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Pharmaceuticals and Hormones in the Environment

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Abstract

Some of the earliest initial reports from Europe and the United States demonstrated that a variety of pharmaceuticals and hormones could be found in surface waters, source waters, drinking water, and influents and effluents from wastewater treatment plants (WWTPs). It is unknown though, at this time, what ecotoxicological effects can be had from pharmaceuticals and hormones that are essentially designed for one purpose (e.g., treatment of human and domestic livestock for illness and disease) and their possible adverse effects on terrestrial wildlife, aquatic organisms, bacteria, and ultimately humans, through unintentional environmental exposure.

One of the challenges the analytical chemistry community faces is the development of robust and standardized analytical methods and technologies that can easily be transferred to laboratories worldwide. While today's analysts can detect pg L^{-1} and ng L^{-1} concentrations of numerous pharmaceuticals, hormones, and their metabolites, in a variety of environmental matrices, there are still analytical gaps that are necessary to fill. We hope that this article will add to the body of knowledge of environmental analytical chemistry techniques regarding pharmaceuticals and hormones; giving environmental scientists a good overview of those analytical techniques that are currently available, and where possible, improvements and new methodologies that can be developed in support of this important, and relevant, environmental issue.

Key words: environmental chemistry, pharmaceuticals, hormones, analytical chemistry, solid-phase extraction, sampling, mass spectrometry, pressurized liquid extraction, green chemistry

1 Introduction

Some of the earliest reports from Europe and the United States demonstrated that a variety of pharmaceuticals and hormones could be found in surface waters, source waters, drinking water, and influents and effluents from wastewater treatment plants (WWTPs).[1,2,3] Regarding pharmaceuticals and hormones in the environment, there are a few insightful reviews covering general topic knowledge, analytical methods, and reports of occurrence of pharmaceuticals and hormones in the literature.[4-16] It is unknown though, at this time, what ecotoxicological effects can be had from pharmaceuticals and hormones that are essentially designed for one purpose - treatment of human and domestic livestock for illness and disease. There are real concerns of adverse impacts on wildlife, aquatic organisms, bacteria, and ultimately humans, through unintentional exposure to pharmaceuticals and hormones via environmental contact. For example, there are reports of feminization of male fish that swim in wastewater effluents, increases in antibiotic-resistant bacteria that are found in wastewater effluents, and acute toxicity and genotoxicity to aquatic organisms upon exposure (via water) to several antibiotics.[17-21] One early paper by Siegel (1959), showed that humans who were unintentionally exposed to environmentally persistent aerosols of penicillin developed anaphylactic shock.[22] A recent paper by Kidd et al. (2007) demonstrated that wild fish populations can collapse in just 2 years when consistently exposed to environmentally relevant concentrations (< 5-6 ng L^{-1}) of ethinylestradiol.[23]

While today's analysts can detect pg L^{-1} and ng L^{-1} concentrations of numerous pharmaceuticals, hormones, and their metabolites in a variety of environmental matrices, there are still analytical gaps that are necessary to fill. With this overview of the environmental analytical chemistry of pharmaceuticals and hormones, we hope to foster the continued advancement of analytical methodologies for chemical contaminants. Improvements in limits of detection and specificity, as well as expansion of the scope of emerging contaminants that are

amenable to detection, are fundamental to advancing our understanding of the sources, transport, and fate of chemical stressors and of biological exposure and effects.

1.1 Historical Perspective

Nearly 30 years ago, Garrison et al. (1976) reported the detection of clofibric acid (the bioactive metabolite from a series of serum triglyceride-lowering drugs) in a groundwater reservoir that had been replenished with treated sewage water using then state-of-the-art technology, gas chromatography coupled to a mass spectrometer (GC/MS).[24] Similar to Garrison's earlier report, Hignite and Azarnoff (1977) reported a year later finding aspirin, caffeine, and nicotine in wastewater effluent.[25] In 1983, Watts et al. reported at the Third European Symposium on the Analysis of Organic Micropollutants in Water, Oslo, Norway the presence of three pharmaceuticals (erythromycin, tetracycline, and theophylline), bisphenol A and other suspected endocrine disrupting compounds (EDCs) in a river water sample.[26] Watts' approach was unique in that this was the first time that the detection of non-volatile compounds were attempted in environmental samples. Watts used high performance liquid chromatography (HPLC) coupled to field desorption mass spectrometry (FDMS), a state-of-the-art mass spectrometry approach for its time. Of note none of these earlier findings were further pursued by the environmental analytical community until almost two decades later.

The earliest review of pharmaceuticals and hormones in the environment was by Richardson and Bowron (1985), who cite Aherne et al. (1985) and note in their review that, at least up until 1985, they (Aherne and English) didn't detect oral contraceptives, therefore "those hormones shouldn't be of environmental concern".[27,28] However, this declaration was somewhat in error, as Aherne et al. did report detecting norethisterone (17 ng L^{-1}) and progesterone (6 ng L^{-1}) in one river and one potable water sample. They also reported methotrexate (an extremely toxic and teratogenic chemotherapeutic agent) in a hospital effluent sample.[28] Of interest is that Richardson and Bowron were seemingly unaware of Garrison's and Watts' earlier

research efforts, as they noted in their overview: "Attempts to analyze for individual pharmaceuticals was not fruitful."[27] However, we now know that is no longer a true statement, in contrast to the findings in Richardson and Bowron's review (1985) over the past decade there have been significant improvements in extraction and detection technologies and upwards of 100 different pharmaceuticals, hormones and steroids, over-the-counter (OTC) drugs, and drugs-of-abuse have been identified in a variety of environmental matrices.[1, 2, 29-45] Current assessments of the literature show a substantial increase in analytical chemistry publications as related to pharmaceuticals and hormones in the environment for the past decade, see figure 1. <Figure 1 near here>

1.2 Rationale for environmental research

Acceptable, reproducible, and sensitive analytical chemistry techniques are necessary to better quantify and support environmental and human-health risk assessments. Pharmaceuticals can find their way into the natural environment after excretion or disposal by end-users.[1, 4, 30, 46] Most pharmaceutical compounds are more polar than pollutants of historic concern [e.g., polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs)] and are not readily sorbed to the subsoil, thereby increasing their potential to enter surface waters or groundwaters. As described by Richardson and Bowron (1985) and Daughton and Ternes (1999), there are several pathways that pharmaceuticals can follow once they are in the environment: 1) biodegradation to basic chemical building blocks, e.g., carbon, nitrogen, water; 2) biotransformation via metabolism, or another mechanism, into another chemical, which can possibly be more harmful than the parent compound: for example, acetaminophen can be transformed into two toxicants (1,4-benzoquinone and n-acetyl-p-benoquinone imine) during the disinfection treatment processes for drinking water;[47,48] 3) persistence in the environment through continual and renewable discharge as from a WWTP (this is considered pseudopersistence); and 4) through compartmentalization into environmental sinks and subsequent re-suspension into the environment, see figure 2. [4, 27, 47, 48] <Figure 2 near here>

1.3 US Federal Agencies, current work and next steps

In 2002 the National Research Council (NRC), in a report to the United States Environmental Protection Agency (USEPA), identified pharmaceuticals as one of a number of chemical pollutants that had not yet received adequate attention as potential pollutants in water, or sewage sludge. The NRC recommended that these classes of compounds be considered among previously unevaluated pollutants for future versions of the Drinking Water Contaminant Candidate List (CCL).[49,50] On December 31, 2003, the USEPA released their Final Action Plan which responded to some of NRC's recommendations (United States Federal Register 68 FR 17379 Wednesday April 9, 2003, pgs 17379-17395). The main emphasis of this action plan included regulatory and non-regulatory activities aimed at strengthening EPA's Biosolids Program. One of the projects listed within the Final Action Plan was the Development and Application of Analytical Methods for Detecting Pharmaceutical and Personal Care Products in Sewage Sludge (United States Federal Register 68 FR 75531 Wednesday December 31, 2003, pgs 75531-75552).

