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Occurrence and Fate of Pharmaceuticals and Personal Care Products within Sewage Sludge and Sludge-Enriched Soils

STRIVE

Environmental Protection Agency Programme

2007-2013

SSTI
Strategy for Science, Technology and Innovation

Comhshaol, Oidhreacht agus Rialtas Áitiúil
Environment, Heritage and Local Government

Environmental Protection Agency

The Environmental Protection Agency (EPA) is a statutory body responsible for protecting the environment in Ireland. We regulate and police activities that might otherwise cause pollution. We ensure there is solid information on environmental trends so that necessary actions are taken. Our priorities are protecting the Irish environment and ensuring that development is sustainable.

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- Office of Environmental Enforcement
- Office of Environmental Assessment
- Office of Communications and Corporate Services

The EPA is assisted by an Advisory Committee of twelve members who meet several times a year to discuss issues of concern and offer advice to the Board.

EPA STRIVE Programme 2007–2013

Occurrence and Fate of Pharmaceuticals and Personal Care Products within Sewage Sludge and Sludge-Enriched Soils

(2005-FS-30-M1)

STRIVE Report

Prepared for the Environmental Protection Agency

by

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in collaboration with

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DISCLAIMER

The EPA STRIVE Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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Executive Summary

1 Scope and Project Aims

The potential threat of pharmaceuticals and personal care products (PPCPs) to the environment has emerged as a topic of concern in recent years. To date, there exists a dearth of analytical methods to empirically determine their occurrence in solid media. This 3-year research and development project focused on a number of topics surrounding the exposure of the terrestrial environment to pharmaceuticals through land spreading of municipal biosolids (sludges) on agricultural land. More specifically, the aims of the project were:

- To identify which PPCPs may occur at significant levels in waste-water treatment sludges
- To develop robust analytical methods for soil and sludge analysis
- To compare any occurrence data with those from a European case study
- To determine the solid–water partition coefficient for all compounds in aqueous sewage sludge and soil suspensions
- To model sorption data in order to identify preferred sorption modes in the environment
- To assess the mobility of such compounds in sewage sludge amended soils after exposure to rainfall.

2 Pharmaceuticals Chosen for Study

A total of 61 PPCPs were chosen to encompass a wide variety of therapeutic classes specifically deriving from antibiotics, analgesics, non-steroidal anti-inflammatory drugs (NSAIDS), beta blockers, antipsychotics and illicit drugs of abuse. Many of these compounds were chosen based on sales data for the Republic of Ireland (Irish Medicines Board) and Norway. Antibiotics were of particular concern and a selection of 14 compounds covering sulfonamides, macrolides, fluoroquinolones, antibacterials, bacteriostatics and antifungals were included.

3 Analytical Methods

An analytical method was initially developed to determine 27 frequently prescribed and consumed pharmaceuticals in biosolid-enriched soils and digested sludges. Using a combination of pressurised liquid extraction (PLE), solid phase extraction (SPE) and liquid chromatography with tandem mass spectrometry (LC-MS/MS), it was possible to detect analytes in each sample type at the low to sub-nanogram per gram level. Solid phase extraction efficiencies were compared for six different sorbent types and it was found that Waters Oasis HLB cartridges offered enhanced selectivities, with 20 analytes showing final method recoveries ≥60% in both soils and digested sludges. The method was validated for linearity, range, precision and limits of detection in both sample matrices. All analytes were then determined in sludge-enriched soils as well as the precursor thermally dried sludge fertiliser produced from a primary waste-water treatment plant. Levels of the antibacterial agent, triclosan, were found to exceed 20 µg/g in digested sludge and 5 µg/g in thermally dried sludge pellets. Significant traces of carbamazepine and warfarin were also detected in the above samples. Alternative separation methods were also investigated and involved the characterisation of ultra-high-pressure liquid chromatography (UHPLC) and long monolithic column technologies to reduce ion suppression effects in mass spectrometry.

4 European Case Study

A broad screening analytical method development study was conducted for the simultaneous determination of 43 PPCPs in sewage sludges from Norway and Sweden. By utilising PLE, mixed-mode SPE (strong cation exchange-reversed phase) and UHPLC-MS/MS, 15 PPCPs were identified in sludges taken from five independent waste-water treatment facilities across Scandinavia. Corroboration with Irish

data was found in the occurrence of selected PPCPs at the low to mid microgram per gram level.

5 Solid–Water Partition Coefficients

A comprehensive analytical investigation of the sorption behaviour of 54 PPCPs to soils and freeze-dried digested sludges is presented. Batch sorption experiments were carried out to identify which compounds could potentially concentrate in soils as a result of biosolid enrichment. Analysis of aqueous samples was carried out directly using LC-MS/MS. For solids analysis, combined PLE and SPE methods were used prior to LC-MS/MS. Solid–water distribution coefficients (K_d) were calculated based on slopes of sorption isotherms over a defined concentration range.

6 Molecular Modelling

The use of artificial neural networks to identify patterns in sorption behaviour was investigated for 54 PPCPs. Molecular descriptors such as logP, pK_a, molar refractivity, aromatic ratio, hydrophilic factor and topological surface area were collected for all solutes and, along with generated K_d data, were incorporated as a training set within a developed artificial neural network to predict K_d for all solutes within both sample types. Therefore, this work represents a novel approach using combined and cross-validated analytical and computational techniques to confidently study sorption modes within the environment. The logarithm plots of predicted *versus* experimentally determined K_d are presented which showed excellent correlation ($R^2 > 0.88$), highlighting that artificial neural networks could be used as an intelligent predictive tool for this application. To evaluate the developed model, it was used to predict K_d for meclofenamic acid, mefenamic acid, ibuprofen and furosemide and subsequently compared with experimentally determined values in soil. Ratios of experimental/predicted K_d values were found to be 1.00, 1.00, 1.75 and 1.65, respectively.

7 Transport and Mobility in Sludge-Amended Soils

Using a glass column packed with soil and under conditions of constant simulated rainfall, the application of sewage sludge contaminated with 12 PPCPs was studied. It was found that some PPCPs showed strong leaching potential after 6 months average rainfall, whilst others displayed higher retention behaviour and showed the potential to concentrate in soils with added exposure. A mass balance of PPCPs in all compartments was carried out and it was found that six out of 12 compounds suffered significant recovery losses, most likely due to biological or chemical transformation.

1 Introduction

Pharmaceuticals and personal care products (PPCPs) have benefited our society as a whole by enhancing both the quality and length of life. Compounds included within this classification are those used either for human or veterinary health reasons. They are categorised predominantly based on their specific biological activity and comprise a diverse range of chemical structures, modes of action and therapeutic classes deriving from over-the-counter/prescribed medications and cosmetics.

The molecular weight diversity of most active pharmaceutical ingredients (APIs) ranges from 100 to 1,000 Da (classed here as small molecules), but some APIs often exist at weights >100 kDa, such as the biopharmaceuticals. In the commercial sector, pharmaceutical companies design, develop and market both generic and patented products. Consequently, several manufacturers of an API for use within similar commercial products may exist. According to the Industrial Development Agency (IDA), the pharmaceutical sector in 2008 was one of the most significant contributors to the Irish economy, with over €29.7 billion in exports (~40% of total manufacturing exports), 17,000 in direct employment and the largest payer of corporation tax (IDA, 2009, available online at http://www.idaireland.com/home/index.aspx?id=64). Furthermore, their statistics show that 13 of the top 15 pharmaceutical companies had significant operations in Ireland, with 83 separate facilities and some companies choosing multiple sites to engage in business. The latest figures from the Irish Medicines Board (IMB) have indicated that 1,082 new human pharmaceutical products were licensed for sale in the Republic of Ireland in 2007 alone, their highest record to date (IMB, 2007a). The trend in recent years has shown a significant increase from 683 new licenses in 2000. In general, this trend has been observed worldwide and there exists much variance from country to country. Over 6,000 human over-the-counter and 1,000 veterinary medicinal products are currently authorised for sale in Ireland.

1.1 Pharmaceuticals in the Environment

The first instance of detectable PPCPs in the environment was described by Daughton and Ternes in 1998/9 (Ternes, 1998; Daughton and Ternes, 1999). It is obvious that since the introduction of pharmaceuticals to the world market, there has always existed a realistic possibility for bioactive APIs to have an effect on our environment. This ultimately may have some effect on humans, target organisms (such as bacteria or other parasites), or unknown non-target organisms. Several routes to the environment may exist, which are outlined in Fig. 1.1. The most significant source of environmental exposure has been identified as arising from municipal sewage. Other routes naturally exist, such as through livestock, fish and poultry dosing within agriculture and aquatic farming. It is interesting to note that the amount of pharmaceuticals entering the environment through industrial manufacturing processes is minimal in comparison with that entering from municipal waste sources.

