

## Analysis of Trace Pharmaceuticals and Related Compounds in Municipal Wastewaters by Preconcentration, Chromatography, Derivatization, and Separation Methods

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A significant portion of pharmaceuticals and other organic chemicals consumed by people and animals are released into municipal wastewater treatment plants. Most of them are degraded during the wastewater treatment processes, but some of them degrade only partially and may be widely transported and dispersed into the aquatic environment. This is why efficient and fast analytical methods are needed for detection of organic compounds in wastewaters at trace levels. Because wastewaters often consist of complex matrices and high-molecular mass materials, e.g., lignocellulosic biomass, which may bring challenges to the sample preparation procedures, efficient preconcentration methods such as solid phase extraction (SPE) solid phase microextraction (SPME), or single-drop microextraction (SDME) are needed. The most common analysis methods are gas chromatography (GC) and liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS). The aim of this review is to give an overview of chromatographic and spectroscopic methods when characterizing low- and medium-molecular weight organic pollutants, mainly focusing on pharmaceuticals, biocides, and personal care products in environmental matrices.

*Keywords: Extraction techniques; Gas Chromatography; Derivatization; Liquid Chromatography; Mass Spectrometry; Municipal wastewater; Pharmaceuticals; Personal care products; Separation methods; Single-drop microextraction; Solid phase extraction; Solid phase microextraction*

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### INTRODUCTION

Organic compounds can be divided according to their molecular weight. Low molecular weight (LMW) usually refers to compounds with a molecular weight lower than 350 Da (Fiorentino *et al.* 2003), including monomeric and often volatile compounds. Compounds with a molecular weight of more than 1000 Da are considered to be of high molecular weight (HMW), while medium molecular weight (MMW) represents compounds with a molecular weight between 350 and 1000 Da. Organic compounds with a high molecular weight include polymers, lignocelluloses, and humic substances, but compounds with toxic properties found in environmental matrices often belong to the MMW class, together with pharmaceuticals, personal care products, and biocides.

Typically, effluent from a municipal wastewater treatment plant contains on the average 200 mg/L COD, 110 mg/L BOD<sub>5</sub>, and 150 mg/L suspended solids (SS), while the amount of total nitrogen and phosphorous are 20 and 2 mg/L, respectively (Champagne and Li 2009). The primary sludge typically has a pH of about 6.9 and

contains 4% SSs of which 29% is cellulosic material. Municipal wastewaters, sludges, and biosolids include fecal materials, scraps of toilet paper, food residues, humic substances, and other organic materials that contain large quantities of cellulosic and lignocellulosic substances.

Municipal wastewaters contain a wide variety of organic compounds in trace amounts, including pharmaceuticals and personal care products (PPCPs). For example, medications, nutraceuticals, detergents, perfumes, and cosmetics have been widely detected in surface and wastewaters (Metcalf *et al.* 2003a,b). Studies have shown that PPCPs may cause behavioral changes in aquatic organisms, even at low concentrations (Kümmerer 2004; Fent *et al.* 2006). Concentrations of pharmaceuticals found in the receiving water reflect the concentrations in wastewaters and the persistence of the compounds. It has been found that pharmaceuticals occur more widely in surface water than groundwater because the surface waters are more often affected by wastewater effluents (Rohweder 2003). However, increasing the water flow in wastewater treatment plants or in rivers does not lead to a proportional dilution of the pollutants (Giger 2009).

Most pharmaceuticals are found in low concentrations, and some of them are polar and not easily degradable. Antibiotics are used in human and veterinary medicine worldwide. They are an interesting subject because their effect on the natural environment is insufficiently known (Giger 2009). As reported by Göbel *et al.* (2007), significant elimination of antibiotics does not occur during primary wastewater treatment. On the other hand, some antibiotics are partially removed in activated sludge systems, fixed-bed reactors, and membrane bioreactors. However, chemicals such as most fluorochemicals are not removed by wastewater treatment plants and can be distributed and conserved within tens of kilometers from the source (Huset *et al.* 2008).

There is a wide range of compounds used as biocides, such as oxidizing compounds (*e.g.*, chlorine and chlorine dioxide), halogenated compounds (*e.g.*, bronopol), and sulfur compounds, including isothiazolone and carbamates. The biological degradation and removal of biocides in wastewater treatment plants have been widely studied (*e.g.*, Okuda *et al.* 2008; Zorita *et al.* 2009), but the process of removal does not always result in a complete mineralization of compounds. The metabolites of pharmaceuticals pass through wastewater treatment plants and end up in rivers and other environmental systems (Wise 2002; Gros *et al.* 2007). It has been shown (Sinclair and Boxall 2003; Juraske *et al.* 2007; Escher *et al.* 2009) that some transformation products of pesticides exhibit a similar or even more toxic effect than their parent compounds.

Transformation products are formed in raw water during processes such as biodegradation, photolysis, hydrolysis, flocculation, oxidation, and disinfection. However, more data about the occurrence and effects of transformation products in the environment and during water treatment are needed (Zwiener 2007). There are various challenges in modern aquatic environmental analysis. Even though most of the pharmaceuticals are designed to be water-soluble and biodegradable, they often have high tendency of bioaccumulation and high affinity to sludge and soil (Aznar *et al.* 2014). Pharmaceuticals in soil have the ability to be taken up by edible plants and enter the food chain (Shenker *et al.* 2011). Because most of the analysis methods are focused on the aquatic samples and polar compounds contained in them, this may lead to incorrect results of concentrations and problems related to sample preparation methods. Therefore, the development of suitable sample preparation and analysis methods plays an important role in the study of the behavior and transportation of chemicals in the aquatic environment.

The aim of this review is to focus on the analysis methods of LMW and MMW organic compounds detected in trace amounts in waste and natural waters. The focus is on detecting and quantifying target compounds in complex environmental matrices. In addition, current alternatives of analysis methods are discussed, including new applications. However, HMW organic compounds and pollutants from industrial wastewaters are excluded. Considering the wide variety of compounds and the importance of their analysis, a review focused on HMW compounds, including lignocelluloses, is recommended for further research.

## Sampling and Sample Preparation

### *Passive sampling*

Pollutants are often found in natural waters at low ng/L levels, but the results may vary depending on sampling conditions such as rainfall, overload at a wastewater treatment plant, or a recent field spreading of manure. Therefore, samples should be collected frequently, but time, costs, and available resources may restrict such sampling (Camilleri *et al.* 2012). Traditionally, water samples have been collected as grab samples, but in that case, information only reflects the sampling moment, and relevant events such as high or low flow can be missed. Continuous on-line sampling can provide information over extended periods of time but is expensive and needs maintenance, which may prohibit its use, especially during the colder months (Vrana *et al.* 2005).

Fortunately, passive samplers have been developed to optimize water sampling by mimicking the accumulation of hydrophobic substances (Kingston *et al.* 2000; Camilleri *et al.* 2012). Passive sampling is usually based on diffusion through a barrier or permeation through a membrane, but living organisms can also be used as samplers. Generally, passive sampling combines sampling and sample preconcentration into a single step. It often simplifies sampling, sample preparation, and any following steps in the analytical procedure. Power requirements are eliminated, and the costs of analyses are significantly reduced. Furthermore, it is possible to combine passive techniques with grab sampling, which reduces the time required for the analytical sequence (Górecki and Namieśnik 2002).

Contaminant concentrations over longer time periods can be sampled with passive samplers such as semipermeable membrane devices (SPMDs) and polar organic chemical integrative samplers (POCISs). They are designed for the accumulation of hydrophilic or lipophilic substances (Södergren 1987; Huckins *et al.* 1990; Vrana *et al.* 2005). The receiving phase of SPMD is made of polyethylene tubing or other polymer-containing hydrophilic or lipophilic thin films, made of, *e.g.*, triolein or hexane. This method is based on an equilibrium state between the phase and the environment, *e.g.*, air or water.

POCISs are based on the adsorption of pollutants with phases made of polymer and used for the sampling of polar and hydrophilic compounds in water (Alvarez *et al.* 2005; MacLeod *et al.* 2007; Söderström *et al.* 2009). POCISs are easy to use and resistant to biofouling (Alvarez *et al.* 2004). In addition, Alvarez *et al.* (2005) reported that, compared to grab water samples, POCIS can improve detection limits, enabling the detection of compounds with low concentrations. POCIS sampling has been employed, for example, for pesticides (diuron and isoproturon), PPCPs (axithromycin, fluoxetine, levothyroxine, and omeprazole), and illicit drugs such as methylenedioxy methamphetamine, and methamphetamine, but the analyses have mostly been qualitative (Jones-Lepp *et al.* 2004). Quantitative analysis of polar compounds in water is limited

because a targeted calibration study is needed for most PPCPs. However, a qualitative and semiquantitative technique has been reported for analyzing common PPCPs in flowing water (MacLeod *et al.* 2007). The time-weighted average concentrations calculated using sampling rates obtained from laboratory calibrations were consistent with those from regular grab sampling.

The Chemcatcher was developed to monitor polar and hydrophobic compounds in water matrices (Kingston *et al.* 2000). The Empore disk receiving phase is covered with a polyethersulfone (PES) diffusion membrane and placed in a sampler housing made of polycarbonate or polytetrafluoroethylene (PTFE). A variety of adsorption phases are available for the polar configuration, such as octadecyl (C18), poly(styrenedivinylbenzene) (SDB-XC), and sulfonated poly(styrenedivinylbenzene) copolymer (SDB-RPS) (Ahkola *et al.* 2013). For example, the C18 Empore disk receiving phase provides mainly non-polar interactions with hydrophobic molecules via octadecyl functional groups bonded to silica surface (Greenwood *et al.* 2007). However, the silica material has also non-substituted silanol groups and molecules with polar functional groups have a high affinity towards them. Thus the sorbent is suitable for collecting compounds with different properties. Passive samplers need to be calibrated under specific conditions of pH, temperature, conductivity, and salinity before use (Zhang *et al.* 2008). Even though the exposure conditions cannot be reproduced in real life and variations between laboratory and *in situ* calibrations occur, analyte concentrations obtained by passive sampling generally are in good agreement with those from grab sampling.

#### *Solid phase extraction*

Sample preparation is one of the most important steps of the analysis procedure. Previously, sample preparation of a variety of environmental samples most commonly was performed by liquid-solid (LSE) or liquid-liquid extraction (LLE) with organic solvent, followed by clean-up and preconcentration steps. Because these methods are labor- and time-consuming, costly, and require discarding toxic solvents, there has been a need to develop faster and more environmentally friendly extraction methods (Jiang *et al.* 2006). Currently, the most widely used preconcentration method for analytes in aqueous matrices is solid phase extraction (SPE) (*e.g.*, Jiang *et al.* 2006; Garcia-Ac *et al.* 2009; Kim *et al.* 2013). Other preconcentration methods include solid phase microextraction (SPME), single-drop microextraction (SDME), dispersive liquid-liquid microextraction (DLLME), and vortex-assisted liquid-liquid microextraction (VALLME). The advantage of these techniques is that they require only small volumes of potentially harmful organic solvents.