Other US federal agencies, for example the Food and Drug Administration (FDA), under the National Environmental Policy Act of 1969 (NEPA), are required to consider the environmental impacts of approving pharmaceuticals as part of their regulatory process. The FDA regulations, at 21 Code of Federal Regulations (CFR) part 25, require environmental assessments to be submitted for certain drug applications unless applications qualify for categorical exclusion.[51]

One of the challenges the USEPA and the European Union (EU) face is the development of robust and standardized analytical methods and technologies which can be easily transferred to U.S. or EU laboratories. With the continued need for occurrence data it is important to have

affordable and standardized analytical methods for use in data collection efforts. However, there must also be balance between cost and the performance of various analytical techniques in environmental matrices. The USEPA's Engineering and Analysis Division (EAD) in the Office of Water (OW) has developed two analytical methods for the detection of pharmaceuticals and hormones in sewage sludge, sediment, and WWTP influent and effluent: Method 1694 *Pharmaceuticals and Personal Care Products (PPCPs) in Water, Soil, Sediment, and Biosolids by LC/MS/MS* and Method 1698 *Steroids and Hormones in Water, Soil, Sediment, and Biosolids by HRGC/HRMS*.[52,53]

2 Environmental Sampling Strategies

A key, and often overlooked, aspect of any analytical measurement is obtaining a sample which is representative of the original matrix of interest and is free of any contamination due to the actions of collecting the sample. If the sample is improperly collected, then all subsequent processing and analysis steps are meaningless.

When planning your sampling trip, considerations must be made to address the following questions: 1) what sampling method will provide a representative sample of the targeted chemicals and medium?, 2) will the collected sample fulfill the objectives of the study?, 3) is the sample size sufficient to satisfy the minimum detection limit requirements of the method?, 4) what types of quality control measures are needed to address any bias from the sampling procedures?, and 5) what safety measures need to be taken? To begin with, the study needs to define a list of chemicals of interest and the needs of the processing and analysis methods for those chemicals. Often, very different procedures are followed for the extraction and analysis of environmental samples. Subsequently steps to isolate the targeted chemicals from potential

interferences may require separate samples, or portions of a larger homogenized sample to be taken for each procedure.

Regardless of the type of sample to be collected, documentation of the sampling event is critical. The specific types of field measurements which need to be taken vary depending on the objectives of the study and they may include pH, temperature, water/air flow, water turbidity, weather, visible point sources of contamination, and surrounding land use. Other documentation which should be taken includes: how were the samples taken; location of the sampling sites with maps, photographs, and global positioning system (GPS) or other locator measurements; names of the personnel who collected the samples; and what types of quality control samples were used.

The use of quality control (QC) measures during the sampling process can provide a measure for some of the bias associated with these steps. Blanks, spikes, and replicates are the common types of QC samples which are used. For these QC samples to be valid, it is vital that identical conditions (e.g., sampling devices, containers, and protocols) are used. Blanks and spikes are created, using a nearly identical matrix as the media of the studies, and not containing the targeted chemicals.[54] To ensure sample integrity, considerations for the proper sample preservation, storage conditions and times, and shipping methods must be made. When organic chemicals are the analytes of interest, the types of sample preservation will vary with specific chemical classes. Although USEPA Method 1694 and Method 1698 (the pharmaceutical methods) do not currently require preservation chemicals, Vanderford et al. (2003) recommends using sulfuric acid as a preservative to prevent degradation of the steroids and hormones in water samples.[52,53,55] They recommend adding enough sulfuric acid to bring the pH < 2. Whether a preservative is used or not, all samples are generally shipped chilled (< 4-6 °C for water and wet sediments) or frozen (< 0 °C for tissues and dry soils), and then kept chilled, or frozen, upon receipt at the laboratory before extraction. Samples should be kept in amber containers or

protected from light to prevent photodegradation of sensitive chemicals. Shipment of the samples to the laboratory should be performed as quickly as possible to maintain the integrity of the samples, and follow-up extractions should occur within several days of receipt. However, at this time no studies have occurred to determine the possible limits of sample holding times for the pharmaceuticals and hormones.

2.1 Water

2.1.1 Traditional - grab sampling (single point and composite)

Although not immediately obvious, water is extremely heterogeneous both spatially and temporally.[56] The hydrodynamics of the water body can play a large role in the mixing and distribution of waterborne chemicals. Concentrations of chemicals can often change dramatically with depth as stratification can occur in lakes and oceans with changes in temperature, water movement, and water composition. Episodic events (i.e., spills, surface runoff, over-spraying of pesticides) can cause pulses of chemicals to be introduced into a waterbody, which can be short-lived or isolated to very small areas. For these reasons, careful determination of the sampling method to be used needs to be made.

Typical surface water sampling methodologies involve standard grab, or spot, sampling, whereby a single sample is taken, or generating a composite sample from several spot sampling events. The simplest type of sample is one collected by hand with an open-mouth container. Thief samplers are used to collect instantaneous discrete samples primarily from lakes and reservoirs.[57] Thief samplers are automated sampling systems, which pump water into a collection vessel or through a chemical trap at designated intervals or at specific changes in stream flow or depth, and are often used in remote areas or when long-term composite sampling

is needed. The collection of depth-integrated samples can be made using either hand-held (in wadeable streams) or cable-and-reel (for non-wadeable bodies of water) samplers.[57]

Selection of sampling methods for monitoring groundwater is largely determined by the type of the well and the depth of the water. Monitoring wells are often sampled using bailers, thief-type samplers (a plastic tube with valves which can be opened to collect and contain water samples), or portable pumps which can be moved from well to well. Supply wells for domestic, industrial, or agricultural use often have large-capacity pumps permanently installed for routine sample collection.[57]

The type of sample, whole water or filtered, must be considered and will be determined by the goals of the study. Whole water samples provide an estimate of the total chemical concentration from the dissolved-phase, chemicals bound to particulate matter and suspended sediment, and any chemicals associated with colloidal and dissolved organic carbon. Filtered samples provide information on the concentration of free or unbound chemicals which are potentially bioavailable for uptake into aquatic organisms. Generally, filtration of samples is performed in the field during or immediately after sample collection, however, in some cases it may be acceptable to filter the samples in the laboratory.

2.1.2 Time-weighted sampling

Information on the identity and time-weighted average (TWA) exposure concentrations of pollutants in environmental waters is often difficult to obtain because of limitations in conventional analytical and biomonitoring approaches. For example, data from widely used grab and composite sampling methods provides information on water concentration only during the brief time of sample collection. Thus, detection of episodic events and estimation of more biologically relevant TWA values requires multiple samples through time. Also, detection of

trace-levels of bioconcentratable organic contaminants is problematic, because standard sampling methods are designed for relatively small volumes of water (≤5 L). Even when large-volume autosamplers are used, major concerns exist with sample contamination, analyte losses, and procedurally mediated changes in the ambient distribution of target compounds due to the collection, filtration, and extraction of large volumes of water. Biomonitoring organisms for assessing exposure of aquatic life to trace/ultra-trace levels of organic contaminants are often used, however, problems of metabolism/depuration of chemicals, avoidance of contaminated areas, and changes in behavior can lead to a lack of proportionality between tissue concentrations and exposure concentrations.[58,59]

Passive samplers provide a means of overcoming the shortcomings of common active sampling approaches. Passive samplers sample only dissolved chemicals, excluding those associated with particulate, suspended sediment, or colloidal matter. During a typical one-month exposure, a passive sampler potentially can sample tens to hundreds of liters of water, allowing for the detection of chemicals present at very low concentrations, or those that are present episodically. This time integration of contaminant presence is not readily achievable using standard sampling methods that collect discrete 1- or 2-L water samples. There are numerous passive samplers which have been developed for a wide variety of chemical species including, but not limited to, the semipermeable membrane device (SPMD), Chemcatcher™, polyethylene strips, polymers on glass, solid-phase microextraction, and the stabilized liquid membrane device. [58,59]

To date, the polar organic chemical integrative sampler (POCIS) is the primary passive sampler used for integratively sampling pharmaceuticals and hormones from water.[60,61] The POCIS consists of a solid-phase extraction sorbent or mixture of sorbents encased between two microporous polyethersulfone membranes. Organic chemicals move from the surrounding water

through the membrane, either by diffusion through water-filled pores or partitioning through the polymer matrix, and are trapped by the sorbent. Chemicals are recovered by extraction of the sorbent with a suitable organic solvent. Numerous prescription and over-the-counter pharmaceuticals have been measured in the extracts of POCIS deployed in streams receiving treated wastewater effluent.[38,62,63] Vermeirssen et al. (2006) found that using the POCIS to determine the estrogenicity related to steroidal hormones in river water provided data which was less variable and easier to interpret compared to data from grab samples collected at the same time.[64]

2.2 Soils, Sediments, and Biosolids

The collection of soil, sediment, and biosolid samples can be as simple as using a hand trowel to scoop soil into a container to as involved as taking sediment cores in deep lakes. Biosolids, or sewage sludge, are the residual materials which remain from the settling and dewatering processes in WWTPs which are often used as fertilizers and soil amendments with a consistency ranging from pourable liquids to solid cakes.[65,53] To overcome the inherent heterogeneity of soil, sediment, and biosolid samples, as large of a sample should be collected as possible. At the laboratory, the large samples can be homogenized and aliquots taken for analysis. Composite sampling can be used to partially overcome sample heterogeneity, however, if a point source contamination is suspected, composite sampling may dilute peak contaminant concentrations.[54] Care must also be taken to remove any rubble such as sticks, rocks, and leaves which are not part of the sample before homogenization.

