Practical overview of analytical methods for endocrine-disrupting compounds, pharmaceuticals and personal care products in water and wastewater

BY ANNA M. COMERTON¹,* ROBERT C. ANDREWS¹ AND DAVID M. BAGLEY²

¹Department of Civil Engineering, University of Toronto, 35 Saint George Street, Toronto, Ontario, Canada M5S 1A4
²Department of Civil and Architectural Engineering, University of Wyoming, 1000 East University Avenue, Laramie, WY 82071, USA

The detection of organic micropollutants, such as endocrine-disrupting compounds, pharmaceuticals and personal care products, in wastewater and the aquatic environment has brought increasing concern over their potential adverse ecological and human impacts. These compounds are generally present at trace levels (ng l⁻¹) and in complex water matrices, such as wastewaters and surface waters, making their analysis difficult. Currently, no standardized analytical methods are available for the analysis of organic micropollutants in environmental waters. Owing to the diversity of physico-chemical properties exhibited by the various classes of organic micropollutants, the majority of established analytical methods described in the literature focus on a specific class of compounds, with few methods applicable to multi-class compound analysis. As such, analytical challenges and limitations contribute to the lack of understanding of the effectiveness of drinking water and wastewater treatment processes to remove organic micropollutants. This paper provides a practical overview of current analytical methods that have been developed for the analysis of multiple classes of organic micropollutants from various water matrices and describes the challenges and limitations associated with the development of these methods.

Keywords: endocrine-disrupting compounds; pharmaceuticals; personal care products; analytical methods; analytical challenges; water matrix

1. Introduction

The detection of organic micropollutants, such as endocrine-disrupting compounds (EDCs), pharmaceutically active compounds (PhACs) and personal care products (PCPs), in wastewater effluent (Lindqvist et al. 2005; Wennmalm & Gunnarsson 2005; Lishman et al. 2006), receiving waters (Kolpin et al. 2002),

*Author for correspondence (anna.comerton@utoronto.ca).

One contribution of 12 to a Theme Issue ‘Emerging chemical contaminants in water and wastewater’.

3923 This journal is © 2009 The Royal Society
drinking water sources (Stackelberg et al. 2004) and some treated drinking waters (Loraine & Pettigrove 2006; Servos et al. 2007) has brought increasing concern over their potential adverse ecological and human impacts (Colborn et al. 1993; Doerr-MacEwen & Haight 2006). The term EDC is used to describe chemicals that are not produced by the body but act by mimicking or antagonizing natural hormones. PhACs consist of pharmaceuticals used by humans and animals, as well as pharmaceutical metabolites. Pharmaceuticals are prescribed to cure or prevent illnesses; therefore, they are designed to interfere with specific biological systems, such as receptors or enzymes. PCPs are synthetic organic chemicals derived for usage by individuals in soaps, lotions, sunscreens, toothpastes and cosmetics. Several studies have investigated the exposure of wildlife to EDCs, PhACs and PCPs and observed detrimental effects, such as feminization and reproductive and developmental problems (Colborn et al. 1993; Leatherland 1993; Purdom et al. 1994; Jobling et al. 1998; Larsson et al. 1999). However, no conclusive evidence of a linkage between exposure to these compounds and human health has been presented in the literature (Solomon & Schettler 2000; Weber et al. 2002; Brody & Rudel 2003; Safe 2004, 2005; Schwab et al. 2005).

Although adverse health effects cannot be attributed with certainty to the low levels of organic micropollutants present in the water environment, a better understanding of the effectiveness of drinking water and wastewater processes to remove these contaminants is of interest to the water treatment community. Wastewater treatment plant (WWTP) effluents are a major source of organic micropollutants in the environment, yet the removal of these compounds in full-scale treatment plants is not well understood, with few studies having measured both WWTP influent and effluent concentrations. Contributing to this challenge is the fact that the absence of specific compounds in effluent water does not necessarily imply complete removal because treatment may have transformed parent compounds to unknown and/or unmeasured degradates (Stackelberg et al. 2004).

Similarly, the removal of EDCs and PhACs by conventional drinking water processes is challenging to study at full scale and has generally been shown not to be completely effective (Kim et al. 2005; Westerhoff et al. 2005; Hua et al. 2006). Pilot- and full-scale studies are challenging owing to the presence of only trace levels (ng l\(^{-1}\)) of organic micropollutants in source waters as well as the limitations in analysing these types of compounds at such levels. Often it is not possible to quantify the removal of individual compounds either because they are not naturally present in the investigated water at a quantifiable level or their concentrations in treatment process effluents are below detection. This demonstrates that analytical challenges and limitations contribute to the lack of understanding of the effectiveness of water treatment processes and their performance under full-scale operating conditions.