To reach low enough detection limits (ng/L) for analytes in environmental samples, a preconcentration step is almost always needed. For the preconcentration, SPE is the most widely used method because, in addition to the extraction of organic compounds, it also removes interfering components from the matrix (Rodriguez-Mozaz *et al.* 2007). However, in order to achieve a detection limit below 10 ng/L, high enough extraction volumes are required. For example, for the extraction of water samples with C18 SPE cartridges a volume of 250 mL is needed (Cherta *et al.* 2012).

The SPE method is based on physical adsorption of organic compounds on the surface of solid supports. Only a small volume of solvent compared to traditional LLE is required because the analyte is directly extracted from the liquid sample onto the sorbent material. SPE combines the enrichment and extraction steps and provides high sensitivity. In addition, it is clean, selective, rapid, and efficient. SPE can be used with

different detection techniques on-line as well as off-line. In on-line applications, no sample manipulation is required between preconcentration and analysis, which reduces the risk of loss and contamination (Das *et al.* 2012). It also improves the precision and accuracy of the analysis and increases the sample throughput (Rodriguez-Mozaz *et al.* 2007).

A wide range of SPE materials is available. The trapping mechanism of SPE is often based on hydrophobic interactions, and the most common SPE sorbent materials for the enrichment of contaminants are alkyl-bonded silicas (*e.g.*, C2 or C18 silicas) and copolymer sorbents. Because the more selective SPE step provides better sensitivity, tailor-made sorbent materials have been developed for the extraction of target materials (Rodriguez-Mozaz *et al.* 2007).

#### *Solid phase microextraction*

Solid-phase microextraction (SPME), originally developed by Arthur and Pawliszyn (1990), has become popular in the analysis of organic compounds because it combines sampling and pre-concentration in one step (López-Blanco *et al.* 2003). SPME is a simple and solventless method based on extraction with a thin polymeric-coated fused-silica fiber, fitted in a syringe-type holder for sampling. When the fiber is exposed to an aqueous solution, organic compounds are extracted. After sampling, the fiber is transferred to the heated injection port of a GC system, where the analytes are thermally desorbed. This procedure takes only minutes and can be easily automated (Psillakis and Kalogerakis 2001b).

Compared to SPE, SPME requires smaller sample volumes and gives higher limits of detection (Hernández *et al.* 2001). In the SPME, the analytes are extracted from various gas or water media by fused-silica fibers coated with a stationary phase. The technique integrates sampling, extraction, concentration, and sample introduction into one step (Wu and Huang 1999). SPME fibers can be divided into adsorbent- and absorbent-type fibers. Adsorbent-type fibers extract the analytes by physical interaction, while absorbent-type fibers extract by partitioning the analytes into a liquid-like phase (Luks-Betlej *et al.* 2001).

SPME sampling can be divided into two categories: immersion sampling, where the fiber is immersed in the aqueous sample, and headspace sampling, where the fiber is exposed to the headspace above the liquid or solid sample (Lord and Pawliszyn 2000). Immersion sampling enhances extraction, while headspace sampling works well for analytes of low molecular mass. In addition, an increase in the sample temperature enhances headspace extraction (Kirkbride *et al.* 1998), but it is not recommended for non-volatile or thermally unstable compounds such as octogen (HMX).

SPME is suitable for the extraction of non-polar organics such as fuel components, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) in water samples. In addition, many pesticides, phenols, organic acids, and aliphatic amines can be extracted because more polar solvents and *in situ* derivatization techniques for SPME have been developed. Ideally, SPME of organic acids and bases can be performed without a derivatization step, especially if no derivatization is needed for the following analysis (van Doorn *et al.* 1998). SPME fibers can be used for up to 100 extractions, but they are fragile and expensive. In addition, a thermal conditioning step is required before using a fiber for the first time, and a partial loss of coating may still occur, resulting in extra peaks during chromatographic analysis (Psillakis and Kalogerakis 2001b).

Van Doorn *et al.* (1998) reported that organic acids and bases can be selectively extracted by SPME by adjusting the pH of water samples before the extraction. The sorption behavior of organics can be predicted on the basis of the acid dissociation constant in water. Many organic acids and bases have dissociation constants in the same range as water samples (*e.g.*, surface water from 5 to 8), but relatively small changes in pH have a dramatic effect on their SPME/water distribution behavior. In addition, quantitative errors can occur when the pH of the sample and that of the calibration water vary significantly. The selectivity of SPME can be increased by buffering the water samples. On the other hand, a pH of the sample solution in the range of 3 to 9 does not have a significant effect on the extraction efficiency when using Carbowax templated resin (CW-TPR) or polydimethylsiloxane-divinylbenzene (PDMS-DVB) fiber, but the absorption decreases if the pH is adjusted to 11 due to the ionization of the weak acids. The temperature, 20 °C has been shown to be optimum for the absorption, while at 10 °C, the absorption and the rate of diffusion decrease. Additionally, the amount of analytes absorbed decreases with increasing temperature due to a decrease of the distribution constant (Wu and Huang 1999).

There are several types of commercially available SPME fibers. A fiber coating should be selected with a polarity close to the polarity of the target analytes (Namieśnik *et al.* 2000). For polar analytes, polar CW-TPR provides the highest extraction yields, while moderately polar PDMS-DVB fiber is more suitable for less polar analytes (Wu and Huang 1999). Generally, polyacrylate (PA) and carbowax-divinylbenzene (CW-DVB) show higher fiber/water sorption coefficients than polydimethylsiloxane (PDMS)-coated fiber. Depending on the water pH, PA fiber is more selective for acid or basic components than CW-DVB fiber (van Doorn *et al.* 1998).

#### *Single-drop microextraction*

To reduce solvent consumption and the time required for extraction, an alteration of the traditional liquid-liquid extraction method called single-drop microextraction (SDME) has been introduced (Liu and Dasgupta 1996). In SDME, a microdrop of a water-immiscible solvent is suspended on the tip of a microsyringe and immersed in the sample solution. Organic contaminants are transferred to the organic phase, and the microdrop is retracted into the syringe after the sampling and used for further analysis. SDME has been successfully used for analyses of, *e.g.*, alcohols (Tankeviciute *et al.* 2001), nitroaromatic explosives (Psillakis and Kalogerakis 2001a), chlorobenzenes (Wang *et al.* 1998), and volatile organic compounds in water (Buszewski and Ligor 2002).

SDME extraction involves only a few microliters (0.5 to 2.5  $\mu\text{L}$ ) of organic solvent and can be viewed as a virtually solvent-free sample preparation technique. Only a small amount of sample is needed, and sample concentrations of a few mg/L are sufficient. SDME eliminates the problems of peak tailing and sample carry-over often encountered in LLE and SPE and also the fiber degradation of SPME (López-Blanco *et al.* 2003). In addition, only inexpensive equipment commonly found in laboratories are needed, thus minimizing the costs of analysis per sample (Psillakis and Kalogerakis 2001b).

SDME and SPME are equilibrium techniques, and the determination is possible in 15 to 20 min, much quicker than SPE, which is an exhaustive technique. It is possible to achieve 100% recovery of the analyte in SPE, but it requires a longer analysis time and larger volumes of organic solvent. Because SDME is dependent on the solubility of target

compounds, the choice of the water-immiscible solvent is essential (López-Blanco *et al.* 2003). For example, for  $\alpha$ - and  $\beta$ -endosulfan, de Jager and Andrews (2000) recommended hexane, but López-Blanco *et al.* (2003) suggested isoctane because of its higher extraction capacity.

In general, the choice of the organic solvent for SDME should be based on a comparison of selectivity, extraction efficiency, incidence of drop loss, rate of drop dissolution, and the level of toxicity. For example, toluene can be used because it has good selectivity and almost no solvent is lost. The main drawbacks of SDME compared to SPME are the prolonged extraction times and the fact that faster stirring rates are not recommended because they usually result in drop dissolution and/or dislodgment. In addition, the microdrop-based method requires careful manual operation (Psillakis and Kalogerakis 2001b).

#### *Dispersive liquid-liquid microextraction*

Rezaee *et al.* (2006) introduced dispersive liquid-liquid microextraction (DLLME) based on a ternary component-solvent system. A water-immiscible extractant solvent dissolved in a water-miscible dispersive solvent is injected into an aqueous donor solution, leading to a cloudy solution consisting of fine droplets of extractant. Target analytes are extracted into these droplets and subsequently separated by centrifugation. However, the method is difficult to automate, and the use of a disperser solvent often decreases the partition coefficient.

A dispersion of extractant into the sample solution can also be achieved using temperature-controlled DLLME, which is based on the ability of an ionic drop to disperse at higher temperature and separate when cooling, followed by centrifugation (Zhou *et al.* 2008). Even though high enrichment factors are gained, analyte losses due to volatilization at high temperatures are possible. Finally, Regueiro *et al.* (2009) explored the possibility of using ultrasound for dispersing the extractant, such as an organic solvent or an organic solvent/ionic liquid mixture, into an aqueous donor phase. The process is also called homogenization and is a known alternative to LLE. Ultrasound speeds up the homogenization step and increases the formation of submicron size droplets and thus the contact surface between the two liquids. However, ultrasound has been found to produce stable emulsions that result in long separation times (Luque de Castro and Priego-Capote 2007).

#### *Vortex-assisted liquid-liquid microextraction*

Vortex-assisted liquid-liquid microextraction (VALLME) is a fast equilibrium-based solvent microextraction technique used for the trace analysis of pollutants in water samples (Yiantzi *et al.* 2010). A mild emulsification is achieved by the dispersion of a low-density organic solvent into an aqueous phase using vortex mixing (Zacharis *et al.* 2012). The fine droplets formed can extract target analytes faster because of a shorter diffusion distance and larger specific surface area. After the extraction, centrifugation is performed to achieve phase separation. The advantage of VALLME is that equilibrium conditions can be achieved within minutes. Only mild conditions are required for emulsification, and problems associated with ultrasound can be avoided. In the case of more complex matrices, analytes can be determined using the addition of a standard. Recently, the method has been extended using a surfactant as a disperser in the emulsification to improve the mass transfer of analytes from the aqueous to the organic phase (Yang *et al.* 2011). However, surfactants often have a low volatility, which may

cause them to contaminate the inlet or the stationary phase of the GC system (Zacharis *et al.* 2012).

#### *Immunosorbent extraction*

Depending on the composition of the sample, sample pretreatment may involve numerous steps. Immunosorbents have been used in the analysis of environmental water samples because they enable extraction, trace enrichment, and clean-up in one step. Immunosorbents have been found to be highly selective (> 90%) and to give nearly quantitative (> 90%) yields (Ferrer and Barcelò 1999; Ferguson *et al.* 2001).

The antibody of the immunosorbent is immobilized onto a silica support and used as an affinity ligand in the extraction of target analytes and similar species from the sample (Ferrer and Barcelò 1999). Even though the stability of the immunosorbent has not yet been established, the sorbent can be used for six times without any noticeable decrease in recoveries (Ferguson *et al.* 2001). In addition, immunosorbents prepared with solid silica supports have a lifetime of more than 100 extraction cycles when used in the isolation of PAHs from sediment and sewage sludge samples (Peréz *et al.* 1998).