Ingested pharmaceutical compounds undergo biochemical action and transformation to varying degrees. This naturally depends on several physiological conditions, which may differ from individual to individual. Excreted compounds therefore consist of the unchanged parent API along with an array of metabolites and/or conjugates. Those PPCPs applied topically, such as creams, ointments or cosmetics, may not undergo any significant metabolic change and may be simply washed directly into sewage systems. This is termed ‘involuntary’ exposure in that individuals do not purposely harm the environment by intentionally releasing pollutants. Alternatively, incorrect disposal of expired medications may occur through this route. Such ‘purposeful’ exposure results where malicious intent or apathy is concerned.

1.2 The OSPAR Convention

PPCPs in general are relatively polar compounds and are designed in many cases to be transformed during

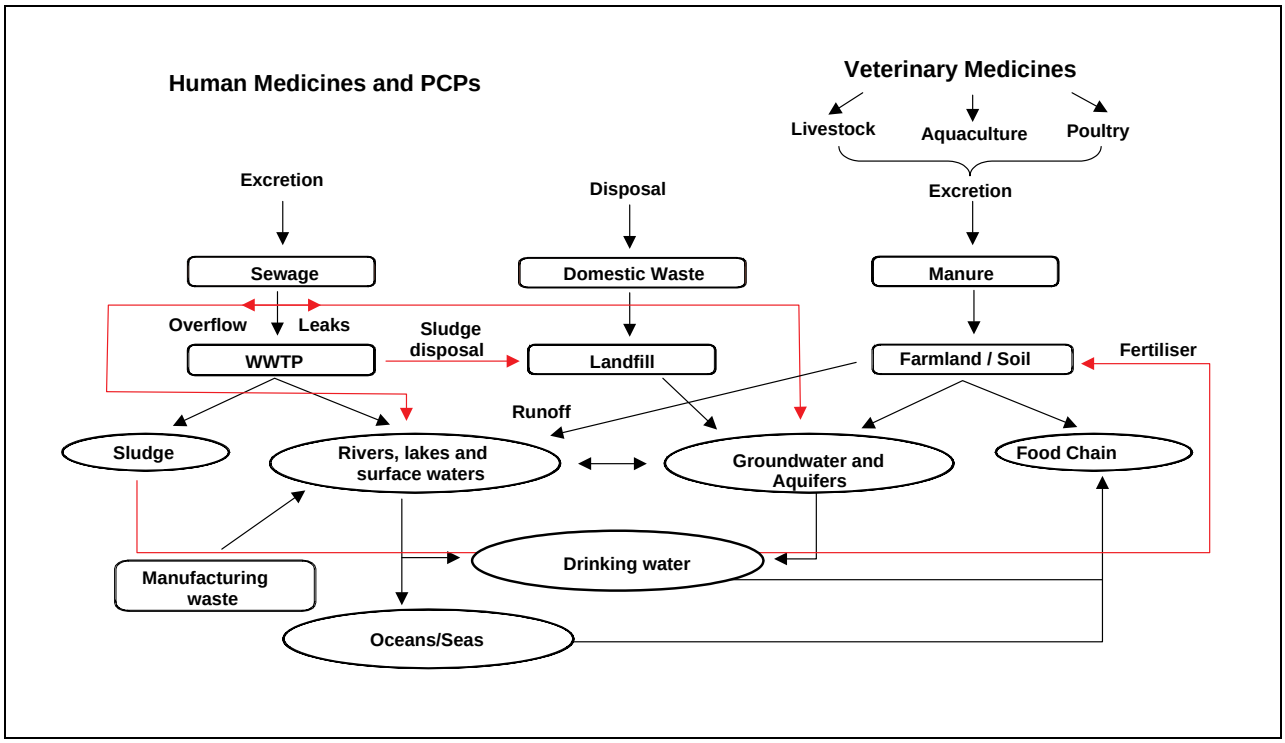


Figure 1.1. Primary routes of pharmaceuticals and personal care products (PCPs) to the environment. WWTP, waste-water treatment plant.

metabolism. Priority pollutants in general must satisfy three main prerequisite characteristics of PBT:

- **P**ersistence,
- **B**ioaccumulation ability, and
- **T**oxicity.

The degree of persistence of a parent PPCP molecule therefore may be low, but, coupled with a chronic exposure through constant reinfusion to the environment, this quantity may not bear any relevance to their categorisation as priority pollutants. Much debate still surrounds this topic. The Oslo Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) provides the current European legislation on the protection of the North-East Atlantic marine environment. The Convention regulates standards on eutrophication, release of hazardous and radioactive substances, and oil and gas industries. OSPAR provides a list of priority chemicals that are considered harmful to the environment due to their PBT. To date, the OSPAR convention stands alone as the only regulatory body to consider pharmaceuticals as a threat to the environment (OSPAR, 2007).

Pharmaceutical agents, clotrimazole, (a common antifungal agent) and diosgenin (steroid), have very recently been listed for priority action whilst 19 other drugs including chlorpromazine (antipsychotic) have been highlighted as of possible concern on a secondary listing which is to be further updated in 2009 (OSPAR, 2008). Additives to personal care products, including xylene (a synthetic musk found in perfumes) and certain phthalates, dibutylphthalate (DBP) and diethylhexylphthalate (DEHP), were also labelled as priority chemicals. These compounds are used to such a large extent in so many products that it is accepted that they are now considered to have a high degree of ubiquity (Takano *et al.*, 2006).

1.3 Waste-Water Treatment and Pharmaceuticals and Personal Care Products Removal Processes

Waste-water treatment plants (WWTPs) receive effluent from many different sources, including domestic and municipal sewage, industrial discharges

and agricultural/landfill run-off. The chemical and biological composition of these waste waters varies greatly (Horan, 1996). Domestic waste water is generally composed of up to 99.9% water and ~0.1% solid matter (Gray, 1989). However, the composition of the waste water arriving from a range of sources at the WWTP will depend greatly on the catchment area. Other components of waste water include bacteria, organic matter, inorganic species containing nitrogen and phosphorus, and pollutants such as pesticides, insecticides and heavy metals. The legislation in Ireland pertaining to waste-water treatment and management was entered into Irish law under the EU Directive 91/271/EEC. This Directive included the Environmental Protection Agency Act, 1992 and the Urban Waste Water Treatment Regulations, 1994, which provide the policies and standards that must be upheld in the treatment of waste water. The treatment plant itself is protected under the 1992 Act, which ensures monitoring of influents so that the performance of the plant is not affected (Environmental Protection Agency, 1997).

The waste-water treatment process is composed of a series of individual processes, each having different functions, but all designed to reduce the concentration of pollutants in the water and prepare it for reintroduction into the environment. Detailed discussions of the fundamentals of WWTP processes lie outside the scope of this review and can be found elsewhere. Of particular interest in this case is the secondary treatment of waste water. Most commonly called the 'activated sludge process', it acts as the primary route for removal of dissolved organic contaminants such as PPCPs. Activated sludge consists of numerous co-inhabiting micro-organisms added to waste water to consume such matter. Removal is facilitated mainly by heterotrophic and autotrophic bacteria but also by some fungi (Chartered Institution of Water and Environmental Management, 1997). However, concerning PPCPs in the environment, the most critical point worthy of discussion is that such facilities may act as 'concentration centres' for bioactive compounds in urban areas with large populations where biodegradation *via* activated sludge is not complete. Many different types of pharmaceutical compounds have been detected in WWTP influents and effluents, including blood-lipid regulators, antibiotics, anti-epileptics, and tranquillisers (Ternes, 1998; Daughton and Ternes, 1999; Ternes *et al.*, 1999; Heberer, 2002). Previous studies have indicated variations in the removal of pharmaceuticals from waste water, with a range of concentrations being released into the environment generally at the nanogram to microgram per litre levels.

1.3.1 Factors affecting PPCP removal

There are a number of operational factors that may affect the removal of PPCP from waste water during treatment. These include:

- Biochemical oxygen demand (BOD)
- Quantity of suspended solids (SS)
- Solids retention time (SRT)
- Activated sludge contact time
- pH
- Temperature.

The SRT is particularly important as a longer period of sludge treatment promotes the growth of micro-organisms with a wider range of metabolising and transforming abilities. This can possibly lead to increased removal of PPCPs before the water is released back to the environment (Drewes, 2007). The physico–chemical properties of each PPCP also govern its fate within a WWTP. Therefore, whether present as the expired product (capsules, tablets, etc.) or within excreta, PPCPs may partition into either solid or liquid phases during secondary treatment. This affinity, coupled with persistence within either phase, will determine overall fate. For example, if the compound is biodegradable then its concentration in effluents may be lower. Moreover, polar compounds such as pharmaceuticals may be more likely to remain in aqueous media and therefore may be more difficult to eliminate from influent streams. Less polar compounds may be more susceptible to adsorption onto sludge particles during treatment and may be subject to biochemical transformation (Drewes, 2007). However, these compounds may survive the treatment process and it is extremely important to note that sorption to complex solid media may depend on

several retention mechanisms and is not solely based on hydrophobic interactions. Therefore, the combined mechanisms that may play a significant role in PPCP sorption to activated sludges are most likely to fall within a selection of one or more of the following (Tolls, 2001):

- Hydrophobic interaction (log*P*)
- Functional group polarity
- Ion exchange
- Chelation
- Cation bridging
- van der Waals forces.