There are several thousand compounds that can be classified as EDCs, PhACs or PCPs along with their metabolites, which represent a wide range of physicochemical properties, making it almost impossible to develop analytical methods for all of these compounds. It has been suggested that the pre-selection of compounds that are environmentally relevant and pose the most potential risk to human and ecosystem health is essential in order to develop multi-compound methods (Ternes 2001). In order to monitor the water environment and evaluate the effectiveness of water treatment processes, the ultimate objective is to develop
reliable and reproducible analytical methods capable of simultaneously measuring a large number of organic micropollutants representing a wide range of physico-chemical properties at trace levels in different water matrices while minimizing sample preparation time and cost (Fatta et al. 2007; Radjenović et al. 2007; Baugros et al. 2008). This paper provides an overview of current analytical methods that have been developed for the analysis of multiple classes of organic micropollutants in wastewater and natural water matrices. The many challenges and limitations associated with the development of these multi-class compound analytical methods to measure the trace levels of organic micropollutants present in various types of water matrices will also be discussed. The goal of this paper is to provide an introduction and practical overview of the available methodology to water treatment engineers and researchers who do not necessarily have a chemistry background but may be applying these methods in their work. Several comprehensive reviews of the analytical methodology for the determination and quantification of organic micropollutants as well as advances in this field have been published recently and should be consulted for a more in-depth analytical chemistry discussion. These include, but are not limited to, papers by Giger (2009), Richardson (2008), Gabet et al. (2007), Fatta et al. (2007), Pietrogrande & Basaglia (2007) and Radjenović et al. (2007).

2. Background

(a) Compound classes

EDCs, PhACs and PCPs can be categorized into several different compound classes representing a wide range of physico-chemical properties that influence the development of sample preparation techniques and analytical methods. Some of the major organic micropollutant classes and their main characteristics are as follows.

(i) Acidic pharmaceuticals

Acidic drugs comprise pharmaceutical compounds that are polar and contain carboxylic moieties and one or two phenolic hydroxyl groups. At acidic pH, no ionic functional groups are present because the hydroxyl and carboxylic moieties are protonated (Ternes 2001; Farré et al. 2008). Examples of acidic drugs include ibuprofen, naproxen, diclofenac and benzafibrate.

(ii) Neutral pharmaceuticals

Neutral pharmaceuticals consist of compounds from distinct medicinal classes that contain no acidic functional groups and, therefore, are not charged at neutral pH (Ternes 2001). Neutral pharmaceuticals include antiepileptics (e.g. carbamazepine), psychiatrics (e.g. diazepam), antineoplastics (e.g. cyclophosphamide) and lipid regulators (e.g. clofibrate).

(iii) Oestrogens

Oestrogen hormones are a group of compounds with steroid structures containing phenolic and sometimes aliphatic hydroxyl groups displaying moderate to high hydrophobicity. They are considered to be the class of

Phil. Trans. R. Soc. A (2009)
compounds displaying the highest potential for endocrine disruption. Synthetic (e.g. 17α-ethinylestradiol) and natural oestrogens (e.g. 17β-estradiol) and their metabolites (e.g. estrone) can be analysed simultaneously because of their similar physico-chemical properties (Ternes 2001; Zuehlke et al. 2005; Gabet et al. 2007).

(iv) Betablockers and β2-sympathomimetics

These two medicinal classes have a basic character and contain a secondary aminoethanol structure as well as several hydroxyl groups. Owing to the elevated number of functional groups, their polarity is relatively high (Ternes 2001; Pavlović et al. 2007; Radjenović et al. 2007). Examples from this class of compounds include metoprolol, atenolol, sotalol and salbutamol.

(v) Iodinated X-ray contrast media

This group belongs to the most frequently applied pharmaceuticals in medicine. They are used for imaging of organs or blood vessels during diagnostic tests, and all contain iodine atoms, which absorb X-rays. They exhibit a high polarity and are very persistent against metabolism by organism and environmental degradation (Pérez & Barceló 2007; Schultz et al. 2008). Examples of compounds used in X-ray contrast media include diatrizoate, iopamidol and iopromide.

(vi) Antibiotics and antimicrobials

These compounds are widely used in human and veterinary medicine to prevent or treat microbial infections. This group comprises a broad spectrum of substances that contain acidic and basic functional groups in their structure, are non-volatile and, at neutral pH, exist in their ionized form (Renew & Huang 2004; Díaz-Cruz & Barceló 2005). Common antibiotics/antimicrobials include sulphonamides (e.g. sulphamethoxazole), tetracyclines (e.g. chlortetracycline), penicillins (e.g. benzylpenicillin), quinolones (e.g. norfloxacin) and triclosan.