Immunosorbent extraction has been used as a selective technique, for example, in the preconcentration of pesticides and polycyclic aromatic hydrocarbons prior to analysis with high performance liquid chromatography (HPLC) or gas chromatography (GC) (Ferrer and Barcelò 1999). In addition, immunosorbent extraction coupled with LC and electrospray ionization mass spectrometry detection (ESI-MS) is also suitable for the determination of other environmentally relevant compound classes, such as pharmaceuticals (Ferguson *et al.* 2001). Currently, pharmaceuticals are often analyzed by LC-MS/MS, and the analyses may be simplified using commercial antibodies (Ternes *et al.* 1998b). Method sensitivity in this case may be enhanced by the extraction of larger volumes (>1 L) of surface water prior to the immunosorbent step.

#### *Molecularly imprinted solid phase extraction*

Molecularly imprinted polymers (MIPs) are synthetic cross-linked polymers that are synthesized in the presence of template molecules (Caro *et al.* 2003; Jiang *et al.* 2006). MIPs are easy to prepare, have high thermal and chemical stability, and can selectively rebind target molecules. In some cases, the MIP also recognizes structurally related analytes in the rebinding step, which is known as cross-reactivity. The two main strategies for the preparation of imprinted polymers are grafting of thin MIP films on the surface of porous silica (Sulitzky *et al.* 2002) and polymerization within the pores of preformed silica beads (Titirici *et al.* 2003). The latter is a simple method because the reagents are added to the pores and heated to allow the polymerization, which leads to a composite material with the same size and shape as the silica beads.

MIPs have been used as sorbents in molecularly imprinted solid phase extraction (MISPE) mostly in an off-line mode (Berggren *et al.* 2000; Martín-Esteban 2001; Lai and Wu 2003). Only a few methods have been developed using an on-line mode with MISPE-LC (Masqué *et al.* 2000; Koeber *et al.* 2001; Martín-Esteban 2001). For example, Caro *et al.* (2003) reported a method for the analysis of polar phenols with SPE coupled on-line to LC. Usually, polar phenols cannot be accurately quantified at low levels because the humic substances in the matrix disturb the quantification of most polar compounds. With the polymer of 4-chlorophenol and 4-vinylpyridine as a selective sorbent, the disturbances are removed and a linear response is gained. MIPs have been used in the determination of various pharmaceuticals in biosamples, but only rarely in environmental



matrices (Koeber *et al.* 2001). However, they are reportedly suitable for the analysis of environmental and biological matrices, and trace analysis as well (Jiang *et al.* 2006).

#### *Extraction of volatile compounds*

Numerous techniques, such as LLE and purge-and-trap (PT), are used in the extraction of volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs) (Antoniou *et al.* 2006). In addition, headspace analysis (HS), SPME, closed-loop stripping analysis (CLSA), and capillary membrane sampling-flow injection analysis (FIA) can be used in their extraction. The extraction and concentration steps of VOCs and SVOCs include active sampling by cartridges with sorbent-packed tubes or passive sampling. The analytes can be recovered by solvent extraction or thermal desorption. VOCs and SVOCs are often separated from sludge by the PT method or taken with a gas-proof syringe. They can be separated from wastewater sludge by SPME, which is fast and simple and eliminates the need for solvents. The amount of extraction medium is very small compared to the sample volume.

The extraction of volatile analytes by SPME can be performed using direct immersion (DI) or headspace (HS) techniques. For semi-volatile compounds, DI-SPME seems to be more widely used, while for volatile compounds, HS-SPME is more appropriate (Guimarães *et al.* 2008). In HS-SPME, the fiber is exposed to the vapor above the solid or liquid sample, while in direct immersion SPME, the fiber is placed directly in the sample (Kotowska *et al.* 2012). HS-SPME is a multi-phase partitioning system where an equilibrium is reached between the sample matrix and the headspace and also between the headspace and the extracting phase. The isolation process is affected by process conditions such as temperature, pH, and extraction time. On the other hand, VOCs and SVOCs can be extracted by SPME from water samples as well. DI-SPME has been used, for example, for the analysis of organic pollutants such as nitrobenzene, nitrotoluene, and triazines (Kotowska *et al.* 2012).

The analytes are concentrated in the stationary phase of the SPME fiber, introduced directly into the GC-injector, and thermally desorbed. Antoniou *et al.* (2006) introduced a fast and simple method using HS-SPME-GC for the determination of halogenated VOCs (*e.g.*, chloroform, bromoform, and chloromethane) in drinking water and wastewater treatment plant effluents. The method is simple and fast, as no complex instrumentation or pretreatment procedure is required, and allows detecting VOCs at ng/L levels. In addition, the method requires no solvent and is inexpensive because the SPME fiber can be used about 60 times.

HS-SPME is also suitable for the extraction of volatile and semi-volatile organic compounds from wastewater sludge (Kotowska *et al.* 2012). HS-SPME coupled with GC-MS enables the detection of a wide range of highly volatile (*e.g.*, methanol and acetone) to moderately volatile (*e.g.*, hydrocarbons with more than 20 carbon atoms) compounds. For example, the method is suitable for quantitative analysis of volatile phenolic compounds derived from lignin in aqueous matrices (Kolb *et al.* 2013). HS-SPME combines the isolation and concentration of analytes in one stage without the risk of contamination, and only small sample volumes (less than 1 mL) are required. To adapt the extraction step for the needs of quantitative analysis, additional isolation techniques such as sorbent tubes need to be considered.

In addition, a method for the determination of phthalates from water samples was reported (Luks-Betlej *et al.* 2001). The application of SPME followed by GC-MS in the single ion monitoring mode (SIM) allows the detection of phthalates in drinking water

with detection limits in the ng/L range. The method is simple, sensitive, portable, and requires no solvent. In addition, the best repeatability in the extractions is gained using CW–DVB fibers.

#### *On-line extraction*

An automated on-line SPE coupled to LC-MS/MS (polar embedded stationary phase C18 + 5  $\mu\text{m}$  PA, mobile phases of water and acetonitrile, mixtures of 0.01% HCOOH in acetonitrile and 0.01% HCOOH in water) has been introduced for analyzing pesticides and their metabolites (Hernández *et al.* 2001). In particular, when analyzing a group of compounds with varying hydrophobicities, the optimization of conditions with the classical reverse phase sorbents is challenging. The automated on-line SPE is suitable for samples with relatively clean matrices, *e.g.*, surface water, but it remains a challenge to adapt the method for wastewater treatment plant effluents. However, it is one of the most promising techniques for the rapid extraction and preconcentration of most pharmaceuticals.

On-line SPE connected to LC-MS/MS has also been used for the detection of sulfonamides in aqueous samples with excellent detection limits (pg/L). The approach affords full automation and minimum sample handling and requires only low sample volumes (5 to 40 mL, depending on the matrix). In addition, reproducibility, throughput, and sensitivity are improved. Compared to off-line methods, the number of samples analyzed by the on-line method is almost twice as high (García-Galán *et al.* 2010).

SPME has been used as a sample preparation method, but its throughput has been low because of a lack of suitable extraction phases and automation (Chia and Huang 2005). However, multiple parallel extractions can be performed with the aid of an automated robotic unit, where the amount of analyte extracted by SPME is proportional to the volume of the extraction phase (Vuckovic *et al.* 2008). For example, a configuration of a robotic autosampler has been reported that enables 96 parallel analyte extractions with detection limits of low ng/mL (Cudjoe *et al.* 2009).

An automated on-line SPME requires relatively expensive instrumentation (García-Galán *et al.* 2010). In addition, clogging may occur when handling matrix-rich environmental water samples without prior filtration. Sample pretreatment procedures are often required, which may result in the loss of matrix-bound analytes. To overcome these challenges, a new SPME method with a C18 extraction phase deposited as a thin film (TF-SPME) has been used for the extraction of pharmaceuticals in biological samples. In the TF-SPME, particles are immobilized on a flat, thin, stainless steel surface that allows a larger surface-area-to-volume ratio (Cudjoe *et al.* 2009). The amount of target analyte extracted with C18 film is improved compared to traditional SPME as a result of a larger volume of the extraction phase in the film geometry. SPME extraction sensitivity is improved because of the film geometry and a higher extraction rate throughput (Togunde *et al.* 2012).

Commonly, MISPE is used in an off-line mode, but there are a few on-line applications as well (Mena *et al.* 2002; Caro *et al.* 2003; Vian *et al.* 2005; Watabe *et al.* 2004). In an on-line mode, an MIP column and an analytical column are connected by a column switching system coupled to an HPLC. The MIP column is used for pretreatment to enrich and extract analytes from the sample, while the actual separation is performed in the analytical column (usually a C18 silica column). Compared to the off-line mode, the on-line mode requires no sample manipulation between the pretreatment and analysis steps, which decreases the risk of contamination and loss of analytes. In addition, the

consumption of organic solvents is lower, while the reproducibility and the limit of detection (LOD) are improved. However, the sorbents in the pretreatment and analytical columns must be compatible. The column switching system increases the complexity of the HPLC system and the analysis costs. Equivalent methods for on-line sample pretreatment have been proposed, such as immunoextraction coupled to HPLC and atmospheric-pressure chemical ionization mass spectrometry (APCI-MS) (Ferrer *et al.* 1997) or injection directly to HPLC-ESI-MS (Ferguson *et al.* 2001).

#### *Effect of polarity*

There are various challenges in modern aquatic environmental analysis. In addition to the detection of a pollutant, the formation, environmental fate, and bioactivity of its transformation products need to be evaluated (Giger 2009). The transformation products can be more persistent, more (eco-)toxic, and more polar than the parent compounds, resulting in an altered environmental distribution behavior, *e.g.*, a higher mobility in aquatic systems (Sinclair and Boxall 2003).

In an aquatic environment, organic pollutants can be dissolved in water or adsorbed to suspended particles, such as humic substances or sediments (Esteve-Turrillas *et al.* 2007). However, they are less available to aquatic organisms due to their limited diffusion capability through the cell membrane. The affinity to adsorption of a compound increases with the water temperature and the octanol-water partition coefficient ( $K_{ow}$ ). The value of the coefficient increases with increasing lipophilicity. Additionally, the lipophilicity of a compound increases with increasing value of  $\log K_{ow}$ .

Even though a large part of pollutants are polar, the slightly polar and non-polar compounds still contribute to aquatic pollution (Fischer *et al.* 2012). For example, pharmaceuticals are commonly designed to be polar (Togunde *et al.* 2012). Generally, polar compounds are adsorbed by polar materials, such as silanol, which contains polar OH groups. On the other hand, non-polar compounds are well adsorbed by non-polar materials, such as those with octadecyl chains. The detection of polar and non-polar compounds requires different preconcentration methods with different materials, but also different methods of analysis. For example, polar compounds are commonly analyzed by LC-MS or LC-MS/MS (Balakrishnan *et al.* 2006), while GC is more suitable for slightly polar and non-polar compounds. However, the less volatile compounds and compounds with polar functional groups and active hydrogens often require a derivatization step prior to GC-MS analysis to enhance their volatility (Togunde *et al.* 2012; Aznar *et al.* 2014). Recently, two dimensional GC combined with time-of-flight mass spectrometry (TOF MS) has been used for the detection of non-polar and slightly polar pollutants *e.g.* organochlorine pesticides and chlorobenzenes (Muscalu *et al.* 2011).