Comprehensive studies of sorption behaviour have not been carried out to any sufficient level thus far and such work is essential in assessing any potential threat of PPCPs to the terrestrial environment. This work attempts to address these unknowns comprehensively in later chapters. Those PPCPs that remain in the aqueous phase may be transported in treated discharges to downstream river catchments or surrounding coastal regions. On the other hand, activated sludge is usually subjected to anaerobic digestion and thermal drying to reduce volume before transport. This dried sludge or biosolid is commonly disposed of through landfill or used as an agricultural fertiliser. According to the latest figures for the Republic of Ireland (Department of the Environment, Heritage and Local Government, 1999; Collins *et al*., 2005; Smith *et al*., 2007), ~121,750 t of dried sludge were produced nationwide in 2004–2005. Of this, ~76% were recycled for use as an agricultural biosolid fertiliser and a further ~17% as landfill. It is unclear from these reports whether biosolid fertilisers were used within food production sites or, if so, how much of them were spread on or injected into such land. Ireland was reported in the period 2001–2003 to be one of seven EU Member States to use >50% of its biosolids for agricultural purposes along with Belgium, Denmark, Spain, France, the UK and Hungary. Sewage sludge spreading on agricultural land is a topic of much debate and is currently prohibited in Counties Offaly, Roscommon, Mayo, Wexford and Longford. There are numerous mechanisms by which pharmaceuticals could be degraded; however, the most likely include aerobic and anaerobic biodegradation, (during secondary treatment), chemical degradation, volatilisation and adsorption onto solid particles (Drewes, 2007). The more hydrophobic pollutants removal pathways may be predictable using the octanol–water partition coefficient (K_{ow}) as previously reported by Rogers (1996). Those compounds with a higher logK_{ow} value could be more likely to adsorb onto solid matrices, but this still needs further investigation in relation to PPCPs.

Studies to date have indicated relatively low removal of pharmaceuticals, antibiotics and diagnostic X-ray contrast media from WWTPs (Carballa *et* al., 2004). Ternes *et al.* reported little affinity of acidic drugs for sludges during primary treatment processes using the solid–water distribution coefficient (K_d) (Ternes *et al*., 2004). Some removal of diclofenac and the fluoroquinolone antibiotics was reported during primary treatment in separate studies (Nasu *et al*., 2001; Ternes *et al*., 2004). Some antibiotics exhibit high log*P* values (also referred to as logK_{ow}), such as diclofenac, which as discussed earlier, may partly explain higher levels of removal (Drewes, 2007). Many studies based on detecting pharmaceuticals in WWTPs have focused on acidic pharmaceuticals with concentrations in influent and effluent ranging from 300 to 23,400 ng/l and from 24 to 2,400 ng/l, respectively (ibuprofen being highest in both cases) (Stumpf *et al*., 1999; Clara *et al*., 2005; Vieno *et al*., 2005). Other studies have focused on determining the amount of adsorption onto sludge which occurs during treatment. In 2001, removal of pharmaceuticals, including diclofenac and carbamazepine, was reported after just 15 min of contact with activated sludge (Moehle and Metzger, 2001). Activated sludge treatment plants with low SRTs have demonstrated low levels of pharmaceutical removal and, in some cases, none at all, as reported in a number of publications (Kreuzinger *et al*., 2004; Clara *et al*., 2005). It has become increasingly clear that the length of the SRT within the plant influences the removal of PPCPs. The sorption component is also subject to an equilibration time and may contribute significantly to PPCP removal efficiency. Clara *et al.* reported an increase in elimination of ibuprofen from 0 to 98% by extending the SRT to 48 days (Clara *et al*., 2005).

Similar results have been reported by Buser *et al.* for WWTPs that employ longer SRTs (Buser *et al*., 1999). The theory that operational aspects of waste-water treatment affect the removal of drugs has also been investigated, with activated sludge plants and oxidation ditch systems both reporting relatively high removals of acidic pharmaceuticals (Stumpf *et al*., 1999; Carballa *et al*., 2004; Vieno *et al*., 2005). A WWTP employing a trickling filter treatment bed was less successful in the elimination of pharmaceutical residues (Stumpf *et al*., 1999). However, some compounds do not adhere to these theories; discrepancies have been reported in removal data for diclofenac from waste waters subjected to similar SRT periods. The concentration of the anti-inflammatory removed during treatment has varied from slight amounts to more than 70% (Ternes, 1998; Buser *et al*., 1999; Clara *et al*., 2005). Investigations into the degradation of clofibric acid in a range of WWTPs with differing treatment techniques were also ambiguous. Wide-ranging levels of biodegradation were reported for conventional activated sludge plants, trickling filter establishments and plants with additional tertiary treatments (Stumpf *et al*., 1999; Tauxe-Wuersch *et al*., 2005). These findings indicate that the performance of sludge in WWTPs varies extensively.

An important study carried out by Jones *et al.* in 2007 investigated the sorption of paracetamol, ibuprofen, propranolol, mefenamic acid and salbutamol to biosolids in a UK WWTP. Although elimination rates were high (~90%) for all target analytes, nanogram per litre concentrations were still detected in effluent except for propranolol. The conclusion drawn from this study was that sorption of the selected analytes to sludge was not the primary mechanism of removal and that microbial metabolism was more likely (Jones *et al*., 2007). More recently, several PPCPs from a wide range of therapeutic classes were detected in the influent and effluent of Irish WWTPs (Lacey *et al*., 2008).

Antibiotic residues have been detected in the influent and effluent waters of a number of WWTPs across Europe, Canada and the USA (Hirsch *et al*., 1999; Miao *et al*., 2004; Karthikeyan and Meyer, 2006). The antibiotics detected included sulfonamides, tetracyclines, macrolides, and fluoroquinolones (Karthikeyan and Meyer, 2006). Influent and effluent concentrations of five antibiotics ranged from 0.04 to 1.30 µg/l in American WWTPs and sulfamethoxazole concentrations as high as 400 ng/l have been reported in Germany (Hirsch *et al*., 1999). Overall, activated sludge treatments have shown relatively successful removals of antibiotics (Kreuzinger *et al*., 2004; Karthikeyan and Meyer, 2006). A study of waste-water samples taken during different seasons demonstrated that lower concentrations of antibiotics were detected in the early spring and summer representing the months receiving the most precipitation (Karthikeyan and Meyer, 2006). These results are in agreement with earlier studies which highlighted reduced removal of pharmaceuticals during periods of high rainfall (Ternes *et al*., 1999). Similar concentrations of the fluoroquinolones have been detected at WWTP sites in Switzerland and Canada (Golet *et al*., 2003; Miao *et al*., 2004). Sorption to sewage sludge is thought to play an important role in the removal of antibiotics from waste water. Tetracyclines are prone to complexation with metal ions forming stabilised complexes which could bind to suspended matter in the sludge (Drewes, 2007). This may explain the low concentrations of tetracycline in some German WWTPs, while other studies with particularly short SRTs reported median concentrations of ~150 ng/l (Miao *et al*., 2004). This result suggests that biodegradation is also an important factor in the removal of antibiotics and should not be ignored. However, recent studies into antibiotic removal from the Pearl River Delta in South China have indicated that fluoroquinolones are eliminated due to adsorption to sludge (Xu *et al*., 2007). This was demonstrated by direct analysis and detection of fluoroquinolones in sludge biomass. Macrolides and sulfonamides were found to survive the treatment process. One possible future problem associated with antibiotic presence during the activated sludge process is inhibition of bacterial function as demonstrated by Dokianakis *et al.* (2004).

Tertiary treatments in WWTPs such as ferric chloride, lime and aluminium sulfate coagulation were shown to remove less than 25% of PPCPs from drinking water (Westerhoff *et al*., 2005). Tertiary treatments involving oxidation with chlorine and ozone showed better results; however, this study focused mainly on endocrine-disrupting chemicals and hormones; further

work is required to investigate the effects of these processes on pharmaceuticals (Westerhoff *et al.*, 2005). New techniques are being developed to improve the quality of waste water before it is released back to the environment. These include membrane bioreactor technology (MBR) and advanced oxidation processes such as ozonation and photocatalysis (Guil *et al.*, 2007). Membrane bioreactor technology has already shown promising results for the removal of acidic, neutral and basic pharmaceuticals; however, there are still issues with some persistent compounds such as carbamazepine (Kreuzinger *et al.*, 2004; Clara *et al.*, 2005).