(vii) Pesticides

Pesticides are widely used to control unwanted pests such as insects, weeds and fungi. They are polar and thermally stable compounds and generally comprise organophosphates, carbamates, pyrethroids and organochlorines (Steen et al. 1999; Costa et al. 2008). Common pesticides include atrazine, simazine, alachlor and metolachlor.

(viii) Personal care products

PCPs consist of a broad range of synthetic organic chemicals used by individuals in products such as soaps, lotions, sunscreens, toothpastes and cosmetics. PCPs include: fragrances, such as nitro- and polycyclic musks; sunscreens and UV blockers, such as oxybenzone; insect repellents, such as DEET; and antioxidants and preservatives, such as phenols and parabens (Pietrogrande & Basaglia 2007).
(b) Overview of current analytical methods

Currently, no standardized analytical methods are available for the analysis of organic micropollutants in environmental waters. Owing to the diversity of physico-chemical properties exhibited by the various classes of organic micropollutants, the majority of established analytical methods described in the literature focus on a specific class of compounds, with few methods applicable to multi-class compound analysis. For example, Zuehlke et al. (2005) developed a method for oestrogens using automated solid phase extraction (SPE), followed by liquid chromatography tandem mass spectrometry (LC–MS/MS) with electrospray ionization (ESI) operated in negative ionization mode. Renew & Huang (2004) analysed antibiotics in wastewater using an anion-exchange cartridge in tandem with a hydrophilic–lipophilic balance (HLB) cartridge to minimize interferences from wastewater organic matter, followed by detection with LC–ESI-MS with selected ion monitoring (SIM). Steen et al. (1999) describe a method for polar pesticides and their transformation products from surface and estuarine waters using SPE with LC–ESI-MS/MS in positive ionization mode. Gómez et al. (2007) achieved simultaneous detection of 10 acidic and neutral pharmaceuticals using SPE (HLB cartridge) followed by gas chromatography tandem mass spectrometry (GC–MS/MS) from wastewater without the use of a derivatization step. Reddersen & Heberer (2003) developed a multi-compound method for the measurement of 21 pharmaceuticals and polar contaminants in environmental waters using SPE (C-18 cartridge), chemical derivatization and detection by gas chromatography/mass spectrometry (GC/MS) with SIM. Finally, Lin et al. (2005) developed a method to analyse selected pharmaceutical residues using SPE (HLB cartridge) followed by GC/MS analysis with on-line derivatization.

Simultaneous analysis of several groups of compounds requires compromises between performance parameters (e.g. shape and separation of chromatographic peaks, analyte sensitivity and recovery, etc.), which often lead to not obtaining the best performance for each one of the compounds analysed. However, many researchers have attempted to overcome these challenges and several multi-compound methods have been recently developed. For example, Gros et al. (2006) presented a sensitive multi-compound analytical method based on off-line SPE (HLB cartridge) followed by LC–ESI-MS/MS for simultaneous analysis of 29 pharmaceuticals in both surface and wastewaters. Zhang et al. (2007) described a method for the simultaneous determination of 56 compounds classified as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and pharmaceuticals and personal care products (PPCPs) with a wide range of polarities using two extraction discs (C-18 and SDB-XC cartridges) in a single extraction step. This was followed by GC/MS in which PAHs and PCBs were analysed directly and PPCPs were analysed after derivatization. More recently, Baugros et al. (2008) developed an analytical method based on a combined method using SPE followed by either GC/MS or LC–MS/MS to determine the trace levels (ng l$^{-1}$) of 33 multiple-class compounds in environmental waters including wastewater effluents and surface and ground waters. These along with other recently published multi-class compound methods are summarized in table 1. At present, a combination of LC/MS and GC/MS techniques appears to be the most powerful and comprehensive approach to multi-class compound analysis because the application of the two complementary methodologies widens...
Table 1. Summary of recently published methods for multi-class compound analysis in water and wastewater.