#### *Matrix effects in analyses*

Matrix effects depend on the type of matrix and analytes. It has been shown that the most polar compounds are most affected by the matrix (Garcia-Ac *et al.* 2009). Most likely, the polar compounds are not sufficiently far away from the solvent front, and a more selective analyte extraction and more thorough sample cleanup before injection may be required. For quantitative analysis, the addition of a standard is required to overcome the problems with a changing matrix. The standard should be similar to the analyte and preferably show a similar behavior in the analysis. For example, pharmaceuticals can be determined using similar pharmaceutical compounds as internal standards. However, pharmaceuticals used as standards may also be present in the

samples. Thus, chemical compounds not often used in neighboring countries are recommended instead (Van De Steene *et al.* 2006).

The matrix effect should be evaluated and eliminated as much as possible. A good way to study matrix effects is to use isotopically labeled internal standards (Benijts *et al.* 2004; Petrović *et al.* 2005; Taylor 2005) or structural analogues that are not present in the sample. The problem with environmental samples is that there often are a number of compounds present. In addition, different matrices, such as river water and wastewater, have to be examined. In LC-MS/MS analysis, a buffer can be used to reduce possible pH effects. However, concentrations of buffer components should be kept low to keep them from suppressing the signals.

The matrix effect can be suppressed using lower flow rates in LC-MS systems (Van De Steene *et al.* 2006). Compared to a common flow rate (1 mL/min), a lower 200 µL/min flow rate shows practically no matrix effects. Especially in the ESI mode, if too many compounds are eluted and ionized at the same time, the matrix effect can have an effect on the reproducibility and accuracy. At a lower flow rate, smaller droplets are formed and the total surface area of the droplets increases. Competition between the analyte and matrix components at the droplet surface is reduced, and more analytes can be ionized (Kloepfer *et al.* 2005).

## METHODS OF ANALYSIS

### Gas Chromatography

Organic environmental pollutants in aqueous matrices can be divided into categories such as volatile, semi-volatile, nonpolar/lipophilic, polar/hydrophilic, and amphiphilic compounds. Typically, chemicals of a particular group can be determined by the same analytical method. The two most important separation techniques are gas chromatography (GC) and liquid chromatography (LC). Both techniques can be coupled to mass spectrometry (MS) for qualitative and quantitative analysis (Giger 2009). A selection of compound groups detected in different environmental matrices, *e.g.*, in surface and wastewaters, and their analysis methods based on GC are described below and finally summarized at the end of this chapter in Table 1.

#### *Antibacterial agents*

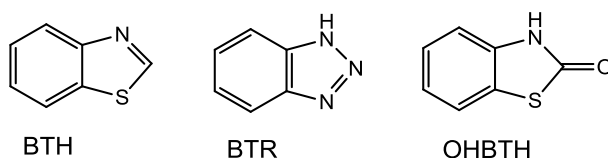
Many compounds found in aqueous samples can be analyzed by GC-MS. The antibacterial agent triclosan (TCS) and its transformation product methyltriclosan (MTCS) are monitored worldwide in a variety of environmental samples such as influents and effluents of municipal wastewater, surface waters, and sediment samples. Generally, TCS and MTCS in aqueous and solid samples can be analyzed, for example, by LLE or SPE combined with LC-MS/MS or GC-MS with electron impact ionization (EI) or electron-capture negative ionization (ECNI) techniques. For example, TCS in municipal wastewater effluents can be pretreated by reversed phase C18 SPE, derivatized with *tert*-butyldimethylsilyl (TBDMS), and analyzed by GC-MS with the detection limit of 1 ng/L (Cheng *et al.* 2011).

Derivatization must be performed to increase the volatility of TCS and achieve high resolution in GC-MS analysis. Silylation is a common derivatization procedure. Cantwell *et al.* (2010) compared two silylation reagents, N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) and N-(*tert*-butyldimethylsilyl)-N-methyltrifluoroacetamide

(MTBSTFA), and found that the latter yields a stable TBDMS-TCS product and a good separation from MTCS. However, derivatization with diazomethane or silylation reagents such as TBDMS or pentafluorobenzoate (PFBA) can be laborious and time-consuming. Cheng *et al.* (2011) showed that direct injection-port derivatization of various organic pollutants reduces solvent waste and simplifies sample preparation. Additionally, to improve detection sensitivity, a large-volume sample injection device can be used.

#### *Benzothiazoles and benzotriazoles*

Benzothiazoles (BTHs) and benzotriazoles (BTRs) are water-soluble compounds used as biocides, corrosion inhibitors, and vulcanization accelerators in rubber production, to name but a few (Fig. 1). They are resistant to biodegradation and only partially removed in wastewater treatment (McNeill and Cancilla 2009) and thus widely distributed in municipal and industrial wastewaters. BTHs and BTRs also can be determined by GC coupled to electron impact mass spectrometry (EI-MS), but the procedure suffers from interference caused by the complex matrices.



**Fig. 1.** Molecular structures of benzothiazole (BTH), benzotriazole (BTR), and 2-hydroxybenzothiazole (OHBTB)

Often, LC-MS or LC-MS/MS is used for the determination of BTHs and BTRs in environmental samples, but ionic suppression in the electrospray ion sources may occur due to charge competition with compounds provided by the matrix (Giger *et al.* 2006). To overcome this problem, BTHs and BTRs can be determined by GC-MS or by two-dimensional GC×GC-TOF MS (Jover *et al.* 2009). However, analysis by GC-MS with conventional polar columns may suffer from column bleeding, where a small amount of diagnostic ions can coelute with coextracted compounds.

Alternatively, BTHs and BTRs can be determined by GC-MS from environmental matrices using ionic liquids (ILs) as a stationary phase (Dominguez *et al.* 2012). ILs have been applied as a stationary phase in GC-MS analysis and in two-dimensional GC×GC (Purcaro *et al.* 2010). They have been used in several applications, such as separation of fatty acids and their methyl esters (Delmonte *et al.* 2011).

ILs have become interesting because of their unique physicochemical properties (Tobiszewski *et al.* 2009). Room-temperature ILs are commonly composed of asymmetrically substituted organic, nitrogen-containing cations such as imidazole, pyrrolidine, or pyridine with inorganic anions (*e.g.*, Cl<sup>-</sup>, PF<sub>6</sub><sup>-</sup>, BF<sub>4</sub><sup>-</sup>). The properties of ILs often include low volatility, high viscosity, and good thermal stability (Sun and Armstrong 2010).

In addition, ILs can separate both polar and nonpolar compounds (Ding *et al.* 2004; Sun *et al.* 2011) and provide increased unique selectivity and high efficiency in the determination of complex mixtures (Bianchi *et al.* 2011). The high selectivity is caused by the resolution of polar compounds from non-polar ones, which is useful when analyzing complex mixtures of both (Sun *et al.* 2011).

### Nitrosamines

Nitrosamines (NAs) belong to the nitrogen-containing disinfection by-products whose main generation source is the final disinfection of drinking water, especially when chloramines are employed as the disinfectant agent (Wang *et al.* 2011). NAs such as caffeine (CAF) are polar compounds, and they are usually water-soluble and difficult to extract with organic solvents. Caffeine is metabolized in mammals by *N*-methylation to dimethylxanthines. The non-metabolized substances are mostly excreted in urine as unchanged substances that can be found in wastewater treatment plant effluents (Halling-Sorensen *et al.* 1998). The contaminants found in the environment are often highly polar and cause low selectivity when analyzed with low-polarity stationary phases which result in high background noise with polar GC-MS columns (Reyes-Contreras *et al.* 2012). Depending on the compound, detection limits of 19.5 to 186 ng/L have been achieved.

For the detection of NAs, analytical methods, such as GC coupled to a thermal energy analyzer (TEA) (Vieira *et al.* 2006), nitrogen phosphorous detector (NPD) (Calderón-Preciado *et al.* 2011), MS (Grebel *et al.* 2006), MS/MS (Munch and Bassett 2006), and high-resolution mass spectrometry (HRMS) (Planas *et al.* 2008) have been used. Commonly, the GC columns used for these analyses are made of cyanopropylphenyl dimethyl polysiloxane, trifluoropropyl methylpolysiloxane, polyethylene glycol, or diphenyl dimethyl polysiloxanes. Alternatively, ILs can be used as a stationary phase. ILs as stationary phases in GC-MS analysis have been reported as suitable for the detection of NAs and CAF metabolites in wastewater samples (Reyes-Contreras *et al.* 2012). CAF and other NA metabolites can also be quantified by GC-MS/MS from wastewater and ground water samples. For the analysis of CAF metabolites, ionic liquid stationary phase SLB-IL59 (1,12-di(triethylphosphonium)dodecanebis (trifluoro-methylsulfonyl) imide) provides elution with the highest peak symmetry in an acceptable analysis time.

### Amines

The analysis of amines with common analytical techniques is often difficult because of their basic character and because highly polar amines are not easily extracted from the polar water matrix (Baltussen *et al.* 1998). The amino group induces a large dipole moment, which is responsible for a strong interaction with silanol groups and siloxane bridges in the stationary phase of the GC capillary column. As a result, nonlinear adsorption effects and strong tailing peaks in the chromatogram may occur. To prevent this, the amines need to be derivatized. The derivatization reagent is selected on the basis of the derivatized functional group and other functional groups in the molecule (Knapp 1979). Acylation, alkylation, and silylation can in principle be used in the derivatization of amino groups contained in alkyl amine structures.

Primary alkyl amines are often determined with GC and derivatized with pentafluorobenzoyl chloride (Baltussen *et al.* 1998) or with pentafluorobenzaldehyde (Ngim *et al.* 2000). In addition, acetyl and trifluoroacetyl derivatives can also be used (Knapp 1979). However, long-chain primary alkyl amines can be determined by GC with simultaneous FID or NPD as well as GC-MS. Trifluoroacetylated derivatives of *tert*-octadecylamines in water samples can be quantitatively determined with GC coupled to FID or NPD. Long-chain primary alkyl amines can be determined with GC-MS, GC-FID, or GC-NPD after derivatization with trifluoroacetic anhydride (TFAA) (Kusch *et al.* 2006). Silylation with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) is not

suitable, but acylation with trifluoroacetic anhydride (TFAA) converts active hydrogen sites (-NH, -OH, -SH) into amides, esters or thioesters.

### *Steroids*

Steroid estrogens can be determined by GC-MS, but their derivatization to more volatile forms is required and the detection limits often remain above the ng/L level. Because the levels of estrogens are likely to be low in the aquatic environment due to dilution, degradation, and sorption, sensitive analytical techniques are required to determine their concentration levels. More sensitive results can be obtained by GC-MS/MS, but a derivatization step is still needed (Huang and Sedlak 2001).

For the derivatization of steroid pollutants, samples are often labeled by a hydroxyl group, neglecting a full derivatization, such as oximation of steroids' keto group(s) (Kolpin *et al.* 2001; Andrási *et al.* 2011b). Bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) is the most frequently applied derivatization reagent. However, it is reported to show considerably lower responses compared to N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) and hexamethyldisilazane combined with trifluoroacetic acid (HMDS+TFA) reagents with certain steroids such as androsterone and norethisterone (Andrási *et al.* 2011a).