Soil grab samples are collected by scooping soil from the surface or shallow depths (generally less than 30 cm) with a stainless steel trowel or small shovel.[52,53,54] To obtain a soil sample at greater depths, a soil punch or auger can be used. Sediment sampling generally

involves the use of coring devices or dredges. Dredges have the advantages that large samples can easily be taken; however, fine particles are often lost as the sample is raised through the water column from the bottom. Sediment cores maintain the sample's integrity and can provide data on a vertical gradient, but suffer from small sample sizes. Collection of a sample from the sediment-water interface is nearly impossible with both of these types of samples as the pressure wave generated ahead of the sampling device can cause dispersion of material. However, the U.S. EPA has developed and tested a sampling device, the undisturbed surface sediment (USS) sampler, to overcome the pressure-wave interference.[66] Biosolids can be collected as grab samples for pourable liquids (e.g., water and liquid biosolids), and as composite mixtures of low moisture cakes found in drying beds, dewatered biosolids, compost piles, and fields.

2.3 Tissue

The methods for collecting biological tissue samples can vary greatly between species. Problems such as limited availability of target organisms, restrictions on the collection of protected species, and difficulty in accessing critical habitats can all limit the scope of the sampling plan. This section will briefly discuss sampling options for fish communities; however, adaptations of these procedures could be applied to other species.

Preferred methods for collecting fish include direct current (DC) electrofishing, seining, or a combination of the two as these methods are the least injurious to the fish.[67,68] Other methods such as hook-and-line, trap-, gill-, or trammel-netting, and alternating current (AC) electrofishing can be used, however, these methods are much more injurious to fish and can bias quantitative health assessments and biomarker analyses.[67] Often large numbers of fish must be collected to obtain fish of the right size and gender. Mature fish are often preferred as it is easier

to determine gender (e.g., male fish may be preferred in an intersex study) and larger fish have a greater potential for bioaccumulation of chemicals aiding with method detection limit issues.

3 Analytical Chemistry of Environmental Analysis of Pharmaceuticals and Hormones

Many analytical challenges are offered to environmental chemists by the variety of environmental matrices that they may encounter, e.g., sediments, water, plant and fish tissue, biosolids/sludges, and soils. Additives and naturally occurring chemicals can also cause substantial interferences during both extraction and detection methodologies that are used. Some example interferents are surfactants in wastewaters, fats and chemical additives (ferric chloride, lime, and cationic polyacrylamide polymers) in biosolids, chlorophyll and pigments in plants, and humic acids in soils and lipids in earthworms. [38,65,69-73]

3.1 Extraction techniques

Typical concentrations of pharmaceuticals found in the environment are in sub-microgram per liter ($\mu g\ L^{-1}$), making pre-concentration prior to detection an important step. There are several methods of extracting and concentrating both aqueous and solid samples. These include:

- liquid-liquid extraction (LLE),
- solid phase extraction (SPE)
- tandem-solid-phase extraction (where two, or more, SPE cartridges are used in tandem for separation and clean-up)
- ultra-sonication
- microwave assisted extraction (MAE)
- molecularly imprinted polymers (MIP)

- pressurized liquid extraction (PLE). [3,39,43,74-79]

3.1.1 Aqueous extraction techniques

SPE is one of the most widely reported methods for the extraction of pharmaceuticals and hormones from environmental aqueous samples. SPE was developed as an alternative to replace LLE, which is labor intensive, difficult to automate, and requires large portions of high-purity solvents, such as methylene chloride, a known carcinogen. Also, hydrophilic pharmaceuticals may not readily partition into the organic solvent resulting in poor extraction efficiencies. SPE offers lower solvent consumption, shorter processing times, options for automation, and simpler procedures than LLE. Another advantage of SPE is that it can be modified for direct sampling in the field thereby eliminating the need for transport and storage of large sample volumes of water to the laboratory. Also, field-portable SPE can reduce the possibility of degradation of target analytes that can occur after sample collection during sample holding times.[80,81]

Physically there are two SPE formats available, thin flat discs (47 and 90 mm) and small cylindrical cartridges (usually < 6mL reservoirs). Both types can be packed with a wide-variety of sorbents, e.g. C_{18}, hydrophobic lipophilic balanced (HLB), mixed cation exchange (MCX), and mixed anionic exchange (MAX). The SPE sorbents are chosen for their ability to retain the pharmaceuticals and hormones of interest, and upon a variety of physico-chemical properties of the analytes of interest (e.g., pK_a, K_{ow}, polarity). For example, C_{18} is used as an universal extraction sorbent, with a pH range from 2 to 8, and its retention mechanism is primarily governed by hydrophobic interactions between the analytes and the carbonaceous moieties of the C_{18} alkyl chains.[82] Currently, the most commonly reported SPE sorbents used for extracting pharmaceuticals and hormones from environmental matrices are HLB sorbent and MCX sorbent.[3,39,43,74-78,83-86] Other less commonly used SPE sorbents include weak cation-exchange

(WCX), weak anionic-exchange (WAX), strong MAX, anion or cation exchange sorbents without mixed mode sorbents, and silica-based sorbents C_{18} and C_8.[87,88] The ion exchange cartridges are useful not only for extraction and concentration, but also for sample clean up. For example, SPEs can be used to separate humic and fulvic acids from basic pharmaceuticals, or separate neutral lipids from charged analytes.

Some pharmaceuticals can be considered as simple or ordinary ampholytes (compounds that have both acid and base functionality) such that they exist in solution as either ionized acids (anions) or ionized bases (cations), depending on pH. As examples, ampicillin, cephalexin, cephaloglycin, fexofenadine, nitrazepam, albendazole and sulfadimidine are ordinary ampholytes; while pyridoxine, niflumic acid and terbutaline belong to the zwitterionic amphoteric compounds (where both acid and base functions are ionized, and the resultant species is neutral at the isoelectric point).[89,90] In ordinary ampholytes, only the acidic or basic group can be ionized at a given time when the difference between the pKa of the acid and base is greater than 3. In cases where the pKa difference is less than 3, portions of both groups can be ionized forming a small amount of the zwitterionic species. The neutral form of the ordinary ampholyte is the dominant species when the pH is equal to the average of the pKa of the acid and base (for a simple diagrammatic explanation see figure 3). The hydrophilic-hydrophobic nature of the compounds of interest can be controlled by adjusting the pH in cases where the hydrophobicity of the neutral species is greater than that of either the associated anion or cation.[91] For a thorough review of the fundamentals of SPE extraction sorbents the reader is referred to Poole (2003).[82] <Figure 3 near here>

Most SPE methods rely on conditioning the SPE cartridge with neutral solvent(s); passing 500-mL to 2-L of water sample through the SPE cartridge, at approximately 7 to 10 mL min^{-1}, using either gravity flow, vacuum-induced, or syringe-pushed; drying the cartridge; and

finally extracting the analytes off of the cartridge using various solvents and solvent mixtures, dependent upon the pK_a's and polarities of the analytes, see figure 4. <Figure 4 near here>

Other types of extraction techniques have been reported. For example, published in the literature are two types of microextraction techniques. The first methodology uses liquid–liquid–liquid microextraction (LLLME) to extract sulfonamides from small volumes (microliters) of aqueous samples.[91] This extraction method depends upon equilibrium, rather than an exhaustive extraction like LLE. LLLME uses a polypropylene hollow fiber to extract the sulfonamides from a donor phase (i.e., a water sample) into several microliters of an organic phase and then from the organic phase into an acceptor phase. Unlike solid-phase microextraction (SPME) the extract phase of LLLME does not come into contact with the sample solution. In using LLLME the organic phase can be freely changed to extract target analytes and it utilizes disposable hollow fibers, thereby eliminating the risk of carryover and cross contamination.

