The LC separation system is equipped with a Waters Symmetry C<sub>18</sub> column (4.8 mm × 75 mm, particle size 3.5 μm). The mobile phase is made of eluent A (0.1% formic acid in ultrapure water), eluent B (acetonitrile), and eluent C (methanol). The flow rate of the mobile phase is 0.2 ml min<sup>-1</sup>. The gradient elution is operated with 10% of eluent B, followed by a 25-minute gradient to 80% of eluent B and a 3-minute gradient to 100% of eluent B, and then changed to 100% of eluent C within 8 minutes, and hold there for 10 minutes. The system is reequilibrated for 10 minutes between runs. Typically the injection volumes are 1 to 10 μl.

A general and well-known problem of HPLC-ESI/MS is ionization suppression due to matrix components eluting at the same time as an analyte. On the one hand, ionization suppression effects can reduce the sensitivity of the method considerably; on the other hand, special care must be taken to achieve reliable quantitation. Ideally, isotopically labeled analytes should be used as internal standards but are available only in few cases for pharmaceuticals. Otherwise, standard addition methods must be applied to obtain correct quantitative data; however, such methods increase the length of the analytical procedure considerably. In all cases it makes sense to optimize the efficiency of the sample cleanup protocols in order to minimize interferences. New-generation MS instruments in some cases allow a dilution of the sample extract before injection into the HPLC system, thereby minimizing any matrix effects. Furthermore, other MS detectors such as time of flight (TOF) are more selective than QqQ, and hence more suited for samples of highly complex matrices, such as wastewater or biological extracts. However, the sensitivity of TOF currently is not as good as QqQ, and requires further development.

## 16.5 ANALYTICAL QUALITY CONTROLS

To ensure data quality, all the analytical processes should be subject to strict QC procedures to determine systematic and random errors. QC measures in relation to estuarine and coastal water analysis include the collection of blank water samples derived from laboratory-grade or organic-free water to determine if sampling procedures, sampling equipment, field conditions, sample shipment and storage (field blank), or laboratory procedures (laboratory blank) introduced target analytes into environmental samples. The spiked water samples are used to check the precision and recoveries. The blank and spiked water samples are typically made by ultrapure water taken from a Milli-Q system. Typically, several blank and spiked samples are produced with each set of real samples (ten samples for each set). In addition, the random errors involved in sampling are assessed by carrying out replicate sampling of water at the same site and the analysis of sample extracts. Internal standards (usually the target compounds labeled by stable isotopes such as <sup>13</sup>C or <sup>2</sup>H) are used to compensate for losses involved in the sample extraction and workup, to further characterize the method performance. Prior to use, all glassware is rinsed twice with dichloromethane and methanol, or is baked at 450°C for 4 hours. All the solvents used are of distilled-in-glass grade. All these processes are carried out to minimize the cross-contamination and the loss of analytes through adsorption onto the surface of sampling vessels and the extraction apparatus. As seawater has a very different matrix to freshwater, more appropriate blanks should be used in the future for seawater studies. As an example, synthetic seawater prepared by dissolving appropriate amounts of key ionic substances (e.g., NaCl) in ultrapure water at a concentration identical to that in real