Steroid hormones in municipal wastewater samples can be extracted by SPE and analyzed with GC-MS (Esperanza *et al.* 2004), but they need to be derivatized by reacting with TMCS and BSTFA before injection into the GC-MS system (Keith *et al.* 2000). Steroid hormones can be analyzed in the SIM mode, which allows detection at the low ng/L range (Sowers *et al.* 2009). On the other hand, it has been shown that concentrations of steroid hormones in wastewaters can be much higher (Martinović *et al.* 2007; Tan *et al.* 2007).

### *Volatile compounds*

During a biological sewage treatment process, various volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs) formed in the metabolism of microorganisms may escape to the atmosphere. VOCs and SVOCs are harmful to humans and the environment. Some of them degrade during the sewage treatment, while others remain unchanged. The main VOCs include *n*-alkanes, branched, cyclic, and unsaturated aliphatic hydrocarbons, aromatic hydrocarbons, and halogenated aliphatic hydrocarbons. The most odorous VOCs include dimethyl sulfide, limonene, and  $\alpha$ -pinene. The SVOCs consist of aromatic hydrocarbons, phthalic acid esters, chlorobenzenes, nitroaromatic compounds, and phenols (Kotowska *et al.* 2012).

VOCs in environmental samples are usually analyzed by GC-MS, GC-FID, or by coupling GC with triple quadrupole mass spectrometry (QqQ-MS/MS) (Guimarães *et al.* 2008; Kotowska *et al.* 2012). By means of MS/MS, adequate precursor and product ions can be selected. In most cases, the use of MS/MS improves the sensitivity and the signal-to-noise ratio. Selected reaction monitoring (SRM) mode with two MS/MS transitions gives the possibility of simultaneous confirmation and quantification.

HS-SPME followed by GC-QqQ-MS/MS is a rapid method for the determination of VOCs (*e.g.*, chloroform, benzene, toluene, xylene) in environmental and wastewater samples (Cervera *et al.* 2011). GC-MS (SIM) and GC-MS/MS (SRM) are both suitable for their determination, but the specific ions cannot be measured in the SIM mode at low concentration levels. On the other hand, a fully automated method for determining aliphatic primary amines in wastewater, such as methylamine, ethylamine, and

cyclohexylamine, is based on the simultaneous derivatization with pentafluorobenzaldehyde (PFBA) and HS-SPME, followed by GC and ion trap tandem mass spectrometry (GC-IT-MS/MS) (Llop *et al.* 2010).

**Table 1.** Examples of GC Analysis Sequences of Selected Compounds and Compound Groups Found in Environmental Matrices

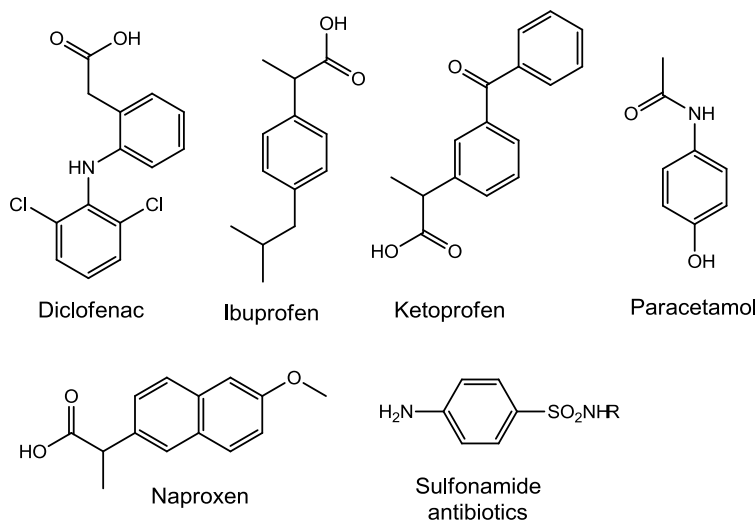
Analyte	Method	Matrix	References
Sulfonamide veterinary drugs ( <i>e.g.</i> , sulphathiazole)	Tishler cleanup-GC-ECD-MS/MS	Bovine and swine liver	Matusik <i>et al.</i> (1990)
$\alpha$ -endosulfan $\beta$ -endosulfan	SDME-GC-ECD	Water sample	López-Blanco <i>et al.</i> (2003)
Primary alkyl amines	SPE-GC-MS, SPE-GC-FID, SPE-GC-NPD	Boiler water, Steam, Condensate	Kusch <i>et al.</i> (2006)
Nitrosamines Nitrosables	Saliva extraction-GC-TEA	Artificial saliva on nipples and pacifiers	Vieira <i>et al.</i> (2006)
Nitrosamines	SPE-GC-CI-MS/MS, SPE-GC-HRMS	Drinking water	Munch and Bassett (2006) Planas <i>et al.</i> (2008)
VOCs (Trihalomethanes, Chlorinated solvents, BTEX)	HS-SPME-GC-MS	Drinking water	Guimarães <i>et al.</i> (2008)
Aliphatic primary amines	HS-SPME-(PFBA)-GC-IT-MS/MS	Wastewater	Llop <i>et al.</i> (2010)
Triclosan Methyltriclosan	SPE-(TBDMS)-GC-MS	Wastewater, Surface water	Cheng <i>et al.</i> (2011)
VOCs ( <i>e.g.</i> , chlorinated and brominated solvents, BTEX)	HS-SPME-GC-MS/MS (QqQ)	Surface water, Wastewater	Cervera <i>et al.</i> (2011)
Benzothiazoles, Benzotriazoles, Benzosulfonamides	SPE-GC $\times$ GC-TOF-MS SPE-IL-GC-MS	Wastewater, River water	Jover <i>et al.</i> (2009) Dominguez <i>et al.</i> (2012)
VOCs and SVOCs ( <i>e.g.</i> , hydrocarbons, alcohols, esters, carbonyls)	HS-SPME-GC-MS	Sludge from wastewater treatment	Kotowska <i>et al.</i> (2012)
Lignin-derived phenolic monomers ( <i>e.g.</i> , guaiacol, vanillin)	HS-SPME-GC-MS	Supernatant of LHW pretreated wheat straw	Kolb <i>et al.</i> (2013)



## Liquid Chromatography

LC-MS/MS has been widely used because of its high sensitivity and selectivity. It is suitable for the determination of a wide range of polar and amphiphilic compounds. Because pharmaceuticals are often polar and non-volatile, they can be analyzed by LC without derivatization. Combined with MS/MS, detection limits of 2 to 13 ng/L can be reached (Togunde *et al.* 2012). Many other semi- or non-volatile classes of organic compounds, such as peptides, proteins, and polycyclic aromatic hydrocarbons, are easier to analyze by LC instead of GC. In addition, excellent selectivity and sensitivity can be achieved by multiple reaction monitoring techniques in tandem or multistage quadrupole and ion-trap spectrometry (MS/MS, MS<sup>n</sup>), which enables the determination of trace compounds in complex mixtures (Giger 2009).

Endocrine-disrupting chemicals (EDCs) are a group of compounds such as natural and synthetic steroid hormones, phthalates, alkylphenols, phytoestrogens, pesticides, surfactants, and polychlorinated biphenyls (Routledge and Sumpter 1997; Clouzot *et al.* 2008). They are known to induce endocrine disturbances and have antagonistic or agonistic properties. EDCs enter the environment from various sources, such as wastewater effluent, agricultural runoff, and landfills. Compounds of interest, such as pharmaceuticals and EDCs, are often present in surface and wastewaters at trace or ultra-trace levels; thus, sensitive analytical methods are required for their detection. A selection of commonly detected pharmaceuticals in environmental samples is presented in Fig. 2, and examples of sequences for analyzing pharmaceuticals, steroid hormones, and other organic compounds found in environmental matrices are summarized at the end of this chapter in Table 2.



**Fig. 2.** Molecular structures of selected anti-inflammatory, analgesic, and antibiotic drugs

### Pharmaceuticals

Pharmaceuticals are widely used as human and veterinary drugs and are commonly excreted in urine or feces discharged into wastewaters (Golet *et al.* 2001). Pharmaceuticals and their metabolites enter the environment primarily *via* wastewater treatment effluents and sludges (Ternes 1998a; Ternes *et al.* 2005). Their transformation processes vary depending on the composition of sewage and the design and operation of the treatment process (Johnson and Sumpter 2001; Roberts and Bersuder 2006). Even though only scant information is available on the behavior of pharmaceuticals in

wastewater treatment (Carballa *et al.* 2004), it has been shown that many pharmaceuticals are not completely removed. This results in their occurrence in effluents, rivers, and lakes, and some of them may remain in the environment for extended periods of time (Carballa *et al.* 2004; Roberts and Bersuder 2006).

Previously, pharmaceuticals such as sulfonamide drugs have been analyzed by HPLC with photo diode array (PDA) detection (Horie *et al.* 1990), but also by GC-MS (Carignan and Carrier 1991), GC-MS/MS (Matusik *et al.* 1990), and GC with electron-capture detection (ECD), or thin-layer chromatography (TLC) (Unruh *et al.* 1990). The enriched wastewater matrix has been found to be too complex for UV detection; tandem mass spectrometry has been shown to be more suitable. Pharmaceuticals such as sulfamethoxazole and sulfadiazine can be analyzed with HPLC-MS in the SIM or SRM mode. SIM is more sensitive than SRM, but it is not as selective (Niessen 1998; Hartig *et al.* 1999).

In analyses of pharmaceuticals in environmental water matrices, HPLC coupled with positive-ion ESI-MS/MS is the method of choice (Hartig *et al.* 1999). An analytical C18 column and a guard column are recommended, coupled with MS/MS detection in the positive or negative ionization mode, depending on the compound. The method shows good reproducibility, sensitivity, and selectivity and attains a low (ng/L) detection range (Roberts and Thomas 2006).

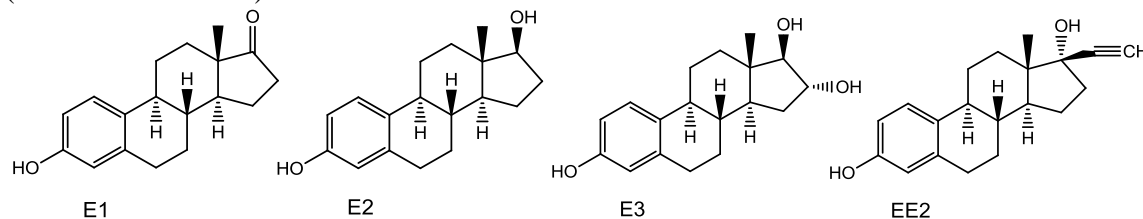
Analgesics and antibiotics are primarily analyzed by LC-MS/MS, and only in some cases by GC-MS/MS after derivatization with TBDMS (Lee *et al.* 2005). However, it is better to avoid derivatization when the thermal stability of the studied compound is unknown. HPLC-MS and HPLC-MS/MS have been used in the determination of pharmaceuticals in surface water, wastewater, and groundwater (Stackelberg *et al.* 2004; Roberts and Bersuder 2006). Acidic pharmaceuticals have previously been detected by GC-MS, but recently, LC-MS methods with ESI have become more important. Also, the most polar compounds and their metabolites can be analyzed by LC-MS with low (ng/L) detection limits (Deng *et al.* 2003).