2 Chemical Analysis and Occurrence

Concentrations of PPCPs observed in solid samples seem to be markedly higher than those in aqueous media, possibly suggesting some form of concentration of these molecules onto solid surfaces (Carballa *et al.*, 2004; Buchberger, 2007; Heidler and Halden, 2007). It has been reported that some metabolites may even revert to the parent molecule after conjugation with sugars or other polar moieties and microbial action (Ternes, 2001). However, as relatively little data are available on the degree of persistence, bioaccumulation and toxicity of every PPCP in every matrix, it is not known to what concentration these residues will cause an effect in aquatic or terrestrial organisms and, therefore, the need for superior analytical method detection limits for a relatively small range of PPCPs in solid matrices may be redundant other than to detail any observed trends until these critical quantities are elucidated. Therefore, there exists a need for a more general approach to determine a broad range of pharmaceutical compounds.

As these compounds span the entire range of chemical diversity in exceptionally complex matrices, they pose a great difficulty for screening at trace levels using one general analytical approach. The majority of methods are currently tailored for a relatively narrow range of structurally and chemically related PPCP residues using either gas chromatography (GC) (Aguera *et al.*, 2006; Trenholm *et al.*, 2006) or liquid chromatography (LC), based usually on reversed-phase retention mechanisms (Panusa *et al.*, 2007; van Tonder *et al.*, 1996). Some methods have also utilised ion chromatography (Ding and Mou, 2000) or capillary electrophoresis (Flaherty *et al.*, 2002) as separation modes. More recent studies have shown an increase in the use of tandem mass spectrometric (MS/MS) detection for selective determination of trace PPCPs in complex environmental matrices combining single ion monitoring (SIM) or selected reaction monitoring (SRM) for increased specificity (Ternes *et al.*, 2001). Alternate detection modes have been investigated such as ultraviolet (UV) (Bones *et al.*, 2006a; Santoro *et al.*, 2006), electrochemical (Hedenmo and Eriksson, 1995) or fluorescence detection, particularly in the determination of the fluoroquinolones (Golet *et al.*, 2002). Preparative techniques for soils (Díaz-Cruz and Barcelo, 2005; Kim and Carlson, 2005) and biosolids (Díaz-Cruz *et al.*, 2003, 2006; Ternes *et al.*, 2004, 2005; Göbel *et al.*, 2005; Xia *et al.*, 2005) have generally employed multi-step procedures involving some form of lyophilisation, pressurised liquid extraction (PLE) or ultrasonication extraction (USE) and then coupled to solid-phase extraction (SPE) for clean-up or analyte concentration. Key considerations for development of high recovery preparative methods were predominantly based on analyte pK_a and degree of molecular hydrophobicity. Validation of analytical methods for soils or sludges has commonly been carried out in the sample matrix to attempt to quantify interference from matrix components, though all were still subject to sample–sample composition variance. Detection limits are generally observed in the mid to low nanogram per gram range, though the more tailored methods for physico–chemically related compounds obviously display enhanced sensitivities (Díaz-Cruz *et al.*, 2006). Reliable quantitation of PPCP residues has been the topic of much discussion, with many researchers opting for internal standard methods using either radio-labelled or deuterated analogues of target molecules (Göbel *et al.*, 2005; Heidler and Halden, 2007). This option has proved to be very expensive however, with relatively few radio-labelled reference standards being commercially available. Conversely, sample throughput for traditional standard addition calibrations can be time consuming and cumbersome for real-time quantifications.

In line with an apparent shortfall in research data on PPCP content in solids combined with investigating a general screening approach, this section outlines the development of an analytical method for the determination of a large selection of structurally and chemically diverse PPCPs in digested sludges and biosolid-enriched soils. The assortment reflected a sample of Ireland's top 100 currently most frequently

prescribed pharmaceutical products in the latest report for 2004 (HSE, 2004), as well as some over-the-counter PPCPs reported regularly in the literature and which displayed pK_a and log$P$ values that ranged from 3.5 to 14.0 and –0.07 to 6.14, respectively (Bones *et al.*, 2006a). This selection was intended to encompass a variety of therapeutic classes such as antibiotics and bacteriostats, non-steroidal anti-inflammatory drugs (NSAIDS), beta blockers, alpha blockers, antipsychotics, blood lipid regulators, anticoagulants, and recreational stimulants.

2.1 Experimental

2.1.1 Chemicals and reagents

Analytical grade paracetamol, salicylic acid, propranolol hydrochloride, clofibric acid, ketoprofen, diclofenac sodium salt, bezafibrate, warfarin, flurbiprofen, indomethacin, ibuprofen sodium salt, meclofenamic acid sodium salt, gemfibrozil, atenolol, salbutamol, sulfamethoxazole, sulfamethazine sodium salt, furosemide, pravastatin sodium salt, carbamazepine, nimesulide, ivermectin, oxytetracycline hydrochloride and clotrimazole were all obtained from Sigma–Aldrich (Steinheim, Germany). Trimethoprim, caffeine, naproxen and triclosan were ordered from Fluka (Buchs, Switzerland). All pharmaceuticals were of a purity of ≥95%. Extra pharmaceutical compounds included within developed methods for Scandinavian sludges were tetracycline, penicillin G, fluoxetine, pivmecillinam, erythromycin, spiramycin, doxazosin, amitriptyline, nortriptyline, nifedipine, budesonide, simvastatin, tamoxifen, papaverine, sertraline, citalopram and phenazone (Aldrich; >97%). The structures of all compounds studied over the course of the entire project are listed in Table 2.1. Methanol, isopropyl alcohol and acetonitrile were purchased from Labscan (Dublin, Ireland) and dichloromethane, dichlorodimethylsilane, ethyl acetate and acetone from Aldrich (Gillingham, UK). All solvents were of high-performance liquid chromatography (HPLC) grade or higher when used in combination with mass spectrometry. Ultra-pure water used for standards, mobile phases and extraction solvents was obtained from a Millipore Milli-Q water purification system with a specific resistance of 18.3 MΩ.cm or greater (Millipore, Bedford, MA, USA). For pH adjustments, dilute solutions of formic acid or ammonia were used (Aldrich). Ammonium acetate used in the aqueous mobile phase component was ordered from Aldrich. For pressurised liquid extraction, analytical grade sea sand was also ordered from Aldrich to completely fill extraction cells.

Stock 1,000 mg/l standards of each parent pharmaceutical molecule were prepared in either ultra-high-purity water or methanol, depending on solubility, and all were stored in the dark and in the cases of nimesulide, carbamazepine and trimethoprim, were stored in a refrigerator at 4°C until required as per their material safety data sheets. Working mixed standards were prepared weekly in either water or, where required, in 90:10 v/v 10 mM ammonium acetate in water/acetonitrile and these were also stored in the refrigerator and in the dark for increased stability.

2.1.2 Glassware considerations and procedures

Glassware to be used in all work was pre-silanised by initial cleaning with a 50:50 v/v methanol/water solution, followed by one rinse with 10% v/v dichlorodimethylsilane in dichloromethane, two rinses with dichloromethane, followed by two rinses with each of methanol and water, respectively. This procedure was applied to all sample bottles, volumetric flasks, storage vials and graduated cylinders bimonthly to minimise adsorption of some pharmaceuticals to glass walls. Amber glass bottles and vials were used where possible to reduce photodegradation of samples during SPE or storage. All vials were used once and discarded after the analysis was complete. Any glassware to be reused for successive spiked or unspiked samples was rigorously washed with a 50:50 solution of methanol/water and then 100% ultra-pure water between preparations.

2.1.3 Sampling sites and collection

Digested sludge samples were taken from two WWTPs by sampling directly from the sludge digestion tanks or from final product silos into sealed storage bags and were frozen immediately until analysis. The first sampling site was located in Leixlip, Co. Kildare, with an average waste-water turnover of 3×10^7 l/day at the time of sampling, ~7,000 t/year digested sludge turnover for 2006 and a population equivalent (PE) of 80,000. This plant only consists of primary and secondary treatment services and resultant sludges

Table 2.1. Name, *m/z* transition and structure of all pharmaceuticals and personal care products studied in this report.