<table>
<thead>
<tr>
<th>reference</th>
<th>number of compounds analysed</th>
<th>compound classes</th>
<th>water matrices analysed</th>
<th>extraction</th>
<th>analytical instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gros et al. (2009)</td>
<td>73</td>
<td>various classes of pharmaceuticals</td>
<td>surface and wastewater</td>
<td>SPE with Oasis HLB</td>
<td>LC–MS/MS with hybrid triple quadrupole-linear ion-trap mass spectrometer</td>
</tr>
<tr>
<td>Baugros et al. (2008)</td>
<td>33</td>
<td>carbanates, organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs), fungicides, alkylphenols (APs), industrial EDCs</td>
<td>wastewater effluent, surface and ground water</td>
<td>SPE with Strata C-18 GC/MS (for OCPs and industrial EDCs) and LC–MS/MS (for carbanates, OPPs, fungicides and APs)</td>
<td></td>
</tr>
<tr>
<td>Zhang et al. (2007)</td>
<td>56</td>
<td>PAHs, PCBs, PPCPs</td>
<td>river water</td>
<td>SPE with two-layer disc (C-18 and SDB-XC)</td>
<td>GC/MS (for PAHs and PCBs) and GC/MS with derivatization (BSTFA) (for PPCPs)</td>
</tr>
<tr>
<td>Gómez et al. (2007)</td>
<td>10</td>
<td>acidic and neutral pharmaceuticals</td>
<td>wastewater</td>
<td>SPE with Oasis HLB</td>
<td>GC–MS/MS</td>
</tr>
<tr>
<td>Gros et al. (2006)</td>
<td>29</td>
<td>analgesics and non-steroidal anti-inflammatories, lipid regulators, psychiatric drugs, antihistaminics, antiulcer agents, antibiotics and betablockers</td>
<td>surface and wastewater</td>
<td>SPE with Oasis HLB</td>
<td>LC–ESI-MS/MS</td>
</tr>
<tr>
<td>Kampioti et al. (2005)</td>
<td>20</td>
<td>eight classes of pesticides</td>
<td>river water and treated drinking water</td>
<td>automated on-line SPE with Hysphere Resin GP cartridge</td>
<td>LC–ESI-MS/MS</td>
</tr>
</tbody>
</table>
the range of compounds that can be reliably measured (Pietrogrande & Basaglia 2007). A detailed overview of the various analytical steps and options for the measurement of organic micropollutants in water samples is provided in the following section.

3. Analytical steps

(a) Sample preparation

Sample preparation prior to injection on the analytical instrument is a time- and labour-intensive step. Although various sample preparation techniques exist, they all have the same major objectives: remove potential interferences from the sample matrix; if necessary, convert the analytes into a more suitable form (e.g. via derivatization or pH adjustment); increase the concentration of the target analytes; and provide a robust, reproducible method that is independent of the sample matrix (Pavlović et al. 2007). The major sample preparation steps include pre-treatment, clean-up and concentration (figure 1).
(i) Pre-treatment

Water samples are collected in amber glass bottles pre-cleaned with ultrapure water and organic solvent (Gómez et al. 2007). They are stored in the dark at 4°C, filtered and extracted as soon as possible (usually within 48 h of collection). Preservatives (e.g. formic acid, hydrochloric acid, sulphuric acid, formaldehyde or methanol) can be added to the sample after filtration in order to avoid changes in sample composition and bacterial activity (Gabet et al. 2007). One of the challenges associated with environmental samples is that they contain interferences, such as natural organic matter (NOM), which can reduce extraction efficiencies in the clean-up step. As such, environmental samples are generally pre-filtered with 0.2–0.5 μm membrane filters to minimize interferences and clogging during extraction (Steen et al. 1999; Renew & Huang 2004; Díaz-Cruz & Barceló 2005; Fatta et al. 2007; Farré et al. 2008).

pH adjustment is often included in the pre-treatment step prior to sample extraction, elution and concentration. Solution pH is a critical parameter because it determines the chemical form (speciation) of the analytes, their stability and their interaction with the sorbent in the subsequent extraction step (Díaz-Cruz & Barceló 2005). For example, in the case of pharmaceuticals containing acidic groups in their structure and existing largely in their ionized form at neutral pH, acidification of water samples is often necessary to achieve good recovery through the extraction process (Fatta et al. 2007). However, acidification of water samples to obtain undissociated or cationic species can also result in the retention of highly negatively charged NOM by the extraction cartridges which can lead to interferences in the analysis of the target compounds (Renew & Huang 2004).

(ii) Clean-up

Sample clean-up, which consists of the extraction and elution steps, is generally performed via SPE and involves first conditioning the sorbent, then percolating the sample, rinsing and cleaning the sorbent to remove interfering compounds, eluting the sample analytes from the sorbent and, finally, recovering the analytes (Pichon 2000). The other most widely used method for sample clean-up is solid phase microextraction (SPME), which is based on the partition equilibrium of analytes between sorbent and sample (Pichon 2000; Radjenović et al. 2007).