Anti-inflammatory drugs can be analyzed by immunoassays, but suitable antibodies are still required for most pharmaceuticals (Deng *et al.* 2003). Their transformation products and metabolites are often more polar and water-soluble than the parent compounds. Because low concentrations require preconcentration and extraction, highly polar compounds in aqueous samples are challenging to analyze (Zwiener 2007).

SPE-HPLC combined with QqQ-MS/MS is a precise method for the determination of pharmaceuticals with long environmental persistence or high potential toxicity, such as estrogens and anti-cancer drugs (Castiglioni *et al.* 2005). SPE is performed with a mixed-mode cation exchange (MCX) and a mixed reversed phase-cation exchange cartridge in which the strong cation exchanger sulfonic acid groups are placed on the surface of a poly-divinylbenzene-co-N-vinylpyrrolidone copolymer. Because the cation-exchanger binds basic, ionized compounds, and the reversed phase binds both acidic and neutral compounds, all acidic, neutral, and basic compounds can be extracted. An HPLC system with a C8 column is employed for the analysis and detection with a turbo ion spray source in the range of ng/L to µg/L.

### Steroid estrogens

The sex hormones (natural and synthetic estrogens (Fig. 3), testosterone, progesterone, and androstenedione), like other EDCs, have frequently been detected in sewage waters (Beck *et al.* 2006), from which they often end up in lakes and rivers (Baronti *et al.* 2000).



**Fig. 3.** Molecular structures of natural and synthetic estrogens; estrone (E1), 17β-estradiol (E2) estriol (E3), and 17α-ethynyl-estradiol (EE2)

Synthetic EE2 is engineered from the natural hormone E2. It is more resistant to biodegradation than the natural E2. According to Johnson and Williams (2004), 43% of EE2 is expected to be metabolized in the human body, 27% is excreted as conjugated molecules, and 30% is in the free form. Biological treatments are suitable for the removal of organic matter such as ECDs, while the activated sludge process removes 75% of natural estrogens and EE2. It has been reported that up to 75% of the natural estrogens are mineralized, but the degree of mineralization of EE2 is considerably less (Layton *et al.* 2000). On the other hand, testosterone, androstenedione, and progesterone can be completely removed during biological wastewater treatment (Esperanza *et al.* 2004). In addition, almost all of the estradiol and more than half of the E1 and E2 can be removed. E2 also generates E1 as an intermediate product when degrading, which increases the amount of E1 detected in the final effluents.

Even though the concentrations of hormones in the aquatic environment are low (low ng/L range), they largely contribute to the estrogens of surface waters (Desbrow *et al.* 1998). For example, EE2 is a contraceptive compound that causes feminization of fish even at low (ng/L) concentration levels (Clouzot *et al.* 2008). The occurrence of EE2 in the aquatic environment comes from WWTP effluent due to insufficient purification. Different treatment processes have been tested for the removal of EE2, such as photodegradation (Liu *et al.* 2003), granulated activated carbon, MnO<sub>2</sub>, sand reactor (Rudder *et al.* 2004), and ozonation (Huber *et al.* 2004), but they were shown to be inadequate.

In sewage treatment plants, estrogen conjugates undergo chemical or enzymatic dissociation and re-form active estrogens (Ternes *et al.* 1999). There are low concentrations of estrogen glucuronides in sewage waters compared to corresponding free estrogens (Isobe *et al.* 2003). This suggests that glucuronides are degraded prior to reaching the sewage treatment plant. In addition, acidic pH prevents the dissociation of steroidal conjugates to free steroids. Methods such as isotopically labeled surrogates are needed to identify conjugates of steroid hormones, pharmaceuticals, and other compounds that may transform and are released into wastewaters and surface waters (Reddy *et al.* 2005).

The lowest concentrations of EE2 are often below the LODs (< 2 ng/L), which limits the evaluation of its removal during wastewater treatment (Laganà *et al.* 2004). However, reductions from 37% to 87% between influents and effluents have been measured (Johnson *et al.* 2000; Cargouet *et al.* 2004; Laganà *et al.* 2004), and 60 to 80%

of EE2 is adsorbed on sludge during the activated sludge process. Chemical treatment such as precipitation is inefficient for the removal of EE2. The removal of EE2 is dependent on the sludge retention time (SRT). The higher the SRT is, the more efficiently EE2 is biodegraded. This synthetic hormone is much more resistant to biodegradation than natural ones, but its highly hydrophobic nature makes sorption a significant removal factor in WWTPs (Clouzot *et al.* 2008).

Salste *et al.* (2007) concluded that it is not only estrogens E1, E2, and EE2 that account for estrogenic activity in wastewater effluents. Their often detected low concentrations account for a low percentage of the whole activity. However, other studies (Aerni *et al.* 2004; Beck *et al.* 2006) have shown that natural estrogens are the major causes of estrogenic activity in surface and wastewaters. Even though there are synthetic non-steroidal compounds such as nonylphenol, phthalates, and animal sterols in wastewater effluents, their estrogenic activity is several orders of magnitude lower than that of estrogens, and they are not therefore likely to account for the residual activity (Fernandez *et al.* 2007). In addition, Björkblom *et al.* (2008) suggested that the activity observed may be due to the synergy of detected compounds when detected as a mixture.

Several analytical methods, such as GC-MS and HPLC-MS, have been employed for the detection of steroid estrogens in environmental samples. However, only a few methods are suitable for quantifying steroid conjugates in wastewaters (Pojana *et al.* 2004). The analysis of estrogen conjugates has mostly focused on applications with biological fluids instead of wastewater samples with more complex matrices (Gentili *et al.* 2002; Isobe *et al.* 2003).

The first method in the analysis of hormones was radioimmunoassay (RIA), which has been used for the detection of E2 and EE2 with detection limits of 0.1 to 0.05 ng/L (Snyder *et al.* 1999). However, compounds structurally similar to the analytes are prone to interfere. In addition, an enzyme-linked immunosorbent assay (ELISA) based on an enzymatic reaction has been reported (Huang and Sedlak 2001). Even though it has an excellent sensitivity (0.1 ng/L), it can suffer from interference of a complex matrix if proper purification is not performed prior to analysis (Almeida and Nogueira 2006).

HPLC is considered more suitable than GC-MS for the detection of estrogens and their derivatives because of a less expensive detector and lower maintenance costs, even though polar interference may occur with environmental samples. In addition, a derivatization step is usually needed prior to the GC-MS analysis. However, the hydroxyl group at C17 of EE2 is difficult to silylate, and EE2 may convert to estrone, resulting in an overestimation of E1 and underestimation of EE2 (Shareef *et al.* 2004). Sulfate derivatives of steroid estrogens cannot be analyzed directly by GC-MS, but they must first be dissociated to free estrogens, chemically or enzymatically. However, the hydrolysis of estrogen conjugates may not be efficient enough, and uncontrolled losses of free steroids may occur. In addition, hydrolysis based on enzymes may not act on all forms of conjugates. To overcome these issues, steroid conjugates have been analyzed by HPLC-MS/MS (Zhang and Henion 1999).

Because of the complex matrices, it is challenging to avoid a loss of target analytes during extraction of EE2. Soxhlet extraction with methanol-acetate (1:1) gives above 70% recoveries, and with ethyl acetate, the recovery rate is over 95%; however, Soxhlet extraction is time-consuming and requires large quantities of solvent (Ying and Kookana 2003). Recently, SPME has been considered a good alternative to SPE due to its higher recovery and accuracy. When longer monitoring is needed, passive samplers appear as promising tools to quantify EE2 because of their lower LODs and ability to

take temporal variations into account (Mitani *et al.* 2005). For analyses of EE2 in WWTP effluents, LC-MS is often used to gain low LOD. The lowest LODs are obtained with tandem mass spectrometry, which is better adapted to environmental matrices.

Highly sensitive (0.1 to 5 ng/L) estrogen analysis can also be achieved using LC with APCI-MS/MS detection. It is an attractive method because no derivatization step is required (Laganà *et al.* 2000). However, the instrumentation is expensive, and its application to environmental monitoring is not yet in routine use (Ferguson *et al.* 2001). In addition, steroid estrogens and their glucuronide and sulfate conjugates in sewage influents have been measured by HPLC-ESI-MS/MS (Reddy *et al.* 2005). The purification can be performed by SPE and separation by diethylaminoethanol anion exchange chromatography (DEAE). Natural and synthetic derivatives of estrogenic compounds in municipal and industrial wastewaters can also be detected by HPLC-ESI-MS in the ng/L range (Pojana *et al.* 2004). The method requires extraction with SPE on a C18 cartridge followed by HPLC with a C8 column coupled with MS *via* an electrospray interface. On the other hand, estrogens E1, E2, EE2, and E3 have been determined with LC-MS/MS from wastewater effluents (Björkblom *et al.* 2008).

Because the concentrations of diverse target steroid compounds in environmental samples are low and combined with complicated matrices, robust *in vitro* methods are needed. *In vitro* yeast estrogen screen assays can be used for the determination of estrogenic activity in environmental samples because they respond to all receptor-mediated estrogenic activity, regardless of chemical structure (Campbell *et al.* 2006). For identification of specific estrogenic compounds, the results of biological assays must be combined with GC-MS or LC-MS analyses. For example, the estrogenic activity of SPE (Oasis HLB) concentrated steroid estrogen in municipal wastewater samples has been determined with bioluminescent yeast assays and with LC-MS/MS (Salste *et al.* 2007). Significant differences in estrogenic activities have been reported (Furuichi *et al.* 2004) when combining the results of chemical analysis and biological assays. However, Salste *et al.* (2007) reported that the results were in good agreement with those of chemical analysis.

### *Illicit drugs*

Residues of illicit drugs can be found in municipal wastewater and surface waters (Jones-Lepp *et al.* 2004). Illicit drugs found in the environmental media are the most widely used ones, such as cannabinoids, amphetamines, opioids, and cocaine. The molecules found in environmental media are mostly urinary excretion products, unchanged parent drugs, or their metabolites (Castiglioni *et al.* 2008).

GC-MS and LC-MS are considered most suitable when quantitative analysis of illicit drugs is needed (Kraemer and Paul 2007). Because the amounts of residues are expected to be low, LC-MS offers the needed selectivity and sensitivity (Castiglioni *et al.* 2008). Amphetamine, opiates, cocaine, cocaine metabolites, and cannabinoids can be simultaneously extracted by liquid/liquid separation, derivatized, and analyzed with GC-MS. Also, HPLC analyses can be improved by derivatization. For example, primary alkyl amines can be derivatized with, *e.g.*, 1-fluoro-2,4-dinitrobenzene (van der Horst and Holthuis 1988) or salicylaldehyde diphenylboron chelate (Class *et al.* 1986) before HPLC analysis. On the other hand, amphetamines and other hypnotics can be extracted by SPE followed by LC-MS/MS analysis (Bogusz *et al.* 2000), which allows the simultaneous detection of several drugs (Castiglioni *et al.* 2008). Other methods have been introduced

as well, such as GC coupled with chemical ionization (CI-MS) and ion-trap mass spectrometry (IT-MS).