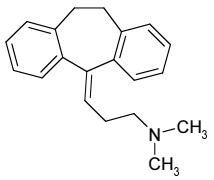
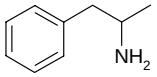
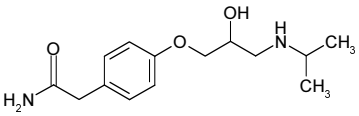
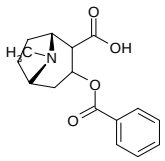
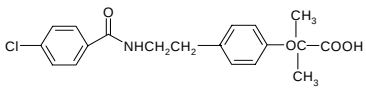
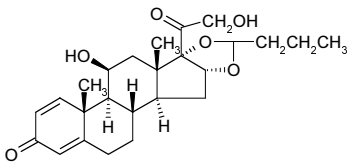
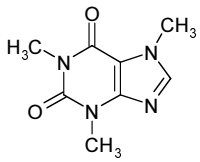
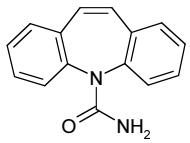
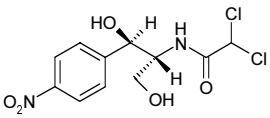
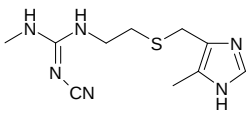
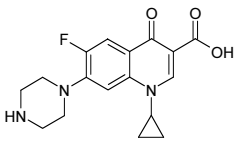
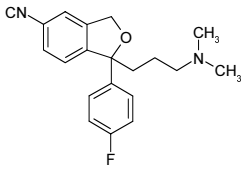
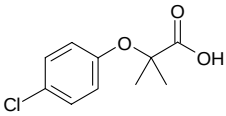
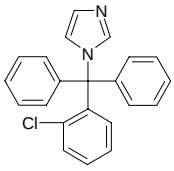
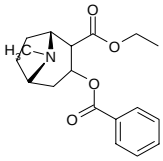
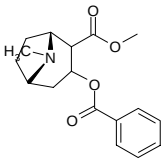
Name, *class* (*m/z* transition)	Structure	Name, class (*m/z* transition)	Structure
Amitriptyline *Antidepressant* (278>233)		**Amphetamine** *Illicit drug* (136>119	
Atenolol *Beta blocker* (267>190)		**Benzoylecgonine** *Illicit drug metabolite* (290>168)	
Bezafibrate *Hyperlipidaemic* (362>316)		**Budesonide** *Glucocorticoid steroid* (431>413)	
Caffeine *Stimulant* (195>138)		**Carbamazepine** *Antipsychotic* (237>194)	
Chloramphenicol *Antimicrobial* (345>275)		**Cimetidine** *Histamine H2-receptor antagonist* (275/253>211)	
Ciprofloxacin *Antibiotic* (332>314) (332>288)		**Citalopram** *Antidepressant* (325>262)	
Clofibric Acid* *Hyperlipidaemic* (213>127)		**Clotrimazole** *Antifungal* (277>165)	
Cocaethylene *Illicit drug metabolite* (318>196)		**Cocaine** *Illicit drug* (304>182)	

Table 2.1 *contd.*

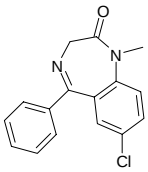
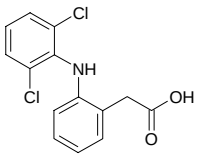
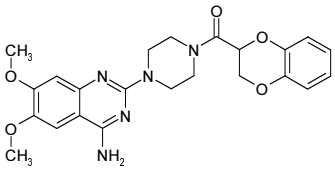
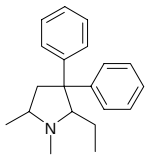
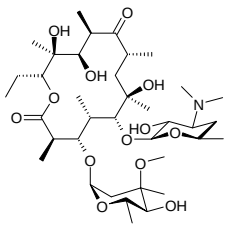
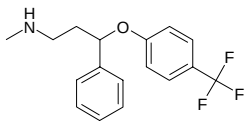
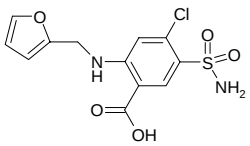
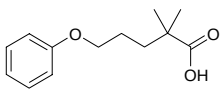
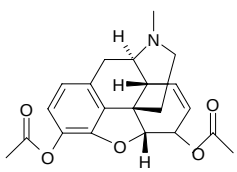
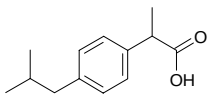
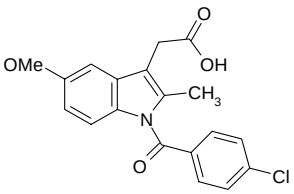
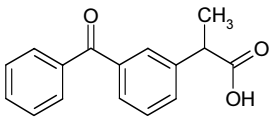
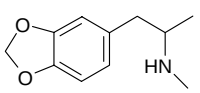
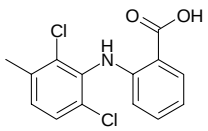
Name, *class* (*m/z* transition)	Structure	Name, *class* (*m/z* transition)	Structure
Diazepam *Sedative* (285>257)		**Diclofenac*** *NSAID* (294>250)	
Doxazosin *Alpha blocker* (452>344)		**EDDP** *Illicit drug metabolite* (278>249)	
Erythromycin *Antibiotic* (734>576)		**Fluoxetine** *Antidepressant* (310>148)	
Furosemide* *Loop diuretic* (329>285)		**Gemfibrozil*** *Hyperlipidaemic* (249>121)	
Heroin *Illicit drug* (370>268)		**Ibuprofen*** *NSAID* (205>162)	
Indomethacin *NSAID* (358>174)		**Ketoprofen** *NSAID* (255>209)	
MDMA *Illicit drug* (194>163)		**Meclofenamic acid** *Analgesic* (294>258)	

Table 2.1 *contd.*

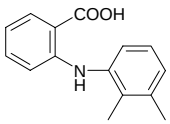
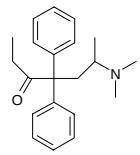
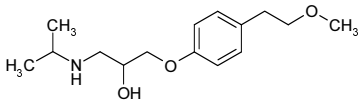
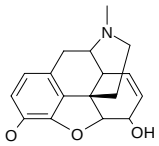
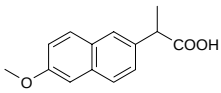
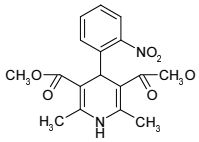
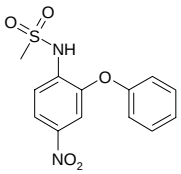
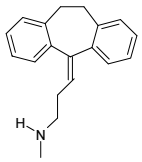
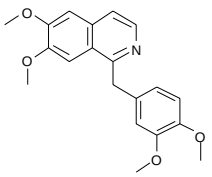
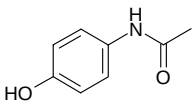
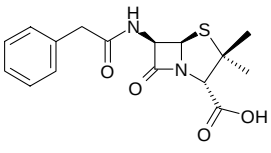
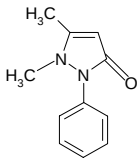
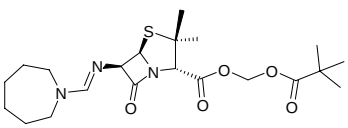
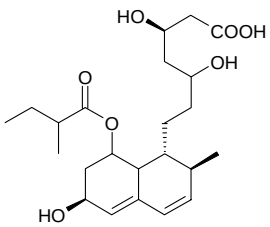
Name, *class* (*m/z* transition)	Structure	Name, class (*m/z* transition)	Structure
Mefenamic acid *NSAID* (194>138)		**Methadone** *Illicit drug* (310>268)	
Metoprolol *Beta blocker* (268>116)		**Morphine** *Illicit drug* (286>268)	
Naproxen* *NSAID* (185>170)		**Nifedipine** *Antianginal* (345)	
Nimesulide* *NSAID* (307>229)		**Nortriptyline** *Antidepressant* (264>233)	
Papaverine *Opiate* (340>202)		**Paracetamol** *Analgesic* (150)	
Penicillin G *Antibiotic* (352>335)		**Phenazone** *Analgesic* (189)	
Pivmecillinam *Antibiotic* (372>199)		**Pravastatin** *HMG-CoA reductase inhibitors* (447>327)	