In SPE, the cartridge sorbent serves as the stationary phase and the mobile phase consists of the aqueous sample during the extraction step and the organic solvent during the elution step. High recoveries through the clean-up process are obtained when analytes are strongly retained by the sorbent in the presence of water, and have a subsequent low retention during elution by organic solvents (Pichon 2000). SPE can be used off-line, where sample clean-up is completely separate from the subsequent chromatographic analysis, or it can be performed on-line, where it is directly integrated into the analytical system (Rossi & Zhang 2000). The advantages of on-line SPE include minimum sample manipulation, full automation, high throughput, ease of application and cost-efficiency (Kamptioti et al. 2005). However, to date most sample preparation methods for organic micropollutant analysis apply SPE off-line, with few studies having reported the use of on-line SPE (Fatta et al. 2007). In addition, on-line coupling of SPE with gas chromatography (GC) is complicated because it is necessary to remove all traces of water before desorption of the compounds.
into the GC instrument (Pichon 2000). An example of a successful on-line SPE method was developed by Kampioti et al. (2005) for the analysis of 20 pesticides belonging to different classes. This method is fully automated and incorporates on-line SPE-LC–ESI-MS/MS with limits of detection (LODs) between 0.004 and 2.8 ng l\(^{-1}\).

The most commonly employed SPE sorbents are: Oasis MCX, a mixed polymeric and cation-exchange sorbent; Oasis HLB, a polymeric sorbent; Lichrolut ENV+, a polymeric sorbent; and C-18, a non-polar alkyl-bonded silica sorbent (Gros et al. 2006; Fatta et al. 2007). The choice of SPE cartridge must consider the various physico-chemical properties of the analytes of interest for multi-compound methods. For example, Oasis MCX offers good recovery (greater than 70%) of acidic compounds but not basic and neutral compounds, whereas Oasis HLB provides good recovery (greater than 70%) of acidic, neutral and basic compounds over a wide pH range (2–7; Gros et al. 2006; Pavlović et al. 2007). Lichrolut ENV+ is appropriate for polar organic compounds at low pH (less than 2.5) as well as neutral drugs at neutral pH (Pichon 2000; Gros et al. 2006). Finally, C-18 is suitable for moderately polar to non-polar analytes (Pichon 2000; Gros et al. 2006; Pavlović et al. 2007).

Once an SPE sorbent material has been selected, the extraction process must be optimized in terms of various parameters, including sample volume and percolation rate, sorbent cartridge volume as well as elution solvent and volume (Baugros et al. 2008). The type and volume of elution solvent are important factors that affect the recoveries of the target analytes. The choice of elution solvent to desorb the target compounds from the SPE cartridge will depend on the physico-chemical properties of the analytes and the elution strength of the solvent. The most commonly used elution solvents, which vary in elution strength and polarity, include ethyl acetate, acetone, methanol and dichloromethane, or mixtures of them (Pietrogrande & Basaglia 2007; Radjenović et al. 2007).

SPME is a relatively new, fast and simple sample clean-up technique based on the partition equilibrium of the analyte between sorbent and sample. SPME uses a short piece of fused-silica fibre support coated with a polymeric stationary phase, which is placed on the tip of a stainless steel plunger. Extraction is performed by piercing the sample septum and then depressing the plunger to allow the fibre to be exposed to the sample. The extraction process continues until equilibrium is reached between coating and sample (Pichon 2000; Pavlović et al. 2007). The combination of SPME and GC is particularly suitable for the determination of volatile and semivolatile, non-polar compounds (Pietrogrande & Basaglia 2007). In fact, an automated system of SPME directly coupled to GC is commercially available (Pichon 2000). When GC is used, analytes are thermally desorbed from the fibre in a GC injector (Pavlović et al. 2007). For example, Suchara et al. (2008) developed an SPME method for simultaneous extraction of pharmaceutical compounds with acidic and basic characteristics (ibuprofen, fenoprofen, diclofenac, diazepam and loratadine), followed by GC/MS with LODs ranging from 0.02 to 0.43 \(\mu\)g l\(^{-1}\). The coupling of SPME and liquid chromatography (LC) is more difficult because thermal desorption cannot completely desorb many non-volatile compounds typically detected by LC from the fibre (Chen & Pawliszyn 1995; Pichon 2000). As such, solvent desorption is necessary, but the challenge is to desorb analytes completely with a minimum amount of solvent to avoid significant impacts on chromatographic performance.
(Chen & Pawliszyn 1995). Monzón et al. (2007) developed a method using SPME coupled with LC using fluorescence detection for the extraction and determination of four benzimidazole fungicides in water. The target compounds were desorbed from the fibre using 50 μl of methanol. However, the LODs were relatively high, ranging from 0.03 to 1.3 μg l⁻¹.