Another microextraction technique uses SPME fibers. SPME, is a technique similar to LLLME. In SPME a hollow-fiber is used to extract compounds from an aqueous sample by absorption in the case of liquid coatings, or adsorption in the case of solid coatings. One of the first reports using SPME fibers to extract pharmaceuticals and steroids from water samples is found in Moeder et al. (2000).[31] The authors compared several different types of SPME fiber coatings, the resultant SPME extracts were derivatized, followed by GC/MS analysis.[31] McClure and Wong (2007) report immersing the SPME fibers (carbowax/divinyl-benzene; polydimethylsiloxane; polydimethylsiloxane/divinylbenzene and carbowax-templated) in 1.5 mL of aqueous sample, then subsequent extraction of the fiber with 10 mL of methanol.[92] Basheer et al. (2005) describe an altered SPME procedure, termed polymer-coated hollow fiber microextraction (PC-HFME), whereby they coated SPME with a new polymer that has a high number of function groups (-OH) more compatible to polar compounds, such as the estrogens.[93]

Briefly, the alcoholic -OH of 2,2-dimethyl-1,3-dioxolanyl-4-methanol was protected using tosyl chloride to give the tosylated derivative, this was reacted with 3-hydroxy benzyl alcohol giving an intermediate, which on reaction with methacryloyl chloride, in the presence of triethylamine, yielded a monomer. Free radical polymerization was carried out in toluene using azobisisobutyronitrile as the initiator and finally deprotonation of the precursor polymer, under acidic conditions, gave the dihydroxylated polymethacrylate (DHPMM) in 70% yield. The SPME fibers were cut in half and soaked in a DHPMM solution to form a thin layer onto the fibers. Using PC-HFME, they extracted diethylstilbestrol (DES), estrone, 17β -estradiol and 17α-ethynylestradiol, from spiked reservoir and tap water samples. The extracts were then derivatized and analyzed by GC/MS.[93] Some obvious advantages to SPME are small sample sizes and small volumes of solvents used. Disadvantages, can be interferents (e.g., surfactants, humic and fulvic acids) competing for limited bonding sites, and extended equilibrium times necessary for ensuring representative extraction efficiencies.

On the horizon is a novel extraction technique that is target class specific, MIPs. MIPs are customized polymer resins that are imprinted with specificity to either a single analyte or to a class of analytes. Once in the realm of the research laboratory there are now several commercially-available MIP sorbents. A simple diagram outlining the making of a MIP is shown in figure 5. A recent publication combines SPME with MIP for the extraction of tetracyclines from animal tissue and milk.[94] While milk and tissue are not traditionally thought of as environmental samples, there is the possibility of transferring this unique extraction methodology to water and wastewater samples. Caro et al. (2006) report the extraction of ciprofloxacin from urine samples using in-tandem SPE (Oasis HLB) and MIP (specific for the fluorinated quinolones) sorbent cartridges.[95] Again, while urine is not traditionally an environmental sample, there is the possibility of transferring this extraction technique to water

and wastewater samples. Four recent publications demonstrate the cross-over from biological samples to environmental samples; Meng et al. (2005), Turiel et al. (2007), Watabe et al. (2006), and Gros et al. (2008).[96-99] In Meng et al., they developed a non-specific MIP to extract ß-estradiol, diethylstilbestrol, estriol, and estrone, from wastewater. The MIP material generated was re-usable for at least 5 times.[96] Turiel et al. (2007) developed a MIP material for extracting fluoroquinolones from soils.[97] One drawback to the MIP template technique is that it is difficult to completely remove the target analytes from the MIP template, and this is especially problematic at the low levels that most pharmaceuticals and hormones are found in the environment, ng L^{-1}, therefore it would be difficult to get an accurate quantitation of the target compound.[98] In Watabe et al.(2006) the authors developed a MIP template to extract only 17ß-estradiol (E2) from river water. They solved the difficulty of interference, from the sloughing off of the residual template material, by using a similarly structured analog of 17ß-estradiol: 6-ketoestradiol (KE2), which has a different chromatographic retention time than that of 17ß-estradiol.[98] In Gros et al.(2008) they developed a method that uses a commercially available MIP template [MIP Technologies (Lund, Sweden)] to selectively extract eight ß-blockers from waste water. They compared the MIP extracts to SPE (HLB) extracts and found that while the recoveries were similar, that the MIP extract provided a lower overall method detection limit due to the specificity of the MIP template.[99] <Figure 5 near here>

Reported in the literature is a simple sonication technique, combined with direct aqueous injection, for the detection of fluoroquinolones in ground water and hospital wastewater (and other non-environmental media, i.e., chicken muscle, urine, and pharmaceutical samples).[100] In this article the authors report directly taking the aqueous sample, degas the sample in a sonication bath, and directly inject it into a liquid chromatograph-ultraviolet detector (LC-UV).

Unfortunately, the authors do not specify how much sample was used, but the data shows ranges 83 to 95% recoveries at 5 ng mL^{-1} spikes.[100]

3.1.2 Solid sample preparation and extraction

While water samples have their difficulties and interferences, such as surfactants, humic acids, variable pH ranges, the more challenging extractions can be had from solid samples. Researchers have reported extracting pharmaceuticals and hormones from such solid matrices as animal lagoon waste, sewage sludges/biosolids, plants, worms, and fish.[65,71-73,101-105]

One of the most commonly reported extraction techniques used for solid samples is PLE. PLE uses organic solvents at high pressures and temperatures to enhance the extraction of organic compounds out of solid matrices. The basic physicochemical properties of this procedure guarantee enhancement of (1) solubility and mass transfer effects, and (2) disruption of surface equilibria.[106]

Briefly, we will outline a simple PLE procedure. Because of the complexity and variable sizes of environmental solids, they usually need to be dried, pulverized and homogenized before extraction. A small amount of the homogenized solid sample (usually < 2 g) is sub-sampled and then mixed with approximately 2x the amount of an inert matrix (e.g., diatomaceous earth). A cellulose filter is inserted at the bottom of a stainless steel extraction cell (capped on one end, sizes are variable according to manufacturer's specifications and size of sample to be extracted) and the homogenate is transferred into the extraction cell using a polytetrafluoroethylene (PTFE) lined funnel. The extraction cell is tapped gently to reduce the air pockets inside the stainless steel cell. The remaining volume is filled with more inert matrix and another cellulose filter is placed on the top of the sample inside the extraction cell, and the remaining cap is screwed on tightly, the sample is now ready for extraction. Depending upon

what matrix and what analytes are being extracted, the proper solvents, pressures and temperatures are chosen. For example, Chu and Metcalf (2007) measured paroxetine, fluoxetine, and norfluoxetine (metabolite of fluoxetine) in fish tissue.[105] For their method, they took 3g of fish tissue, mixed it with 6 g of an inert matrix, and placed the homogenized mixture into a 34 mL stainless steel extraction cell containing a glass-fiber filter in the cell outlet. Methanol was selected as the optimal PLE solvent and the PLE conditions were as follows: oven temperature, 100^{o} C; pressure, 1500 psi; 5 min heat-up time; three static cycles; static time, 5 min. The flush volume amounted to 100% of the extraction cell volume and purge time was 1.5 min using pressurized nitrogen (125–150 psi). The resultant PLE extract was subsequently cleaned-up using SPE (MCX) and analyzed by LC-atmospheric pressure chemical ionization-tandem mass spectrometry (LC-APCI-MS/MS).[105] Nieto et al. (2007) developed a PLE LC-electrospray ionization-mass spectrometry (LC-ESI-MS) method for the determination of three macrolides, five sulfonamides, ranitidine, omeprazole and trimethoprim in sewage sludge samples.[103] Their extraction solvent and operational parameters, such as temperature, pressure, extraction time and purge time were optimized as follows: an extraction solvent of water(pH3):methanol(1:1,v/v); temperature of 80◦C; pressure of 1500psi; sample weight of 5g; extraction time of 5-min; one cycle; flush volume of 60%; and a purge time of 120s.[103]

Another extraction technique that can be used for solids is MAE.[35,107,108] MAE is based upon using microwave radiation (2450 MHz) interacting with a mixture of polar solvent(s) and a solid matrix. The rapid heating that occurs in the mixture is driven by ionic conduction and dipole rotation of the polar solvents, and the thermal order of molecules and subsequent return to disorder (at 4.9 x 10^{9} times per second). The amount of energy absorbed and released into heating the sample is proportional to the dielectric constant (ε') of the solvent chosen, and proportional to the solvent polarity. The overall efficiency of the heating is expressed by the

dissipation factor (tan δ). The ultimate effect is to heat up the water entrained in the matrix, and as the water heats it forms gas bubbles under pressure, resulting in destruction of the matrix macrostructure, which liberates the surface of the matrix (e.g., soils, sediments, tissue) for interaction with the solvents. Two types of commercially MAE's are available: closed vessels (controlled pressure and temperature), termed pressurized MAE (PMAE); and open vessels (atmospheric pressure) termed focused-MAE (FMAE).[109]