Table 2.1 *contd.*

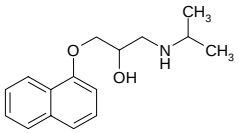
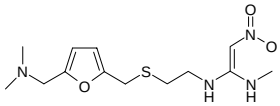
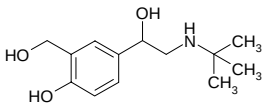
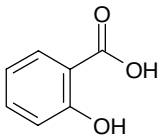
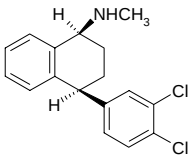
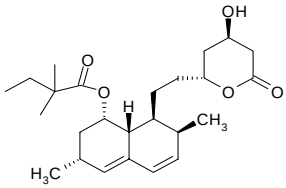
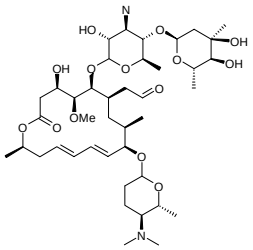
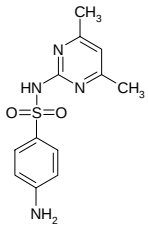
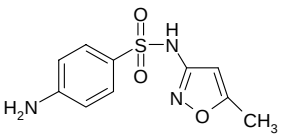
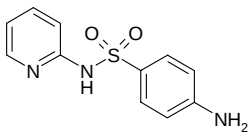
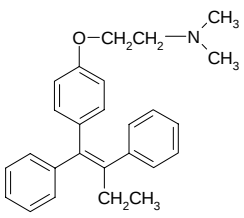
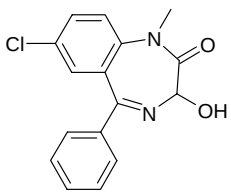
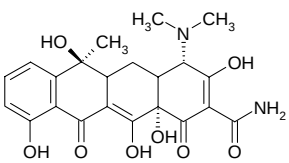
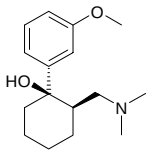
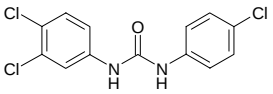
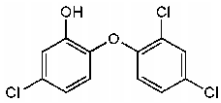
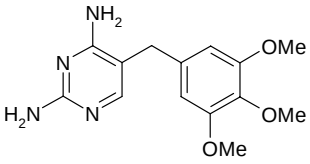
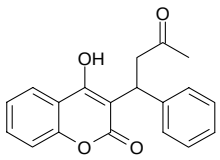
Name, *class* (*m/z* transition)	Structure	Name, *class* (*m/z* transition)	Structure
Propranolol *Beta blocker* (260>116)		**Ranitidine** *Histamine H2-receptor antagonist* (315>270)	
Salbutamol *Antiasthmatic* (240>166)		**Salicylic acid*** *Analgesic metabolite* (137>93)	
Sertraline *Antidepressant* (275)		**Simvastatin** *HMG-CoA reductase inhibitors* (441)	
Spiramycin *Antibiotic* (438>174)		**Sulfamethazine** *Antibiotic* (279>156)	
Sulfamethoxazole *Antibiotic* (254>156)		**Sulfapyridine** *Antibiotic* (250>156)	
Tamoxifen *Anti-cancer drug* (372>327)		**Temazepam** *Sedative* (301>283)	
Tetracycline *Antibiotic* (445>410)		**Tramadol** *Analgesic* (264)	

Table 2.1 *contd.*

Name, *class* (*m/z* transition)	Structure	Name, *class* (*m/z* transition)	Structure
Triclocarban* *Antimicrobial* (313/315)		**Triclosan*** *Antimicrobial* (287/289)	
Trimethoprim *Antibiotic* (291>123)		**Warfarin*** *Anticoagulant* (307>161)	

*Negative mode electrospray ionisation.
EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; MDMA, 3,4-methylenedioxymethamphetamine; NSAID, non-steroidal anti-inflammatory drug;

are spread on forestry land after digestion (~17–21% dry solids). This matrix was used for analytical method validation. The second Irish sampling site at Ringsend, Dublin, handles an average waste-water throughput of 5×10^8 l/day and serves a PE of ~1.7 million. This plant consists of permanent primary and secondary waste-water treatment processes. The location of the plant with respect to many Blue Flag beaches requires temporary tertiary UV treatment of effluent waste waters and this is performed only during the recreational bathing season from May to September. Two biosolid fertilisers are available from this facility, which are currently in the trial phase. These comprise hydrolysed and digested sludge cakes, with 26% dry solid content, as well as a second granular product which is subject to further thermal drying at average temperatures of 450°C with reported annual yields of ~25,000 t/year and ≥90% dried solid material. According to one biosolid spreading company, based in Co. Wicklow, which currently serves agricultural land across the province of Leinster, the dried granular biosolids were applied in 2005/2006 typically at 3.3 t/ha and the sludge cake at 11 t/ha. During this time ~45% was for grassland and 55% for tillage. There are currently no available data on the PPCP residue content within this fertiliser in either form.

Samples of digested sludge cake and thermally treated biosolids from Scandinavia were taken from five WWTP sites in 2007 and served the following PEs:

- Site 1: Sweden (PE: 23,000)
- Site 2: Sweden (PE: 50,000)
- Site 3: Sweden (PE: 20,000)
- Site 4: Norway, Bekkelaget (PE: 350,000)
- Site 5: Norway, Arendal (PE: 40,000).

As before, samples were taken in polytetrafluoroethylene (PTFE)-lined containers and frozen until analysis. The locations of these five sites are shown in Fig. 2.1. Site 2 was responsible for receiving waste waters from a nearby cosmetics factory and it was suspected that levels of triclosan may have been present in the sludge layers.

Grab samples of soil were taken from the DCU grounds and used for analytical method development and validation, as well as from three agricultural sites from north Co. Dublin which had some months previous to this study been enriched with commercial biosolid fertilisers from the Ringsend site. Sites were enriched with granular biosolids or sludge cake about three to four times yearly for the past number of years. Samples were stored in amber silanised glass containers and transferred to a freezer (–5°C) until analysis was carried out. Initial work with soil samples from the DCU grounds involved air-drying on 27 cm diameter filter paper for ~1 week (Macherey–Nagel GmbH & Co., Düren, Germany) in a dark, dry,

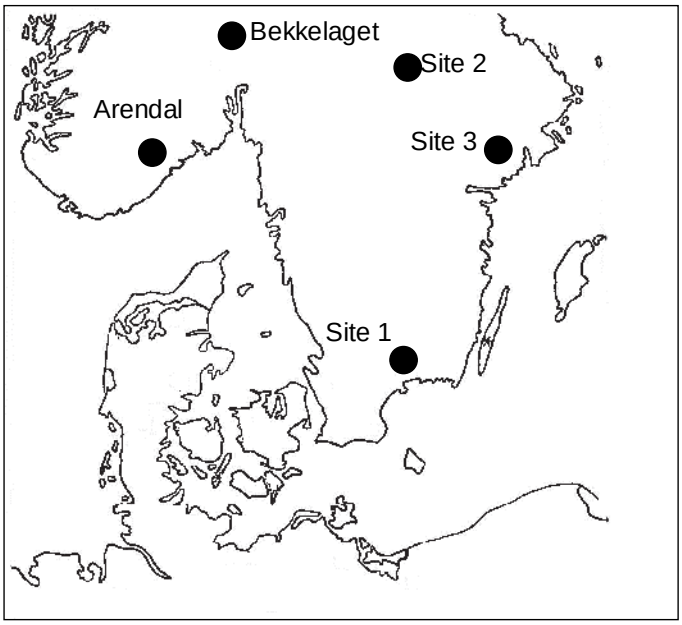


Figure 2.1. Locations of five waste-water treatment plants in Scandinavia from which samples of digested sludge and dried biosolids fertilisers were taken.

refrigerated laboratory. Later, soil samples from agricultural sites and sludges were freeze-dried, finely ground in a pestle and mortar and passed through a 700 µm sieve before pretreatment and analysis.

2.1.4 Sample pretreatment

For PLE, 2.7 g of the dried soil, or 1.0 g of dried sludge, were weighed and transferred into a 33 cm^3 extraction cell partially filled with analytical grade sea sand. Spiking was carried out directly on the soil or sludge prior to mixing and cells were then filled to capacity with more sand. Filters were placed at the bottom end of each extraction cell. Pressurised liquid extraction was carried out on a Dionex ASE-200 (Dionex Corporation, Sunnyvale, CA, USA) supplied with ultra-high-purity nitrogen (Air Products, Dublin, Ireland) from a cylinder fitted with a regulator capable of delivering ~10 bar pressure. The optimised extraction solvent was 50:50 v/v methanol/water. The system was purged and then rinsed three times (~3 × 4 ml) prior to running a sequence and once between each successive extraction (~1 ml). Optimised temperatures and pressures for extraction of target analytes were 60°C and 1,500 psi, respectively. No cell preheating was utilised prior to solvent introduction and both heat time and static times were set to 5 min each. Total flush volume was set to 100% of cell volume with a final purge time of 60 s (nitrogen). The final extraction volume was ~53 ml collected in 60 ml Dionex amber glass vials after two successive cycles.