SPME has been applied owing to several advantages it has over SPE, such as requiring less sample volume, minimizing solvent use, being relatively quick, being easily automated leading to high compound recoveries and its ease of sample handling (Fatta et al. 2007; Radjenović et al. 2007). However, SPME has some limitations when compared with SPE owing to the limited choice of sorbent coatings on the market to obtain selective adsorption. This can lead to poor recoveries and high LODs not suited for the analysis of these types of compounds (Pietrogrande & Basaglia 2007). In addition, the sorption capacity of the fibre sorbent coatings is often insufficient to accomplish sufficient recovery of target analytes (Radjenović et al. 2007). This indicates a need for further optimization of this extraction technique if it is to become a suitable alternative to SPE in the analysis of trace levels of organic micropollutants from environmental samples. The extraction efficiency of SPME depends on several factors, including: fibre coating, sample pH, extraction mode, ionic strength, temperature and extraction time. As such, the optimization of these variables is necessary to achieve better sensitivity and more widespread applicability of the SPME technique (Suchara et al. 2008).

(iii) Concentration

Following the elution step, the sample extract is further concentrated by evaporation under a gentle stream of nitrogen. Samples to be analysed by GC are typically evaporated to a final volume of between 50 and 500 μl and a fraction of this concentrated extract is injected for analysis (Pichon 2000; Farré et al. 2008). For analysis by LC or for a solvent switch, extracts are evaporated to dryness and then reconstituted with the appropriate solvent (Pichon 2000). For samples to be analysed by LC, the use of a solvent corresponding to the composition of the mobile phase at the beginning of the gradient is typical (Benijts et al. 2004; Jahnke et al. 2004; Gros et al. 2006; Farré et al. 2008). One of the challenges associated with evaporation to dryness is that the residue from the dry extracts can be difficult to solubilize. For example, the addition of water is required for more polar compounds, whereas very hydrophobic analytes can only be dissolved using non-polar organic solvents (Pichon 2000). As such, common solvents for the reconstitution of dry extracts prior to LC analysis include mixtures such as methanol/water and acetonitrile/water (Benijts et al. 2004; Gros et al. 2006). Internal standards are usually added following the evaporation step and prior to injection on the analytical instrument (Gros et al. 2006; Farré et al. 2008).

(b) Sample analysis

LC–MS/MS is typically used to determine more polar and less volatile compounds, while GC/MS or GC–MS/MS are used for volatile or volatizable compounds and metabolites (figure 1). Derivatization can be used to broaden the applicability of GC/MS and GC–MS/MS analyses to more polar compounds (Pietrogrande & Basaglia 2007).
(i) Liquid chromatography tandem mass spectrometry

LC should preferably be applied for the analysis of organic micropollutants only when using tandem MS because this combination is able to produce fragment ions that are necessary for the explicit identification of the analytes (Reddersen & Heberer 2003). LC–MS/MS allows separation and detection of compounds having the same molecular mass but different product ions, even if they co-elute. MS/MS detection is therefore preferred for increased analytical sensitivity and selectivity in complex water matrices (Díaz-Cruz & Barceló 2005). The most commonly used LC–MS/MS interfaces are atmospheric pressure ionization technologies, such as ESI and atmospheric pressure chemical ionization (APCI). ESI is well suited for the analysis of polar compounds, whereas APCI is very effective in the analysis of medium-polarity and low-polarity substances (Radjenović et al. 2007). LC is generally suitable for the determination of compounds that are polar, non-volatile or thermodegradable, which cannot be analysed by GC (Díaz-Cruz & Barceló 2005; Baugros et al. 2008). For example, the detection of some pharmaceuticals, such as antibiotics or the extremely polar betablocker compounds, can only be achieved using LC–MS/MS (Ternes 2001; Reddersen & Heberer 2003; Díaz-Cruz & Barceló 2005).

Matrix effects are one of the major drawbacks of LC–MS/MS, particularly when working in the ESI mode. Matrix effects can result in the suppression or, less frequently, the enhancement of analyte signals, leading to the erroneous interpretation of results (Zrostlíková 2002; Benijts et al. 2004; Jahnke et al. 2004; Gros et al. 2006). For example, the analysis of very contaminated samples, such as wastewaters, is expected to result in the suppression of the ESI and requires efficient sample clean-up if LC–MS/MS is to be applied (Ternes 2001). Optimizing both the LC separation and MS parameters also contributes to obtaining reliable results. Chromatographic separation is generally optimized by performing a series of preliminary experiments involving testing of mobile phase composition to determine the conditions that provide the best compromise for all target analytes (Gros et al. 2006; Baugros et al. 2008). Optimization of MS parameters, including cone voltage and collision energy, is performed via flow injection analysis for each compound of interest (Steen et al. 1999; Gros et al. 2006; Baugros et al. 2008).