In MAE pre-extraction sample preparations are dependent upon which analytes are being targeted. For example, Liu et al. (2004) does not pre-dry the sample, but both Cueva-Mestanza et al. (2008) and Rice and Mitra (2007) pre-dry their samples.[35,107,108] Liu et al. (2004) and Rice and Mitra (2007) both added an optimized amount of solvent(s) to their samples before MAE.[35,107] In Liu et al. (2004) they studied many various MAE conditions for the optimization of the extraction of 17-estradiol, estrone, 17-ethynylestradiol, 16-hydroxyestrone from river sediments. They found that the optimal conditions to obtain the best efficiency and recoveries of the estrogens from sediments were: wet (not dried) samples, 25 mL of methanol, 100% power at 600W, 110^{o}C, and a 15 min extraction time.[35]

The procedure described by Cueva-Mestanza et al. (2008) was novel in that they added 8-mL of a non-ionic surfactant, polyoxyethylene 10 lauryl ether (POLE) to 2-g of dried, and homogenized, sediment sample, to enhance the microwave energy, terming this method microwave assisted micellar extraction (MAME).[108] They proceeded with MAE (radiation power of 500 W), and subsequent SPE (Oasis HLB) clean-up of the extract. This procedure gave them comparable results to Soxhlet extraction procedures. Relative recoveries for spiked sediment samples were over 70% and relative standard deviations (RSDs) were under 11%, and detection limits between 4 and 167 ng g^{-1} were obtained. The MAME procedure holds promise for less solvent usage, an important factor in developing green chemistry techniques.[108]

Another, simpler, methodology reported uses ultra-sonic sonication, followed with a SPE and/or gel-permeation (GPC) clean-up procedures.[110-113] In general, a small amount of sample is placed in a container, solvent is added and then the vessel is sonicated for several minutes, the solvent supernatant is decanted and cleaned through SPE or GPC, reduced to a smaller volume, and analyzed by either GC/MS or LC/MS.

3.2. Detection techniques

The majority of detection techniques for pharmaceuticals and hormones are mass spectrometry based. In the previous section on extraction, most of the extraction procedures described end up using a mass spectrometer as the detector. This is due to the reality that most environmental matrices are "messy", and only the mass accuracy and specificity given by mass spectrometry can overcome the large amounts of interferences found in real-world matrices. For example, one of the first reports of finding estrogens in the environment used HPLC-fluorescence detection, but the authors reported many polar interferences in the estrogen-containing fraction, making identification difficult.[114] Later work, by the same principal investigator (Snyder) utilized the mass accuracy and specificity of a mass spectrometer detector for the same analytes, plus they were able to characterize other pharmaceuticals in the same lake water matrix.[55] Another recent publication uses SPE coupled to capillary electrophoresis-UV/diode-array detector (CE-UV/DAD) for the detection of naproxen (an over-the-counter analgesic) in tap water samples.[115] The authors first had to clean-up the sample with off-line SPE; their limit-of-detection (LOD) for naproxen was 200 ng L^{-1}, which is less than what had been reported by Bones et al. (2006) who used an on-line SPE-LC-UV-MS method (LOD 8 ng L^{-1}).[42,115] A drawback to the CE-UV/DAD methodology is that there is no specificity to determine what else is present in the electropherograms generated. What was initially gained by

this CE-UV/DAD method, an 1820-fold increase in sensitivity, can't make up for the lack of specificity gained by using mass spectrometry as a detector. In Bones et al. (2006) the authors point out that although UV detection gives the sensitivity, it cannot give the specificity of mass spectrometry, as both fluoxetine and warfarin were obscured in the UV trace, but were not obscured when coupled to the LC/MS.[42] There are distinct advantages to coupling UV and MS in overcoming background interferences for ultra-trace analyses in environmental matrices.

There are a variety of mass spectrometers now being used as detectors coupled to either GCs or LCs. There are quadrupole mass spectrometers, ion traps (ITMS), time-of-flight mass spectrometers (TOF), triple quadrupole mass spectrometers (QqQ), magnetic sector mass spectrometers, and most recently orbitrap mass spectrometers. Depending upon the type of separation technique used (GC or LC), information needed, mass accuracy necessary, and specificity dictated by regulation, will determine what type of mass analyzer should be used for environmental analyses. The reader is referred to several references for gaining a better understanding of mass spectrometry and its application to environmental analysis: Herbert and Johnstone 2003, Grayson 2000, McLafferty 1980, Barcelo 1996, Busch et al 1988.[116-120]

GC/MS was initially the detection method that had been widely reported as the detection method for non-polar pharmaceuticals, and steroids and hormones. LC/MS was the detection method of choice for most polar and non-volatile pharmaceuticals. However, recent publications are showing an increase in the use of LC/MS and LC/MS/MS detection techniques for the detection of most pharmaceuticals and hormones. In the next section we will explore both the use of GC/MS and LC/MS detection techniques.

3.2.1 Gas Chromatography-Mass Spectrometry

The first two publications regarding pharmaceuticals as contaminants in the environment reported using GC/MS as the detection technique for the metabolites of clofibrate and aspirin (chlorophenoxyisobutyrate and salicyclic acid, respectively), in sewage effluents.[24,25] These polar, and thermally labile, metabolites were only amenable to gas chromatography due to derivatization with diazomethane before analysis, and the fact that GC/MS was no longer only in the realm of research laboratories, but had at that time become commercially available to the environmental analytical community.[25,117]

In general, substances that vaporize at < 300 °C (and therefore are stable up to that temperature) can be measured by GC/MS. Unlike non-specific detection techniques (e.g., UV/DAD, or fluorescence), GC/MS offers the ability to produce multiple fragment ions (via electron ionization) from a given analyte, giving the chemist an unequivocal identification technique.

Pharmaceuticals and hormones that are polar, and/or thermally labile need to be derivatized in order to pass through a GC, and most early attempts to identify these compounds in the environment used derivatization.[31,110,121] There are usually two methods of derivatization used to methylate the H-acidic functional groups of the ions, e.g., COOH and OH groups. One method uses diazomethane, and another uses trimethylsilyl (TMS) derivatization. As an example, several derivatizing agents are used to derivatize the hydroxyl groups contained in estrogen, and estrogen-like compounds: N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA), bis(trimethylsilyl) trifluoroacetamide (BSTFA), and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA).[31,35,110,121,122]

The first two papers to describe the use of TMS are Moeder et al. (2000) and Kelly (2000).[31,121] In Moeder et al.(2000), they developed a SPME extraction technique for determining ibuprofen, clofibric acid, caffeine, paracetamol, phenazone, carbamazepine,

gemfibrozil, naproxen, indomethacine, norethisteron, propranolol, and metaprolol in water. The authors describe adding 100 μL of (BSTFA) to the 500 μL SPME extract, heating for 1-hr at 40°C, evaporating to 250 μL, then injecting 1 μL into a GC/MS for analysis.[31] In Kelly (2000), the author extracted three hormones: estrone, 17β-estradiol, and the synthetic contraceptive steroid 17α-ethinyloestradiol, from water samples. The samples were extracted using SPE (C_{18} disks) and the subsequent 200-μL extract was derivatized with MTBSTFA, before analysis by GC/MS or GC-tandem mass spectrometry (GC/MS/MS).[121] Ternes et al. (2002), describe the extraction of estrone, 17β-estradiol, 17α-ethinylestradiol, and mestranol from sewage sludge and sediments using lypholization (freeze-drying), ultrasonication, then gel-permeation cleanup. The subsequent "clean" extract is then derivatized by adding 50 μL of a derivatization mixture: MSTFA/trimethylsilylimidazole (TMSI)/dithioerytrol (DTE) (1000:2:2; v/v/w); to 1-mL of extract, and then analyzed by GC/MS/MS.[110]

Quintana et al. (2004) lists the various experimental conditions that can affect derivatization: time, temperature, volume of derivatizing agent, and proportion of catalysts (if used). They also state that the differences among the various derivatizing reagents are based: (1) on their reactivity towards aromatic and aliphatic hydroxyl groups; and (2) on the stability of the obtained derivatives.[123]

Derivatization methods have disadvantages. For example, incomplete derivatization can occur, leading to lower recoveries, and subsequently underestimation of contamination. More specifically, the use of diazomethane is not a preferred derivatization method due to its dangerous properties (toxicity and explosivity). Other common methods of derivatization include the use of sulfuric acid-methanol, trifluoroborane (BF3)-methanol, and TMS. The use of TMS, while not "dangerous", can lead to the formation of mono- and di-TMS derivatives, which can cause problems with identification and quantitation. Because of the limitations of

derivatization, there is an increasing trend to use LC/MS as a determinative method in analyzing for polar, non-volatile, and/or thermally labile pharmaceuticals and hormones in environmental matrices.

3.2.2 Liquid Chromatography-mass spectrometry

As discussed in the previous section conventional GC/MS methods have limitations as to the types of analytes that are amenable to that detection technique. Many pharmaceuticals and hormones are polar, thermally instable, hydrophobic, and have low volatility, making them ideal candidates for LC/MS.