Extracts were transferred from collection vials to a 1 l silanised volumetric flask with three to four washes of ~80–100 ml of ultra-pure water in total, reducing the methanol content to <5% once made to the mark. This solution was then transferred to a silanised amber glass bottle and adjusted to pH 5.5 with dilute formic acid or ammonia solutions. Solid-phase extraction was carried out on Waters Oasis HLB cartridges of 200 mg sorbent and 6 ml barrel format (Waters Ireland, Dublin, Ireland) in conjunction with a Phenomenex vacuum manifold (Phenomenex, Macclesfield, UK) under a vacuum of –60 kPa. Cartridges were labelled and conditioned with 6 ml methanol followed by 6 ml water prior to enrichment and clean-up. After loading, the cartridge was washed with 1 ml of ultra-pure water and eluted in 10 ml of 50:50 ethyl acetate/acetone into clear glass 11 ml vials (Agilent Technologies Ireland, Dublin, Ireland). This extract was then dried down to microlitre volumes under a cool stream of nitrogen and reconstituted in 1 ml of 90:10 v/v 10 mM ammonium acetate in water/acetonitrile, the starting liquid chromatography mobile phase composition. These 1 ml extracts were then transferred to 2 ml Agilent

amber snap-top vials before injection onto the LC-MS system, or chilled until analysis.

2.1.5 LC-ESI-MS/MS methods

An Agilent HP1100 liquid chromatograph was used throughout initial work and consisted of a binary pump with an in-line degasser, programmable injector and autosampler. An Agilent diode array UV detector G1315B module set to 225 and 270 nm was initially used to develop standard separations before switching to a Bruker Daltonics Esquire LC electrospray ionisation (ESI) octopole ion trap MS (Bruker Daltonics Coventry, UK) for neat and spiked soil or sludge samples in both positive and negative ESI modes. For the analysis of Scandinavian sludges, a Waters Quattro Premier XE ESI triple quadrupole mass spectrometer was used with alternate polarity switching enabled. All analysis of Irish sludges was performed on a Waters Sunfire 150 × 2.1 mm octadecylsilica analytical column with a particle size of 3.5 µm. The flow rate was set to 0.2 ml/min throughout and injection volumes were 10 µl. All instrument control was carried out using a Hewlett Packard (Palo Alto, CA, USA) personal computer with Agilent Chemstation version A.06.01 and Bruker Daltonics Esquire Control version 6.08 installed. Data analysis was carried out off-line on a Dell Optiplex GX520 computer (Dell Inc., Dublin, Ireland) with Bruker Daltonics Data Analysis version 2.0 installed. Two mobile phase (MP) reservoirs contained 100% 10 mM ammonium acetate in water (A) and 100% acetonitrile (B) and separations were carried out under gradient conditions. Mobile phase composition was maintained at 10% B for the first 5 min after injection and then linearly ramped to 45% B at 28 min. Over the range 28–35 min, mobile phase composition was linearly ramped to 80% B and maintained for a further 10 min. Re-equilibration time was 15 min. For the analysis of Scandinavian sludges at the Norwegian Institute for Water Research, a Waters ACQUITY UPLC was used at a flow rate of 0.2 ml/min (Waters Corp., MA, USA). Analytical columns used for the efficiency studies were the Waters Sunfire 150 × 2.1 mm, 3.5 µm, a Waters Sunfire 50 × 2 mm, 2.5 µm, and a Waters ACQUITY 50 × 1 mm, 1.7 µm, all C_{18} particulate phases. Van Deemter plots were constructed using a 70:30 10 mM ammonium acetate/acetonitrile eluent. For the investigation of long columns, five 100 × 3 mm Phenomenex octadecylsilica monoliths were coupled in series using column couplers (Phenomenex Ltd. Macclesfield, UK)

Optimisation of electrospray MS/MS conditions was carried out by direct infusion using a Cole–Parmer 74900 Series syringe pump set to deliver 300 µl/h of analyte solution from a 250 µl glass syringe. Solutions of ~10 mg/l of each analyte in the approximate mobile phase composition at elution were infused separately and conditions systematically optimised for all analytes in both positive and negative modes without using MS/MS. Base peak ions were noted for each analyte and fragmented to the second degree with MS/MS in both ESI polarities. Of the peaks observed, the most abundant was chosen for monitoring of the MS/MS signal. When used in conjunction with LC, all extracted ion chromatograms (EICs) were smoothed using a 3.8-point (soil) or 4.8-point (sludge) Gaussian averaging algorithm to allow for enhanced sensitivity with minimised peak distortion.

2.1.6 Development of extraction methods

To initiate development of a suitable method for extraction of target analytes from solid matrices it was first necessary to subject PLE sample extracts to a working SPE method prior to LC-MS/MS. A method used previously for the extraction of PPCPs from aqueous samples was adapted here to establish a starting point in the development (Bones *et al.*, 2006a). The only alteration to this procedure was that the reconstitution solvent for final extracts was 90:10 10 mM ammonium acetate in water/acetonitrile. The sorbent used was a Phenomenex Strata X (200 mg; 6 ml syringe barrel format). Extracts from soil were diluted to 1,000 ml and adjusted to pH 4.0 prior to the SPE procedure. Initial experiments with regard to PLE conditions were as follows: temperature, 100°C; pressure, 1,500 psi; solvent, 50:50 v/v methanol/water; cycles, 1; preheat time, 5 min; heat time, 5 min; static time, 5 min; flush volume, 50% of cell volume; and purge time, 100 s. Temperatures investigated during optimisation were from 40 to 120°C ($n = 5$), heat time from 5 to 15 min ($n = 3$), cycles from 1 to 3 (5 min segments), flush volume from 50 to 100% ($n = 3$) and preheat time from 0 to 2 min ($n = 2$). Pressure was maintained at 1,500 psi throughout, as was nitrogen

purge time at 100 s. Extraction solvents investigated were 50% aqueous solutions of methanol, acetonitrile, acetone and isopropyl alcohol as well as one solution of 50:50 v/v ethyl acetate/acetone. Soil sample size was increased from 1 to 5 g (n = 5) to observe any loss of analyte recovery due to matrix effects. Rather than carry out time-consuming optimisation studies for both sample types, optimisation of all conditions was carried out for the sample type expected to contain the least amount of target analyte, namely soil. All optima were chosen from that condition setting which offered the highest number of analyte recoveries above 70% when compared with a standard in ultra-pure water.

Following optimisation of all PLE conditions, the SPE method was further investigated with respect to sorbent type and extraction pH. Sorbents from four manufacturers were studied, all based on reversed-phase retention mechanisms. These were Phenomenex Strata X – 200 mg, 3 ml and 6 ml barrels; Waters Oasis HLB – 200 mg, 6 ml barrel; Merck LiChrolut EN – 50 mg, 3 ml barrel (Merck Darmstadt, Germany); and Varian Focus – 50 mg, 6 ml barrel. One weak cation exchange cartridge (Phenomenex Strata X-CW) was also investigated. For Scandinavian sludges, a strong cation exchange cartridge (Phenomenex Strata XC) was investigated in conjunction with Oasis HLB. All extractions for this study were from the same soil sample and were each spiked with 1 µg/g of analyte and compared with a standard in ultra-pure water. Recoveries for each are therefore quoted as relative recoveries to elucidate the most efficient sorbent type (see Table 2.3). A workflow diagram of the entire PLE, SPE, LC-MS/MS method is shown in Fig. 2.2.

2.1.7 Method validation

For both matrices, the quantities of linearity, range (including a brief cartridge breakthrough study), limits of detection (LODs), limits of quantification (LOQs), percentage recovery and precision (method and instrumental) were investigated using MS, MS/MS signals or both for each analyte. With respect to linearity, n = 10 spiked soil or digested sludge samples were prepared with PPCP concentrations ranging from 0.05 to 10 µg/g and were analysed in triplicate. Acceptable linearity was taken for correlation coefficients greater than 0.98 over a defined range for