### *Surfactants*

Conventional surfactants are degraded to a great extent in the biological wastewater treatment process, but poly- or perfluorinated surfactants (PFS) are stable against biodegradation and oxidation agents. Because PFS compounds are stable also in advanced oxidation processes, physical methods, such as adsorption or membrane treatment, are the most promising options for their removal (Schröder 2003; Berger and Haukås 2005). Fluorinated surfactants can be almost completely (78 to 99%) removed from wastewater by separation and adsorption on, *e.g.*, activated sludge or granulated activated carbon (GAC) (Schröder *et al.* 2010). However, the disposal of sewage sludge in an environmentally friendly way still needs to be addressed.

The degradation of surfactants can be monitored by HPLC combined with HRMS or MS/MS. Extraction and concentration can be performed with SPE and LC separation with a precolumn combined with a reversed phase C18 column. Anionic perfluorinated surfactants, *e.g.*, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), can be easily determined with high-resolution LC coupled to MS. Degradation products can be determined using MS/MS in a collision-induced dissociation (CID) or high-energy collision dissociation (HCD) mode (Schröder *et al.* 2010).

### *Other groups of compounds*

Resin acids and pimaranes are found in the wood and bark of conifers. They are tricyclic, diterpenic carboxylic acids that act as a natural antimicrobial defense system in trees. When municipal wastewater treatment is integrated with the treatment plant of a pulp mill, resin and fatty acids may also be present in the receiving water body. It is believed that resin acids contribute most of the overall toxicity of typical pulp and paper effluents (Zender *et al.* 1994; Valto *et al.* 2012). They can accumulate in fish and affect their reproduction. In addition, toxic effects have been observed even at low concentrations (20 µg/L) of resin and unsaturated fatty acids (Kostamo *et al.* 2004; Valto *et al.* 2012). HPLC-ESI-MS is a fast, highly sensitive method for the quantitative analysis of resin acids and pimaranes in river waters (McMartin *et al.* 2002). It is suitable for the analysis of resin acids when isomeric speciation is not required. However, the resin acid isomers cannot be separated under the conditions used in HPLC, which cannot be recommended for the environmental monitoring of resin acid isomers.

Resveratrol is a polyphenol with strong anti-oxidative properties. It belongs to a group of phytoalexins that are produced in plants during environmental stress, such as insect or pathogenic attack. They exist as a glycon in wine and are called piceid when bound to glucoside. There are numerous methods to determine the resveratrol derivatives, such as SPE or direct injection followed by GC, HPLC (Chu *et al.* 1998), or capillary electrophoresis (McMurtrey *et al.* 1994). However, most GC methods require a derivatization step to increase the volatility, which is time-consuming and may alter the target compound, for instance by converting the *trans* to the *cis* form (Trela and Waterhouse 1996). The most appropriate method for the separation of *cis*- and *trans*-resveratrol is an RP18 column with diode array detection (Adrian *et al.* 2000), but it requires a relatively long analysis time (Martínez-Ortega *et al.* 2001). Piceid isomers and *cis*- and *trans*-resveratrol can be analyzed by direct injection of the sample, followed by an HPLC step with two monolithic columns (Vian *et al.* 2005). A monolithic column

operates at a higher flow rate than a conventional reverse phase column and thus gives shorter washing and re-equilibration times. The molecules can be separated in a single run without a purification step.

HPLC has been used for the detection of pesticides such as DDT. For example, DDT can be extracted from water samples by SPE followed by reverse phase HPLC analysis with UV detection (Asi *et al.* 2008). The method provides detection of DDT and its various metabolites (*o,p'*-DDD, *o,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, and *p,p'*-DDT) at ng/mL levels.

**Table 2.** Selection of LC Analysis Sequences of Pharmaceuticals, Steroid Estrogens, and Other Compound Groups Found in Environmental Matrices

Analyte	Method	Matrix	References
Sulfonamide drugs ( <i>e.g.</i> , sulfamethazine)	SPE-HPLC-PDA	Meat	Horie <i>et al.</i> (1990)
Nonylphenol, octylphenol, nonylphenol ethoxylates, E2, EE2	SPE-HPLC-RIA	River water, Lake water, Wastewater	Snyder <i>et al.</i> (1999)
Estrogen sulfates ( <i>e.g.</i> , E1-3S, E3-3S, E2-3S, E2-17S, E2-3,17S)	SPE-HPLC-MS/MS (SIM)	Human urine	Zhang and Henion (1999)
Antibiotics ( <i>e.g.</i> , Norfloxacin, Tetracycline)	CLLE-HPLC-ESI-MS, CLLE-LC-MS	Raw water, Drinking water	Stackelberg <i>et al.</i> (2004)
Estrogens E1, E2, E3, EE2	SPE-LC-APCI-MS/MS (MRM), SPE-(PFP)-GC-MS (SIM), SPE-LC-(QqQ)MS/MS	Wastewater effluent	Laganà <i>et al.</i> (2000), Björkblom <i>et al.</i> (2008)
Estrogens E2, EE2	SPE-HPLC-ELISA, SPE-HPLC-GC-MS/MS	Wastewater, Surface water	Huang and Sedlak (2001)
Antibiotics ( <i>e.g.</i> , ibuprofen, naproxen) Estrogens E1, E2	SPE-(TBDMS)-GC-MS, SPE-(PFP)-GC-MS	Sewage influent and effluent	Lee <i>et al.</i> (2005)
Estrogen, glucuronide and sulfate conjugates of estrogen	SPE-HPLC-ESI-MS/MS (MRM)	Sewage influent and effluent	Reddy <i>et al.</i> (2005)
Pharmaceuticals ( <i>e.g.</i> , cardiovascular, anti-cancer drugs)	SPE-HPLC-MS/MS	WWTP effluent	Castiglioni <i>et al.</i> (2005)
Pharmaceuticals ( <i>e.g.</i> , hypnotics, analgesics)	SPE-HPLC-ESI-MS/MS	WWTP effluent, Surface water	Roberts and Bersuder (2006)
Nitrosamines ( <i>e.g.</i> , NDMA, NMEA, NDEA, NDPA, NDPhA)	SPE-HPLC-(dansylation)-fluorescence detector, SPE-HPLC-MS, SPE-HPLC-(QqQ)MS/MS, SPE-UPLC-ESI-MS/MS	Drinking water, Wastewater	Cha <i>et al.</i> (2006), Krauss and Hollender (2008), Wang <i>et al.</i> (2011)

Phthalates such as dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) have been determined from sludge and sediment samples by SPME (CW–DVB fibers) followed by HPLC-ESI–MS (Möder *et al.* 1998). Diethyl phthalates have also been extracted by SPME (with CW–TPR and PDMS-DVB fibers) and analyzed by HPLC-UV (Kelly and Larroque 1999). Phthalates and an adipate ester have also been extracted by SPME (PA fiber) and analyzed by GC–MS (Peñalver *et al.* 2000).

## Detection

The most commonly used ionization methods are atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI), and atmospheric pressure photoionization (APPI) when analyzing, for example, pharmaceuticals with HPLC or UPLC coupled to MS (Cahill *et al.* 2004). In the APPI system, a photon beam is created by a high-intensity discharge lamp, with the vapors of a nebulized solution entering the MS source (Marchi *et al.* 2009). This technique can ionize compounds with various polarities regardless of matrix compounds. It has been successfully applied in the analyses of environmental and pharmaceutical samples (Viglino *et al.* 2008).

ESI and APCI have been the most widely used methods for analyses of polar molecules (Cahill *et al.* 2004; Castiglioni *et al.* 2005) in spite of their limitations. Because ionization efficiencies depend on charge affinity, some nonpolar compounds (*e.g.*, PAHs) are difficult to ionize. During ESI, cations such as Na<sup>+</sup> and K<sup>+</sup> are formed, which can increase the chemical background noise (Hanold *et al.* 2004). Target molecules may not ionize if there are competing compounds present in the sample. Therefore, chemical derivatization has been used to increase sensitivity, even in the case of LC-MS analysis (Palmgrén *et al.* 2005).

GC-MS, LC-MS, and inductively coupled plasma mass spectrometry (ICP-MS) coupled with different separation and extraction methods have been used in the analysis of environmental samples (Richardson 2004). In addition, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is suitable for characterizing biomolecules, polymers, and compounds with a high molecular weight, such as humic substances (Gajdošová *et al.* 2003). It has been used for fingerprinting analysis and the sequencing of protein biomarkers (Stump *et al.* 2003). However, the detection of small molecules in organic matrices is difficult due to detector saturation.

For highly polar compounds such as paraquat and cacodylic acid, very low (pg/L) limits of detection can be reached. MALDI-TOF-MS has mostly been used for qualitative analysis of high-molecular weight compounds, but there are also methods for low-mass compounds (Guo *et al.* 2002). For a quantitative analysis, an isotope-labeled analyte molecule or one structurally analogous to the analyte can be used as an internal standard. MALDI-TOF-MS is a rapid and simple method for quantitative analysis compared to GC and HPLC. Because of its high throughput, it is suitable as a screening method in environmental pollutant monitoring when handling large amounts of samples.

Carbon nanotubes as a matrix for MALDI-TOF-MS eliminate the background ion interference and offer a method for the detection of low-mass compounds (Xu *et al.* 2003). However, carbon nanotubes are poorly soluble, which leads to poor resolution and reproducibility of low-mass analytes. In addition, their impurities can interfere with their function as energy receptacles and transporters (Pan *et al.* 2005). Oxidized and purified carbon nanotubes are water-soluble, and simpler handling procedures with good reproducibility are available. Hu *et al.* (2005) reported a MALDI-TOF-MS method for analysis of low-mass compounds in environmental samples. In particular, highly polar



chemicals can be measured with high sensitivity. In addition, the method has relatively high tolerance for interfering molecules, and only a simple pretreatment, or none at all, is needed before MS detection.

Cationic surfactants are surface-active compounds with one or several hydrophobic alkyl chains and a hydrophilic group with a positive charge. Previously, they were determined by capillary electrophoresis (CE) (Piera *et al.* 1997), HPLC (Radke *et al.* 1999; Norberg *et al.* 2000) or GC (Ding and Tsai 2003) from environmental and industrial samples. However, the methods require large quantities of reagents, are time-consuming, and often need derivatization and chromatographic separation of quaternary ammonium surfactants before analysis. In addition, cationic surfactants form micelles and are strongly adsorbed on the capillary surface, which leads to peak tailing and insufficient separation by the CE method.