The coupling of LC to MS has been utilized for over 30 years.[124] Briefly, the mobile phase of the LC is nebulized [these days most LC to MS interfacing is via electrospray ionization (ESI)] into a MS source. The MS source is at atmospheric pressure, and through various combinations of heated capillaries (e.g., ion cones, hexapoles, quadrupoles, and ion filters) the charged analytes are brought into the high vacuum range of the mass spectrometer detector region. Some of the early LC to MS interfaces (e.g., moving belt) were not very stable nor was complete ionization of the analytes possible. One of the unique aspects of LC/MS is that the technique usually creates only a single ion in the source, allowing for identification of the molecular weight of a compound. The ion created is typically the molecular ion plus a hydrogen, if in the positive ionization mode $(M+H)^+$, or the molecular ion minus a hydrogen, if in the negative ionization mode, $(M-H)^-$. This can be a limitation, for without more than one ion for identification it would be easy to misidentify analytes in complex environmental matrices. For example, some surfactant ions are isobaric interferents with some of the macrolide antibiotic ions, as described in Jones-Lepp et al. (2004).[38] The analyst could mistakenly think that they have identified an antibiotic, when in fact it was a surfactant ion (of which there are many more

surfactants used daily than antibiotics), giving a false positive value. Therefore, for more specific identification the analyst must go to what is referred to as tandem MS, or MS/MS techniques. This is a MS technique whereby the precursor ion formed in the LC/MS source [typically the $(M+H)^+$ or $(M-H)^-$ ion] is energized and collided, either in a triple quadrupole, ion trap, or a magnetic sector mass spectrometer, thereby producing product ions. Product ions are typically the loss of various functional groups from the analytes, for example $(M+H-OH)^+$ or $(M+H-CH_3)^+$. When using LC/MS techniques for identifying known, and unknown, chemicals, it cannot be emphasized enough that the analyst must use a LC/MS/MS technique in order to accurately identify the analyte. The reader is referred to three very good books that can give an in-depth review of the history of LC-MS and its wide-spread applications: Liquid Chromatography-Mass Spectrometry (2006 edition), W. Niessen; Liquid Chromatography-Mass Spectrometry: Applications in Agricultural, Pharmaceutical, and Environmental Chemistry, ed. M. Brown (1990); and Applications of LC-MS in environmental chemistry, ed. D. Barceló (1996).[119,124,125] In this article we will only focus on the application and analytical chemistry aspects of LC/MS as applied to the detection of pharmaceuticals and hormones.

Hirsch et al. (1998) was one of the first authors to report using LC/MS for the analysis of several antibiotics in a small river. They extracted the water samples using either lypholization and re-suspension in phosphate buffer for the tetracyclines, or SPE (C_{18}) for the other antibiotics (macrolides, sulfonamides, trimethoprim, penicillins, and chloramphenicol). The extracts were analyzed via three different sets of LC conditions, dependent upon the analytes, and tandem MS (triple quadrupole) detection.[29] The following year Hirsch et al. (1999) expanded and improved their original method, and applied it to a wider variety of water samples, e.g., sewage effluents, river waters, and drainages.[30]

In the earlier GC/MS section we saw how Moeder et al.(2000) developed a SPME

extraction method, with subsequent derivatization of the extract so that it was suitable for GC/MS/MS analysis, detecting ibuprofen, clofibric acid, caffeine, paracetamol, phenazone, carbamazepine, gemfibrozil, naproxen, indomethacine, norethisteron, propranolol, and metaprolol, in water.[31] In 2001 Farré et al., developed a SPE extraction method for some of the same analytes; ibuprofen, ketoprofen, naproxen, diclofenac, the decomposition product of acetyl salicylic acid: salicylic acid, and gemfibrozil in water.[33] However, they subsequently analyzed the extracts directly by negative ionization LC-ESI/MS, without any derivatization step. Farré et al. (2001) compared their LC/MS methodology with their GC/MS methodology (first derivatizing the extract), and found that the LC/MS method was an improvement over the GC/MS method for these particular compounds, since the derivatization step was avoided.[33] All of the compounds selected by Farré et al. (2001) are acidic substances, and very polar, thereby making them amenable to LC/MS techniques.[33] Bones et al. (2006) developed an on-line SPE(C_{18}) LC-UV-MS method, in water, for some of the same Moeder et al. (2000) analytes (i.e., ibuprofen, gemfibrozil, clofibric acid, naproxen), plus several antibiotic classes, fluoxetine, triclosan, and ivermectin.[31,42] In this methodology a 500-mL water sample, adjusted to pH 4, is pumped through a SPE column (at 10 mL min^{-1}), then the elution of the analytes is performed by back flushing the SPE column with mobile phase into the LC-UV-MS.[42] This on-line extraction methodology was found to be very pH dependent for efficient extraction recovery of the polar analytes. In fact no appreciable recovery was obtained of the very polar analytes, due to the use of SPE C_{18} sorbents, whereas, gemfibrozil, a non-polar analyte, exhibited only a slight variation in extraction recovery over a wide pH range.[42] At this time the reader is reminded of the importance of pH selection when extracting polar and non-polar analytes from environmental matrices, see section 3.1.1, no matter what detection technique is chosen.

In 2002, a nationwide (US) reconnaissance of over 60 pharmaceuticals and hormones was

undertaken in the nation's waterways by the US Geological Survey (USGS).[2] In this survey a combination of extraction methods, SPE (using MCX, HLB and C_{18} sorbents) and continuous liquid-liquid extraction (CLLE), combined with either LC/MS (single ion monitoring) or GC/MS detection methods were utilized. This paper, through the use of five different analytical extraction and detection schemes, clearly shows the complexity of analyzing for a variety of pharmaceuticals and hormones in a seemingly simple matrix, natural waters. However, this thorough study demonstrates one of the flaws of LC/MS, namely its lack of specificity without MS/MS as a complimentary identification technique. The authors used single ion monitoring (SIM) LC/MS to detect the polar analytes, specifically erythromycin, which they report detecting in many water samples.[2] However, one of this article's authors (Jones-Lepp) would like to speculate, that without the specificity of MS/MS to accurately identify erythromycin-H_2O (via more than one specific ion) that it wasn't erythromycin detected, but instead an overlapping isobaric surfactant ion(s). To support this supposition the minimal prescriptive use of erythromycin in the US is low, overwhelmed by other macrolide antibiotic prescriptions (i.e., azithromycin and clindamycin) (http://drugtopics.modernmedicine.com/drugtopics/data/articlestandard/drugtopics/072008/491181/article.pdf), and in Hirsch et al. (1999) the authors clearly delineate the problems with detecting erythromycin, and its degradation products, in seemingly neutral aqueous environments.[30] More recent LC/MS articles show the utility of LC/MS, but all of them emphasize the need for MS/MS techniques for positive identification of pharmaceuticals and hormones.[55,126-129]

Choosing the proper LC column is important for proper MS detection. Most of the newer (> 2005) LC/MS methods use some type of reversed phase, small-bore C_{18}-silica bonded packed chromatography column, capable of withstanding high backpressures (2000 to 5000 psi). These types of LC columns typically have particle sizes ranging from 3 to 10 μm, and can handle a wide pH range, usually pH 2 to 8. Most HPLC columns used today are termed small-bore (~ 2.1

mm dia) or micro-bore (< 2.0 mm dia). Newer LC/MS instruments can handle the very low flow rates that these columns use (typically 10 μL min^{-1} to 300 μL min^{-1}) and they are coupled directly into the electrospray source without splitting the flow. Recently, another new type of LC column has been introduced, ultra-performance liquid chromatography (UPLC), that is capable of high-speed analysis, greater resolution, and sensitivity. The use of UPLC first showed up in 2005 for use in the field of metabonomics.[130] Since then several environmental researchers have applied this new type of chromatographic column towards the analysis of pharmaceuticals and hormones in environmental matrices.[41,85,88,131-134] The advantages of UPLC over microbore-LC, or even capillary (nano)-LC, is better resolved analytical peaks, while having enough mass scans across each peak, in the shortest timeframe possible, while retaining acceptable peak shape.[85] Due to the fast elution times (most UPLC chromatographic runs are under 10 min) a fast scanning mass spectrometer, such as TOF or triple-quadrupole (QqQ) mass spectrometer, and an HPLC system capable of high backpressures (~ 6000psi), is necessary to achieve optimization of the UPLC separation technique.