(ii) Gas chromatography/mass spectrometry

GC/MS can be applied for the analysis of non-polar and volatile or volatizable compounds; however, the analysis of polar compounds requires a derivatization step (Fatta et al. 2007). GC/MS has the advantage over LC–MS/MS in that analyte quantification is less prone to water matrix interferences (Reddersen & Heberer 2003). In addition, GC/MS generally has lower LODs when compared with LC–MS/MS but requires more complicated and time-consuming sample preparation owing to the need for derivatization of polar analytes (Díaz & Barceló 2005; Redjenović et al. 2007). Derivatization converts polar substances into less polar analogues and also increases volatility and thermal stability, making the compounds more accessible to GC analysis and improving sensitivity (Gómez et al. 2007; Radjenović et al. 2007). However, the advantages of better sensitivity can sometimes be offset by loss of sample during the additional
manipulation required for derivatization (Gómez et al. 2007). Another drawback of derivatization is that it requires the use of reagents that may be highly toxic, carcinogenic and potentially explosive, such as diazomethane (Lin et al. 2005; Fatta et al. 2007). An ideal derivatization reaction permits the detection of compounds containing polar functional groups, leads to complete derivatization (greater than 90%) and is time efficient. Many variables are involved in optimizing derivatization, including the derivatizing agent and its dose, as well as the reaction temperature and duration (Pietrogrande & Basaglia 2007; Radjenović et al. 2007).

An example of the successful application of GC/MS with derivatization is reported in the method developed by Reddersen & Heberer (2003) for the analysis of 26 pharmaceuticals and polar contaminants from wastewater and surface water using SPE, derivatization with MTBSTFA (N-[(t-butyldimethylsilyl)-N-methyltrifluoroacetamide), followed by GC/MS with SIM. Analyte recoveries were between 70 and 110 per cent and LODs ranged from 1 to 10 ng l⁻¹. One of the risks with multi-compound methods involving derivatization is that harsh chemicals or the high temperatures of the derivatization process can result in thermal breakdown or transformation of underivatizable parent compounds. As such, one solution is to split samples into two fractions prior to the GC/MS analysis, where one-half is submitted to derivatization for the analysis of polar target compounds and the other is directly analysed by GC (Pietrogrande & Basaglia 2007). For example, Zhang et al. (2007) developed a method involving one extraction step, followed by the division of the sample extract into two fractions. Sixteen PAHs and 28 PCBs were analysed by GC/MS with no derivatization and 12 PhACs and PCPs, which were non-volatile and/or thermally unstable, were analysed by GC/MS with derivatization using BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide).

4. Analytical challenges

(a) Sample preparation

There are many challenges associated with the extraction and elution of compounds exhibiting a wide range of physico-chemical properties. For example, polar compounds may be lost during SPE sample extraction owing to their low affinity for hydrophobic sorbents, whereas non-polar compounds are highly retained but may, in turn, be difficult to desorb during elution (Pichon 2000; Gros et al. 2006; Pavlović et al. 2007). Non-polar compounds are also more likely to adsorb on flask walls and connecting tubing, leading to diminished analyte recoveries (Pichon 2000). In addition, the extraction of ionizable compounds, such as acidic pharmaceuticals, requires lowering the sample pH, which can lead to the co-extraction of undesired interferences, such as NOM (Renew & Huang 2004; Fatta et al. 2007). Finally, loss of volatile compounds can occur during the evaporation step, but can be minimized by carrying out the evaporation under mild conditions, such as with a gentle stream of nitrogen (Pichon 2000). The reconstitution of dry extracts containing compounds with large differences in solubility can also lead to loss in recovery (Zhang et al. 2007).
(b) Water matrix effects

As mentioned, water matrix effects are one of the main disadvantages associated with LC–MS/MS, especially when working in the ESI mode. The ESI source is highly susceptible to other components present in the matrix, which can result in signal suppression or enhancement leading to erroneous results (Gros et al. 2006; Gabet et al. 2007). This is because in ESI, ions are released from electrically charged droplets. The limited amount of charge per droplet is often exhausted for the conversion of water matrix components to free ions and is, therefore, no longer available for the formation of free ions of the target analytes (Steen et al. 1999). Signal suppression is greater as NOM concentration increases and can also be influenced by sample pH owing to its impact on the co-extraction of NOM components during the sample clean-up process (Steen et al. 1999; Renew & Huang 2004). For example, Renew & Huang (2004) reported that the biggest challenge associated with analysing antibiotics in wastewater samples was that the relatively high concentration of organic matter resulted in reduced sample extraction efficiency and suppression of the signal intensity with LC–MS analysis. In addition to exhausting the limited number of charge sites on the electrospray droplets, the reduction in LC–MS signal intensity was hypothesized to be contributed by the antibiotic compounds sorbing to the NOM as well as contaminants in the wastewater matrix, masking analyte peaks by raising the chromatographic baseline.