MALDI-MS has been used for the investigation of large molecules such as proteins (Sudhir *et al.* 2005), nucleic acids (Gut 2004), drugs (Sekar and Wu 2006), and synthetic polymers (Marie *et al.* 2000). The analysis of small molecules is difficult because of the interference caused by the matrix ions in the  $m/z$  range below 500 Da. However, Shrivastava and Wu (2007) reported that cationic surfactants including cetyltrimethyl ammonium bromide (CTAB) and cetylpyridinium chloride (CPC) can be determined from river and municipal wastewater by single-drop microextraction (SDME) with atmospheric pressure/matrix-assisted laser desorption ionization mass spectrometry (AP-MALDI-MS) without pre- or post-treatment or separation by HPLC, GC, or CE. Even with river and municipal wastewater samples, a clean spectrum can be obtained by SDME followed by AP-MALDI-MS (Shrivastava and Wu 2007). Intense unwanted peaks and interferences arise without SDME, caused by the matrix or suspended materials. SDME coupled to AP-MALDI-MS gives results comparable to hollow fiber-based liquid-phase microextraction (HF-LPME).

#### *Tandem mass spectrometry*

Numerous analytical methods have been used for analyzing pharmaceuticals from aqueous environmental matrices. Mass spectrometry has traditionally been used to measure substances in biological fluids, but residues of illicit drugs can also be found in urban wastewater and river water. Because of the complex matrices of environmental samples, LC-MS/MS is often the most suitable method for the analysis. For example, cocaine and its metabolites have been measured from urban wastewater treatment plants with this technique (Castiglioni *et al.* 2008). When compounds such as synthetic steroids and cholic acids are identified and quantified with GC-MS/MS, multiple reaction monitoring (MRM) gives the best selectivity and sensitivity compared to full scan (FS), multiple ion monitoring (MIM), and selective ion monitoring (SIM) (Andrási *et al.* 2011b).

For the analysis of pharmaceuticals such as bezafibrate, methotrexate, and orlistat, ESI is the best ionization source for LC-MS/MS (Garcia-Ac *et al.* 2011). Protonated target molecules can be detected with a higher relative abundance by ESI compared to APPI or APCI. In addition, it provides better detection limits, increased signal-to-noise ratios, and higher peak areas. Using flow injection analysis (FIA), about 95% higher signal intensities can be obtained. In the presence of a complex matrix, ESI gives about an 80% higher signal compared to APPI and APCI.

In the LC-MS/MS analysis of pharmaceuticals in wastewater samples, the magnitude of the discharge current of the APCI corona needle does not significantly

affect the appearance of the mass spectra, but it has an effect on the intensity of the ion peaks. As stated earlier, (Hanold *et al.* 2004) and Garcia-Ac *et al.* (2011) found that the addition of a photoionizable substance such as toluene to the LC flow can increase the ionization yield of the target compounds and enhance the signal intensity. It can also increase the background noise and decrease the signal-to-noise ratio. The signal-to-noise ratio and the peaks gained by ESI are higher than those with APPI and APCI. In addition, when using on-column ESI, the peak areas of all analytes are higher than those with APPI and APCI. The same trend can be observed when using FIA compared to on-column analysis.

The current understanding about the identity and quantity of transformation products from biocides and pharmaceuticals is fairly limited because there are only a limited number of reference standards for the many plausible transformation products. One recently introduced analytical tool is high-resolution tandem mass spectrometry (HR-MS/MS), including ion trap-time of flight (IT-ToF) or linear ion trap-orbitrap (LTQ-Orbitrap). These techniques provide highly sensitive detection and accurate mass measurements and are thus suitable for the structural elucidation of analytes at low concentrations (Krauss *et al.* 2010). An ion trap tandem-in-time mass spectrometry detection system can perform simultaneous quantitative analysis and characterization of trace level compounds. Compared to single MS, MS/MS increases the selectivity of the determinations in complex matrices such as wastewater (Llop *et al.* 2010). In addition, studies of capillary electrophoresis coupled with MS, MS/MS, and MS/MS/MS have been reported (Bateman *et al.* 1997).

Finally, Kern *et al.* (2010) reported a method for the detection and analysis of transformation products from the degradation of different pharmaceuticals and biocides such as atenolol, bezafibrate, ketoprofen, metoprolol, and venlafaxine. The method is based on HR-MS/MS followed by quantitative analysis. The suggested procedure captures the formation of biotransformation products of micropollutants both qualitatively and quantitatively during activated sludge treatment. The transformation products were detected by high-resolution mass spectrometry and were shown to be also present in the effluent water of a municipal wastewater treatment plant.

#### *Matrix effect in detection*

In quantitative analysis, the matrix has an influence on the ionization process. The matrix effect may differ between ionization techniques, ionization modes, or the equipment used. When analyzing wastewater treatment plant effluent samples, the matrix causes variations in the results depending on the source. Garcia-Ac *et al.* (2011) observed strong matrix effects with APCI and APPI when analyzing pharmaceuticals. However, opposite results have also been reported (Zuehlke *et al.* 2004; Theron *et al.* 2007). In addition, it has been concluded that the matrix effect is dependent on the LC-MS interface, as the ionization mechanism is different, and this may have an effect on the formation of the desired ions in the presence of matrix compounds.

Earlier studies have shown that matrix effects may differ between various ionization techniques, modes (positive or negative), or instruments (Antignac *et al.* 2005). The matrix of wastewater treatment plant effluent gives varying results, depending on the source (Garcia-Ac *et al.* 2011). In addition, it has been shown (Robb *et al.* 2000) that APCI and APPI can be less sensitive to matrix effects than ESI, but according to Garcia-Ac *et al.* (2011), strong matrix effects have been observed with both methods.

The source of the matrix effect is the presence of endogenous substances, *i.e.*, co-extracted organic or inorganic molecules, in the sample. Therefore, it has been suggested that the solution might be a more selective analyte extraction (Benijts *et al.* 2004; Van de Steene *et al.* 2006). The matrix effects on the ionization process can be compensated for by standard additions when analyzing environmental samples. This method is a relatively fast and efficient way to correct signal distortion effects. However, the internal standard does not bind to the sample matrix in a similar way as the analyte which may lead to incorrect results. The extent of error depends on the analyte and the matrix. If the internal standard addition method is performed correctly with sufficient mixing and equilibration, relatively reliable results can be gained.

Ferguson *et al.* (2001) reported a highly selective method for the determination of steroid estrogens that uses a selective immunosorbent in the sample preparation and allows the removal of the interfering sample matrix present in wastewater. Immunoextraction removes much of the isobaric noise, increases the signal-to-noise ratios of the analytes, and gives lower detection limits. Coupled to HPLC followed by ESI-MS, it has been shown to be a sensitive method for the analysis of steroid estrogens in aqueous matrices (Ferguson *et al.* 2001).

Signal suppression or enhancement effects have been widely reported in atmospheric pressure ionization (API) when analyzing samples with complex matrices (Viglino *et al.* 2008). Matrix effects can be evaluated according to equation 1, where  $RW_s$  is the analyte peak area in the water sample spiked with target compounds,  $RW_{ns}$  is the peak area in the original water sample, and  $DI_s$  is the peak area of the deionized water spiked with the same amount of analyte (Garcia-Ac *et al.* 2009).

$$\text{matrix effect (\%)} = \left( \frac{RW_s - RW_{ns}}{DI_s} \right) \cdot 100\% \quad (1)$$

When analyzing samples with complex matrices, co-eluting compounds can result in signal enhancement or suppression. Methods for the determination of analytes in environmental matrices have to be suitable for detecting parent compounds, as well as their metabolites and transformation products. The chosen method of analysis should be able to diminish matrix effects (Van de Steene *et al.* 2006). Often, LC-MS/MS with triple quadrupoles or time-of-flight is considered the best choice (Petrovic *et al.* 2005). Ion trap instruments are more suitable for screening purposes.

Large numbers of compounds are often present in environmental samples such as river water and wastewater. Co-eluting compounds originating from the matrix may cause signal enhancement or suppression. Matrix compounds entering the ion source at the same time with analytes may decrease the ionization efficiency (Taylor 2005). The best way to study matrix effects is to use isotopically labeled internal standards (Benijts *et al.* 2004; Petrovic *et al.* 2005; Taylor 2005) or structural analogues that are not present in the sample.

Van De Steene *et al.* (2006) reported that a lower flow rate injected into the LC-MS system has a beneficial effect on matrix suppression. Especially in the ESI mode, too many compounds are eluting and ionized at the same time, and the matrix effect can affect the reproducibility and accuracy of the method. At a lower flow, smaller droplets form and the total surface area of the droplets increases. The competition between analyte

and matrix components at the droplet surface is reduced, and more of the analyte can be ionized (Kloepfer *et al.* 2005).

Compared to the common flow rate (1 mL/min), a lower flow rate (200  $\mu$ L/min) gives practically no matrix effect. However, a standard addition is required for the quantitative analysis to overcome the problem of a changing matrix. The best result can be achieved using structural analogues as internal standards (Van De Steene *et al.* 2006). In this way, a method such as the SPE–LC–MS/MS gives a minimal matrix effect without significant loss of analytes. Depending on the type of ionization used in the LC-MS/MS analysis, the matrix can have an effect on the quantification. For example, ESI is more likely to produce interference compared to APCI (Dams *et al.* 2003).

## SUMMARY STATEMENTS

1. There are multiple sample preparation methods suitable for the analyses of organic compounds. For example, SPME requires a small volume, and SDME needs practically no organic solvent. These methods are fast and can be easily automated. In the new TF-SPME method, a C18 extraction phase is deposited on a flat, thin, stainless steel surface. The immobilized particles have a larger surface-area-to-volume-ratio and offer a faster analysis rate and an improved sensitivity.
2. On-line extraction methods have been proposed as well. On-line SPE has been reported as one of the most promising techniques for the rapid extraction and preconcentration of most pharmaceuticals, even though the analysis of groups of compounds with varying hydrophobicities is challenging. In addition, a fast TF-SPME analysis can be performed using a robotic autosampler that enables 96 parallel analyte extractions.
3. Currently, analysis methods based on LC-MS/MS are commonly used. Because large proportions of pollutants in the aquatic environment are polar, no derivatization is required. However, in case of slightly polar and non-polar compounds analysis methods based on GC cannot be omitted. For example, the trend towards use of two-dimensional GC is recognizable. Analysis with GC may require the derivatization of analytes to improve their volatility or to suppress competing compounds present in the sample.
4. The most common ionization methods are APCI, ESI, and APPI. The APPI technique can ionize compounds with various polarities, regardless of matrix compounds. It has been successfully applied in the analyses of environmental and pharmaceutical samples. It has been observed that peaks gained by ESI were higher and their signal-to-noise ratios were better than those with APPI and APCI.
5. The compounds with high molecular mass, *e.g.* humic substances commonly found in environmental aquatic matrices especially in Nordic countries, are known to affect the ionization of polar analytes with lower molecular weight. In order to reach reliable analysis results, the matrix effects must be minimized. Matrix effects can be evaluated by using isotopically labeled internal standards or structural analogues not present in the sample. In addition, matrix effects can be suppressed using a lower flow rate in the system of analysis.

6. Often, LC-MS/MS with triple quadrupoles or time-of-flight is considered the best choice to diminish matrix effects. Ion trap instruments are more suitable for screening purposes. Matrix effects may vary between different instruments, ionization techniques, or modes (positive or negative). APCI and APPI can be less sensitive to matrix effects than ESI, but strong matrix effects may occur with both methods.

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