3.2.2.1 Matrix interferences and ion suppression

Although SPE can significantly reduce the amount of interfering matrix material from complex environmental samples, LC/MS is susceptible to matrix effects. Remaining components of the matrix can result in a suppression or enhancement of the signal from the analytes during LC/MS analysis. These can be compensated for by either the addition of a surrogate compound, or internal standard to correct for signal changes, or by the use of the method of standard addition. An internal standard is added after sample preparation, and will account for differences in instrument signal and injection volume, while a surrogate compound is

added before sample preparation and will account for any losses of the analytes during extraction. In the case of either a suitable surrogate compound or internal standard, one is chosen that is similar in chemical structure to the analyte(s), will not interfere with the analysis, nor is found in the environment. The most ideal surrogate or internal standard for LC/MS analysis is an isotopically labeled version of the analyte. However, it is not always feasible, nor economical, to find an isotopically labeled version of the analyte(s) of interest. Another way to overcome matrix interference and correct for ionization suppression is to use the method of standard additions (MSA). Although labor intensive and time consuming, MSA can be used to correct for recovery, matrix interference, and calculate concentration, when an appropriate surrogate compound or internal standard is not available. MSA is a technique by which a known amount of analyte (of interest) is added to a sample extract and is measured under identical instrumental conditions that the original unspiked extract with an unknown amount of analyte (of interest) was measured. Chu and Metcalfe (2007) use this approach to overcome matrix effects to accurately measure triclocarban and triclosan in municipal biosolids[135]

In the analysis of sulfonamides and tetracyclines with LC-ESI-iontrap MS/MS, Yang et al. (2004) found ionization suppression of tetracyclines in wastewater to be significant, while no suppression or enhancement of the signal for sulfonamides was observed. The matrix suppression of tetracyclines was accounted for using an internal standard.[136] Matrix interferences in Swedish hospital wastewater was also found to be negligible for sulfamethoxazole by Lindberg et al. (2004), while ciprofloxacin was found to be highly susceptible to matrix ionization suppression.[137] However, the use of another fluoroquinolone antibiotic, not approved for use in human medicine in Sweden (enrofloxacin), as an internal standard for the analyzed fluoroquinolones provided the same analyte to internal standard signal

ratio in wastewater as distilled and tap water, which sufficiently accounted for the ionization suppression.[137]

4.0 Summary: Future analytical directions

We have tried to cover a broad range of analytical techniques applicable to extracting and detecting pharmaceuticals and hormones from the environment in this article. Many of the techniques discussed are new technologies, built upon the old methods, although there is always room for improvement.

One example of a new technology, is a novel mass spectrometer, the orbitrap, that has recently been developed.[138] It couples a linear ion trap mass spectrometer to an orbitrap mass analyzer, while operating in the LC/MS mode (1 spectrum $scan^{-1}$) with a nominal mass resolving power of 60,000 [at full width at half maximum (fwhm)], to provide mass accuracy within 2 ppm. This new design, coupled with the increasing speed of chromatographic separations (UPLC and fused-core-LC), will allow for fast separations and mass accurate identifications of analytes in complex environmental matrices, including those with isobaric interferences that require the higher resolving power. One recently published paper explores the use of the orbitrap, along side TOF and Fourier transform ion cyclotron resonance, for the screening and detection of Clenbuturol-R in drug residue analysis.[139] So far no publications, but only two presentations have been made regarding orbitrap and the detection of pharmaceuticals and hormones in environmental matrices.[140,141]

In analytical separations one commercial supplier has recently released a different type of LC column that has the efficiency and resolution of UPLC, but half the backpressure of UPLC (~2000psi), based on their Fused-Core™ technology. The core of the silica particulate is solid and is surrounded by a porous shell. The major benefit is a small diffusion path (0.5 um), which

reduces axial dispersion and minimizes peak broadening. This type of column may be worth experimenting with for faster and more efficient chromatography for environmental separations, essential for faster scanning mass spectrometers, e.g., TOFMS.

We have discussed quite extensively the MIP extraction technology, and again put this forth as a new approach to extraction of classes of analytes from complex environmental matrices, without extracting the interferents.

Another technique that seems to be coming more prevalent is on-line SPE coupled to LC/MS, and one report of SPE coupled to capillary electrophoresis. [42,45,79,115,123,126,128,142]

Finally, we would like to discuss "green" chemistry techniques. The goal of every analyst should be the better use of newer, environmentally friendly technologies (e.g., power saving, recyclable materials) without increasing the use of toxic, or chemically hazardous substances. For example, the typical SPE extraction usually only requires 20 to 50 mLs of solvent for preparation to extraction of the cartridges, while LLE methods can use liters of solvent; SPE can be automated, and theoretically has a higher extraction efficiency. Analysts can also choose to go from large-bore LC columns for LC/MS, which typically have 1 to 2-mL flow rates to small-/nano-bore LC columns that have optimal flow rates of 100 to 300 μL min^{-1}. Or we can conserve energy by purchasing EnergyStar™ efficient computers, and environmentally-friendly components of mass spectrometers and gas and liquid chromatographs. All of these seemingly little aspects add up to a larger environmental savings and smaller carbon footprint when taken over the analytical chemistry community at large. We would like to point our readers to a good review of "*Green Chemistry*" by Armenta et al. 2008 for more insights and suggestions for making a more environmentally friendly laboratory.[143]