Common strategies to reduce matrix effects include optimizing sample clean-up to selectively recover the target analytes while minimizing the co-extraction and co-elution of matrix components such as NOM, as well as optimizing chromatographic separation (Pichon 2000; Gros et al. 2006). Isotopically labelled surrogate compounds can be spiked into samples to evaluate target analyte recovery through the clean-up process as a function of sample water matrix (Pietrogrande & Basaglia 2007). In addition, LC–MS signal suppression can be compensated with the use of isotopically labelled internal standards, standard additions and/or matrix-matched calibration. The use of internal standards as an approach to assessing the loss of signal intensity in individual samples is relatively simple, efficient and less time-consuming than the standard addition and matrix-matched calibration methods (Gros et al. 2006; Gabet et al. 2007). However, appropriate structurally similar, isotopically labelled compounds for use as internal standards are not always commercially available (e.g. antimicrobials) and can be very expensive (Díaz-Cruz & Barceló 2005; Gros et al. 2006; Pietrogrande & Basaglia 2007). In addition, more than one internal standard may be needed in multi-class methods (Díaz-Cruz & Barceló 2005). Although more time-consuming and labour-intensive, standard additions have been found to be the most reliable and efficient method to minimize matrix effects (Steen et al. 1999; Baugros et al. 2008).

(c) Method validation

In addition to the lack of standardized analytical methods available for the analysis of organic micropollutants in environmental waters, there are also no standard method validation protocols. A recent interlaboratory comparison of the analysis of non-steroidal anti-inflammatory drugs (ketoprofen, naproxen, ibuprofen, diclofenac) from freshwater and wastewater by 13 European...
laboratories revealed that generally good agreement was obtained for the mean values reported by the participants (Farré et al. 2008). However, the study did identify the need for a standard validation method in order to minimize potential sources of variation among laboratories. Gabet et al. (2007) performed an extensive review of reported analytical methods for oestrogens in environmental matrices over the past decade. They reported that the majority of the reviewed publications did not completely validate their method. For example, LODs and recoveries were estimated in laboratory-grade water rather than using the same water matrix as the analysed samples. They concluded that validation should ideally include the following: sensitivity should be evaluated not only with laboratory-grade water but also using real water matrices; within-day and between-day repeatability of the overall analytical method should be reported; and isotopically labelled surrogate standards and internal standards should be used.

The issue of method validation is being addressed by European researchers through the recently established NORMAN (Network of Reference Laboratories for Monitoring of Emerging Environmental Pollutants; http://www.norman-network.net). The objective of the NORMAN project is to create a network of reference laboratories in order to improve the exchange of information and data on emerging environmental contaminants as well as to encourage the validation and harmonization of analytical methods and monitoring tools in Europe. Validation protocols will be developed for these analytical and monitoring methods, which can be applied for internal laboratory validation, basic external validation and interlaboratory validation.

5. Conclusions

Currently, no standardized analytical methods are available for the analysis of organic micropollutants in environmental waters. Owing to the diversity of physico-chemical properties exhibited by the various classes of these contaminants, the majority of established analytical methods described in the literature focus on a specific class of compounds, with few methods applicable to multi-class compound analysis. Simultaneous analysis of several groups of compounds requires compromises between performance parameters that often lead to not obtaining the best performance for each one of the compounds analysed. At present, a combination of LC–MS/MS and GC/MS techniques appears to be the best approach to multi-compound class analysis because the application of the two complementary methodologies widens the range of compound properties that can be reliably measured. Also contributing to the analytical challenge are the trace levels (ng{l} \cdot {l}^{-1}) and complex water matrices in which these contaminants are typically present in the water environment. These analytical difficulties can be reduced by optimizing sample clean-up to minimize the co-extraction and co-elution of interferences and maximize target analyte recovery. Isotopically labelled surrogate and internal standard compounds can also be used to compensate for water matrix effects. Finally, standard validation protocols, such as those being developed in Europe by the NORMAN network, for the analysis of organic micropollutants are needed to ensure consistency in reported environmental values.
References


Kampioti, A. A., Borba da Cunha, A. C., López de Alda, M. & Barceló, D. 2005 Fully automated multianalyte determination of different classes of pesticides, at picogram per litre levels...


*Phil. Trans. R. Soc. A* (2009)
Zhang, S., Zhang, Q., Darisaw, S., Ehie, O. & Wang, G. 2007 Simultaneous quantification of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pharmaceuticals and personal care products (PPCPs) in Mississippi river water, in New Orleans, Louisiana, USA. Chemosphere 66, 1057–1069. (doi:10.1016/j.chemosphere.2006.06.067)