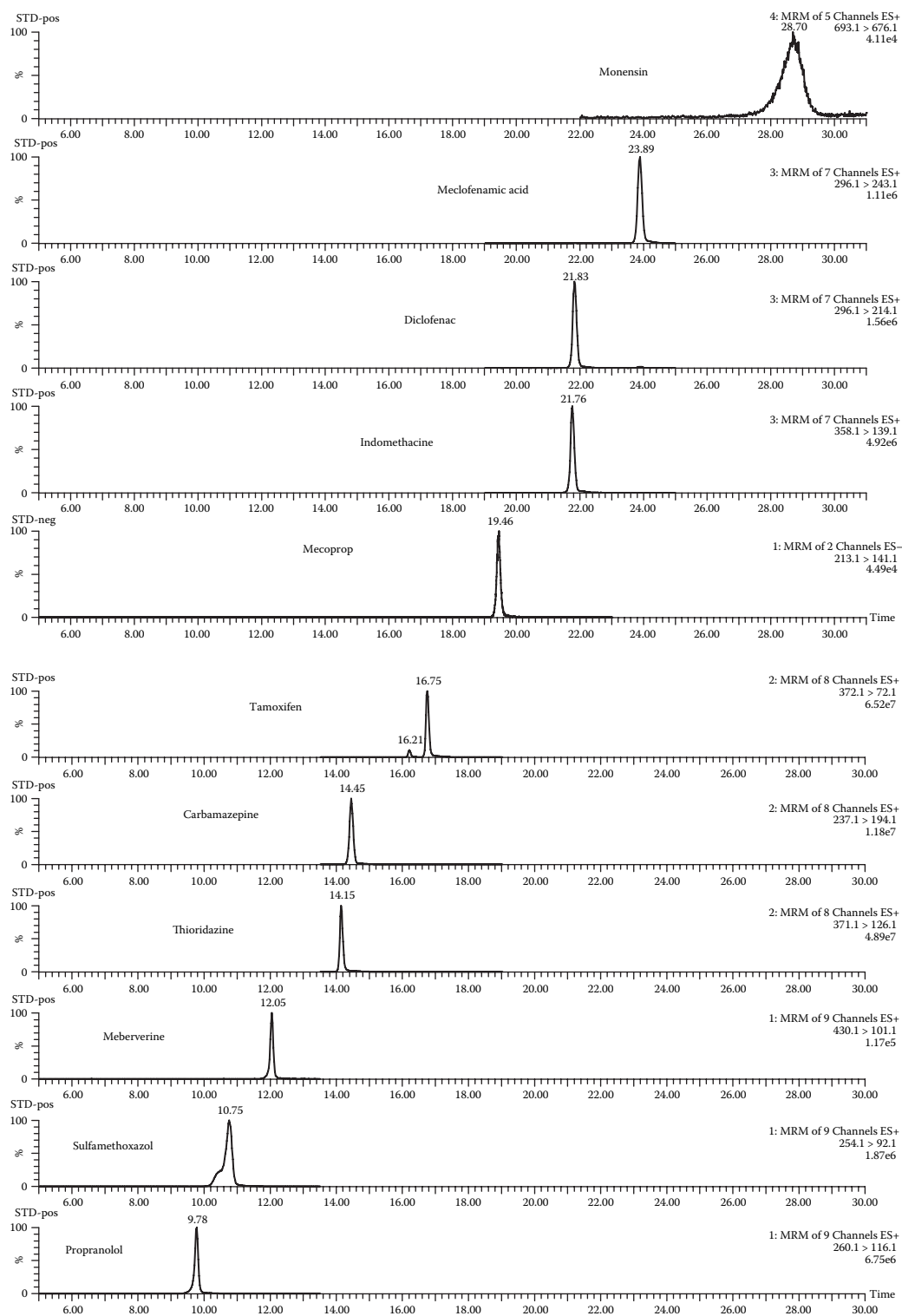


FIGURE 16.4 Chromatograms for the extracted and overlapped MRM of selected pharmaceutical compounds.

seawater of 35‰ salinity should be a more appropriate matrix for making blank samples and spiked recovery samples.

To assess systematic errors, most would use so-called recovery experiments by spiking known amounts of each target compound in seawater, followed by extraction and analysis. This gives a good indication of how reliable your measurement values are (see Chapter 1). In addition, certified reference materials (CRMs) for pharmaceuticals in seawater should be prepared in the future that can identify the closeness between a measured value (from an individual laboratory) and a certified value (from the supplier). As CRMs are vigorously tested under varying environmental conditions by the supplier and independently verified by laboratories worldwide, they become a calibration tool for the international community on pharmaceutical research. Ideally, the relative difference between measured and certified values should be as small as possible (e.g.,  $\pm 10\%$ ). Such materials will ensure that everyone follows the right procedures and generates data of highest quality. In addition, this practice will ensure monitoring data obtained from any marine samples anywhere can be compared against each other, so as to identify hotspots of pharmaceutical pollution, emergence of new pharmaceuticals, and temporal variation of pharmaceutical concentrations in the estuarine and marine environment.

## 16.6 IMMUNOANALYTICAL TECHNIQUES

Immunoassays show attractive features for organic trace analysis due to the fact that they require little sample pretreatment, exhibit high sensitivity, and are inexpensive in comparison to the instrumental analysis described above. A considerable number of immunoassays have been developed and used for residue analysis of pesticides in water samples, but immunoassays for pharmaceuticals in the aquatic environment are still quite rare. Although test kits for pharmaceuticals are commercially available, these kits are in most cases optimized for samples like blood, urine, or food. The applicability to environmental samples has not been investigated in the majority of cases. Deng et al. (2003) developed a highly sensitive and specific indirect competitive enzyme-linked immunosorbent assay (ELISA) for the determination of diclofenac in water samples. When they applied the assay to analysis of diclofenac in tap and surface water as well as wastewater in Austria and Germany, they showed that ELISA-derived diclofenac concentrations in wastewater samples were about 25% higher than those using GC-MS. The technique should be equally applicable to seawater matrices, although the sensitivity and selectivity should be further improved.

## 16.7 SUMMARY AND PERSPECTIVES

Analysis of emerging pollutants such as pharmaceutically active compounds in the aquatic environment, including seawater, was reviewed in this chapter. As pharmaceutical compounds are usually present at trace levels (e.g.,  $\text{pg L}^{-1}$  to  $\text{ng L}^{-1}$ ) in complex matrices (e.g., seawater), it is common practice to develop extraction and analytical methods that can concentrate the target compounds while minimizing matrix interference. The analytical procedure involves many interrelated steps, including sample pretreatment (e.g., filtration), preconcentration (e.g., SPE), and analysis by advanced techniques (e.g., HPLC-MS). Residues of pharmaceuticals have most probably been present in our aquatic environment since their application, but only recently have advances in analytical chemistry and instrument performance allowed analysis of compounds at low  $\text{ng L}^{-1}$  concentrations. The development of advanced mass spectrometric detectors for chromatography has made a significant contribution to these achievements. The limits of detection of analytical methods may be improved even further during the next few years. Residues of pharmaceuticals in aquatic systems are not yet included in regular monitoring programs. The high costs of instrumental analysis may be prohibitive to more extended studies. A focus on a limited set of pharmaceuticals that are representative in regard to toxic effects may be advantageous (but a final selection of such a set has not yet been

done). The importance of reliable and inexpensive biosensors may increase in the future, provided that they meet the criteria of analytical QC in the same way as traditional techniques do.

In addition, general QC procedures must be followed, including appropriate replicate sampling, sample preservation at 4°C, application of isotopically labeled internal standards, suitable blanks, satisfactory recovery of the target compounds, and eventually use of [certified reference materials](#) (either in-house or commercial ones). Although pharmaceutical residues in the environment are a major concern and have been widely studied in freshwater systems, the study of their occurrence, behavior, and impacts in coastal waters has been quite limited. In addition, the residues of pharmaceuticals in aquatic systems are not yet included in the regular monitoring programs of regulatory bodies. Further research on emerging pollutants such as pharmaceuticals should be extended to coastal and marine ecosystems, including polar regions, where our understanding of pharmaceutical occurrence and behavior is very limited. The low cost and robustness of passive sampling such as POCIS is recommended for routine monitoring of pharmaceuticals and other similar pollutants by governmental agencies.

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