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Figures

Figure 1. Trend of pharmaceutical environmentally-related publications

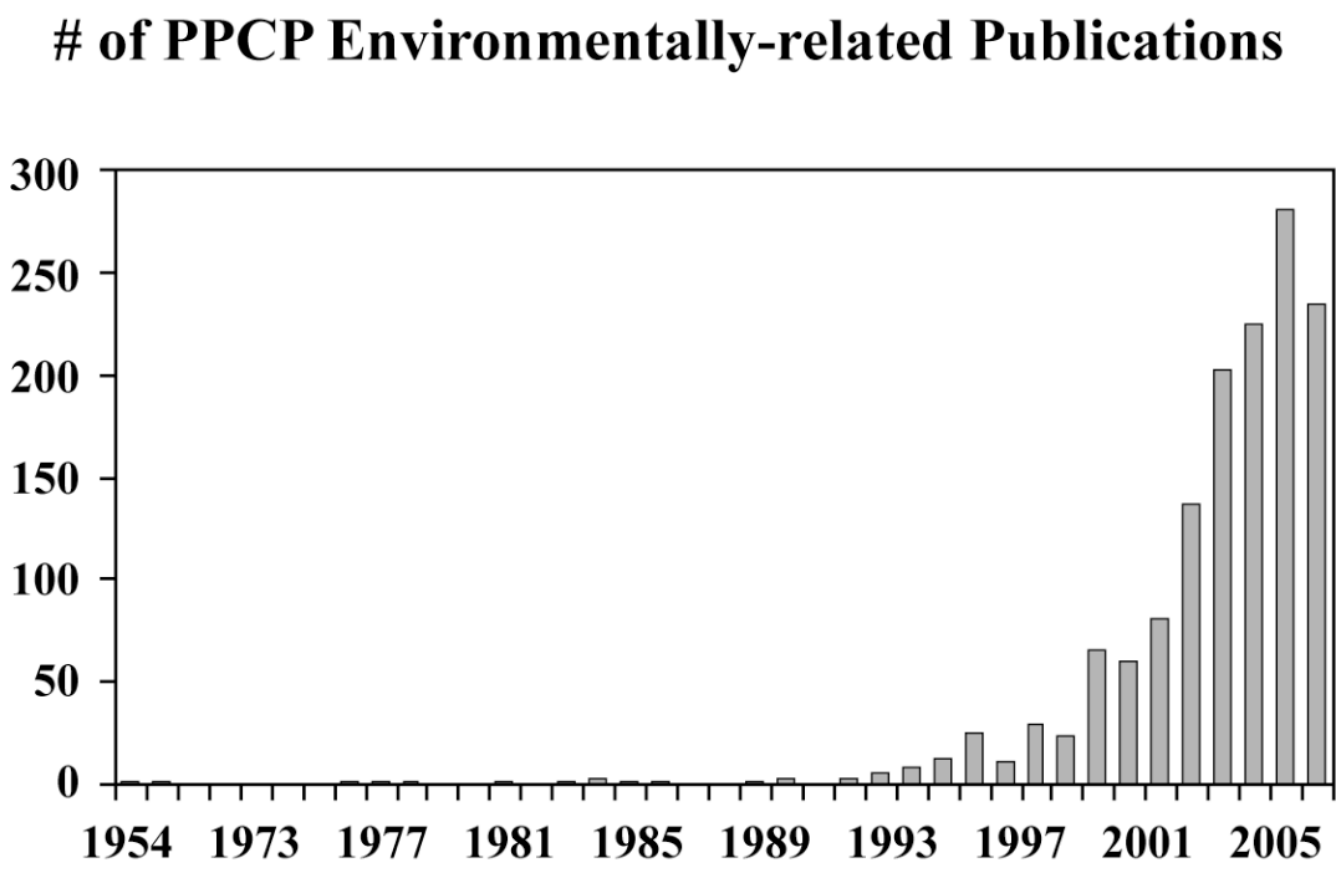


Figure 2. Origin and fate of PPCPs in the environment

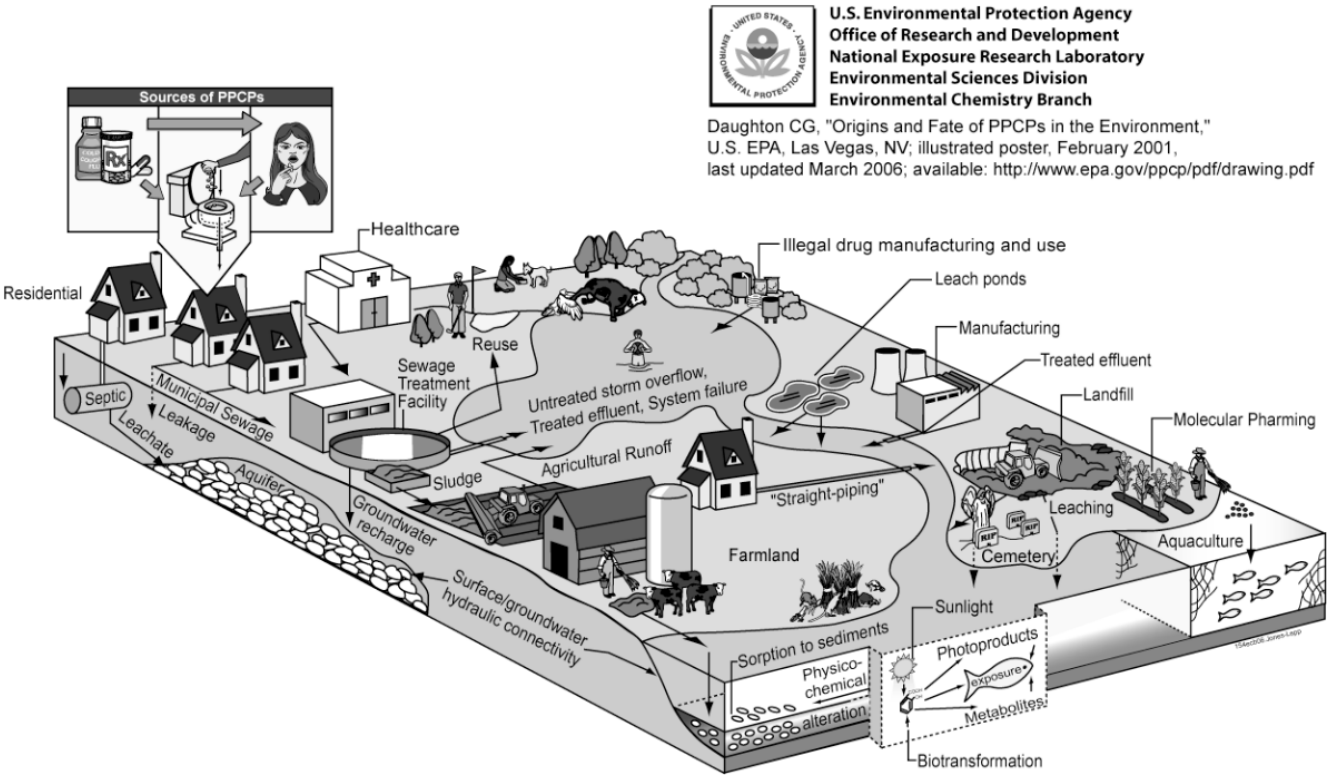
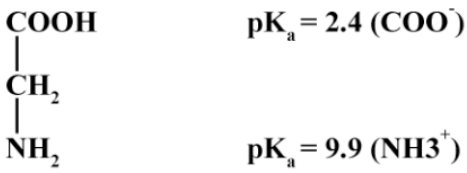
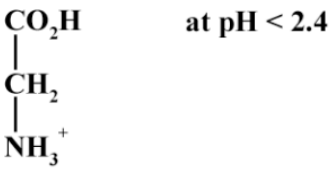


Fig. 3 - Simple diagram of *ampholytes* and *zwitterions* for SPE consideration

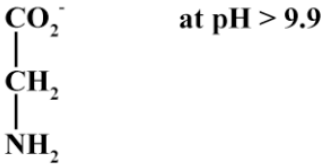
Neutral species



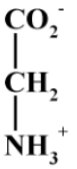
Cationic species



Anionic species



Therefore pH: 2.4 + 9.9 = 12.3 / 2 = 6.15



This is the *zwitterion* at the isoelectric point.

Fig. 4 An outline of the steps used in a SPE method.

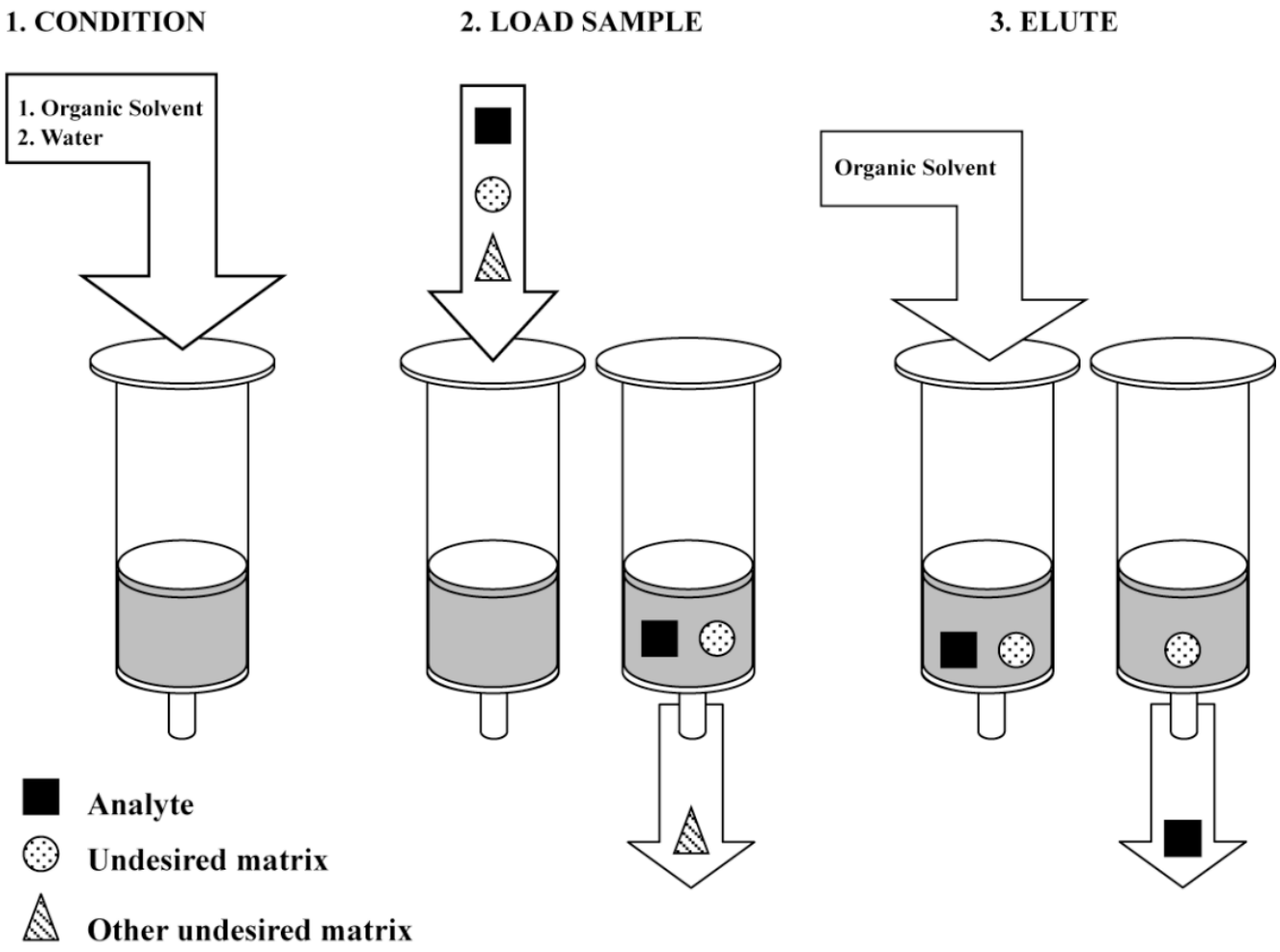


Figure 5. Simple MIP template