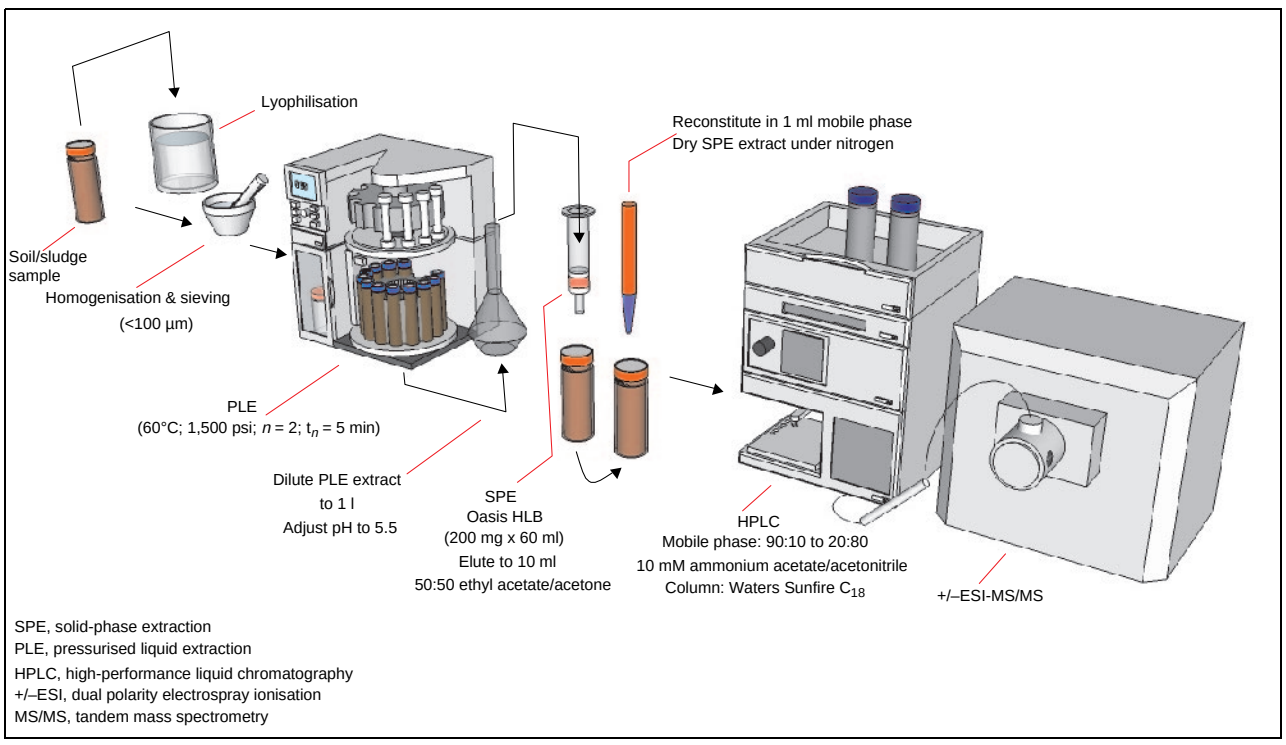


Figure 2.2. Workflow schematic for the extraction and analysis of 27 pharmaceuticals and personal care products from soils and sludges.

each analyte for $n \geq 6$ data points. Cartridge breakthrough, where applicable, defined the upper limit of range and the lower linearity limit was defined as a concentration corresponding to a signal-to-noise ratio of ≥10:1, with instrument relative standard deviation of ≤20% of analyte peak height for $n = 3$ replicates (limit of quantification). Limits of detection were carried out by determining the lowest observable concentration to give a signal-to-noise ratio of close to 3:1 and calculation based on triplicate injections of three low-level spiked samples. Overall method precision studies were carried out by spiking both sample types with 1 µg/g of each PPCP for $n = 6$ replicates. These data were also used to calculate percentage recoveries for all analytes for the complete PLE, SPE, LC-MS/MS method by extracting a sample of unspiked soil or sludge using the optimised conditions. Final dried extracts were reconstituted in 1 ml of the 90:10 ammonium acetate/acetonitrile solution described earlier containing the expected 100% recovery concentration. Background subtraction was carried out for soils and sludges containing PPCP residues where possible to calculate validation data. Instrumental retention time precision was carried out for $n = 11$ replicate injections of a 10 mg/l standard in ultra-pure water, and $n = 6$ replicates of a 1 mg/l standard were integrated manually to determine peak height precision.

2.2 Results and Discussion

2.2.1 LC-ESI-MS/MS

The use of SIM with first- or second-degree MS/MS fragmentation allowed selective determination of each analyte at trace levels. In both ionisation polarities, under direct infusion conditions, relatively simple mass spectra were recorded in most cases.

Direct infusion of standards with no MS/MS fragmentation yielded mostly $[M–H]^-$ and $[M–COOH]^-$ ions in negative mode, or $[M+H]^+$, $[M+Na]^+$, or $[M+K]^+$ adduct ions in positive ion mode. In concurrence with previous studies in the DCU laboratory, clotrimazole seemed to fragment under positive ESI-MS conditions to yield a product ion less than an imidazole moiety at *m/z* 277 (Bones *et al.*, 2006b). Pravastatin was observed mainly as its sodiated adduct ion. In negative mode, the only analytes displaying base peak ions as decarboxylated fragments were meclofenamic acid and naproxen. Extremely weak signals in either mode were obtained for any ions corresponding to ivermectin or furosemide and only marginally stronger signals were obtained for the pseudo-molecular ions of pravastatin, paracetamol and meclofenamic acid. Triclosan was observed at *m/z* 287, 289, 291 and 293 with superposition of peaks corresponding to a trichlorinated species with *m/z* 287 and 289 showing the highest intensities.

Under MS/MS conditions, most fragmentation patterns could be explained in most cases with the simple loss of specific moieties. The sulfonamides, sulfamethoxazole and sulfamethazine, were both predominantly observed in positive ESI-MS/MS mode at *m/z* 156, with cleavage occurring at the sulfur atom and formation of the $[H_2N–C_6H_4–SO_2]^+$ ion. Other less intense fragments observed at *m/z* 124 (sulfamethazine), 108 and 92 corresponded to $[NH_3–C_4HN_2–C_2H_6]^+$, $[H_2N–C_6H_4O]^+$, and $[C_6H_6N]^+$ ions, respectively, with some resulting from the typical molecular rearrangements reported previously (Hartig *et al.*, 2001; Göbel *et al.*, 2004; Díaz-Cruz *et al.*, 2006). Pravastatin underwent loss of water following cleavage of $–C_6H_{10}O_2$ and was observed as the sodium adduct ion at *m/z* 323. The protonated form of this fragment ion has been reported previously by some researchers, though was not observed here (Mulvana *et al.*, 2000; Miao and Metcalfe, 2003; Kawabata *et al.*, 2005). Similar fragmentation pathways were also observed for propranolol and metoprolol in which cleaved moieties shared similar chemical structures and so, not surprisingly, shared comparable fragmentation patterns (Gómez *et al.*, 2006; Spyridaki *et al.*, 2006). While positive ion mode yielded MS/MS data for all observed analytes in single MS, negative ion mode yielded no fragment ions of noteworthy intensity for paracetamol, triclosan or flurbiprofen and poor sensitivity for salicylic acid. As such, sensitive monitoring of these three compounds was limited to the observed base peak ion using no MS/MS fragmentation. Furosemide appeared as a more intense peak at *m/z* 285 as its $[M\text{-}COOH]^-$ ion under MS/MS conditions than any ion under MS conditions. Please refer to Table 2.1 for observed ion mass transitions and single ion masses.

Individual ESI-MS conditions for the dry gas flow, drying temperature, nebuliser pressure, capillary voltage, end plate offset, skimmers, capillary exit offset, octopole voltage, octopole delta, octopole RF, trap drive and lenses were optimised for each analyte. An average was taken of all optima to fully represent the different species under study. Optimised conditions for both positive and negative ion modes are given in Table 2.2. Since this method was developed for a wide variety of analyte chemistries, MS sensitivity for some ions was enhanced using one or other of negative or positive ion mode polarities, though most could be determined in most cases using one polarity. In most cases, the chosen ionisation mode for a compound could be predicted by its acidity, though some more obvious compounds like bezafibrate ($pK_a = 3.6$) or ketoprofen ($pK_a = 4.45$) did not adhere to this assumption (Bones *et al.*, 2006b). These were observed with more intensity and with an enhanced signal-to-noise ratio in positive ion mode. Naturally,

Table 2.2. Liquid chromatography and electrospray ionisation tandem mass spectrometric conditions.

Liquid chromatography conditions		
Column	Waters Sunfire 150 × 2.1 mm, 3.5 µm particle size	
Flow rate	0.2 ml/min	
Injection volume	10 µl	
Mobile phase	A	10 mM ammonium acetate (aq.)
	B	100% acetonitrile
Gradient	0–5 min	10% B
	5–28 min	10–45% B (curve = 5)
	28–35 min	45–80% B (curve = 5)
	35–45 min	80% B
Re-equilibration time	15 min	
Electrospray ionisation tandem mass spectrometric conditions		
	Negative ion mode	**Positive ion mode**
Capillary (V)	4,500	4,500
End plate offset (V)	–764	–561
Skimmer 1 (V)	–15	28.1
Skimmer 2 (V)	–4.4	6.7
Capillary exit offset (V)	–50	63.7
Octopole (V)	–1.0	2.51
Octopole radio frequency (Vpp)	103.3	155.3
Octopole delta (V)	–1.36	1.98
Lens 1	3.4	–2.4
Lens 2	30	–37.8
Trap drive	22.8	34.7
Dry gas flow (N_2, l/min)	8	8
Nebuliser pressure (psi)	30	30
Dry gas temperature (°C)	300	300

separations with two sequential positive and negative ESI-MS/MS runs were required to obtain increased sensitivity for all target compounds at trace concentrations.

2.2.2 *Pressurised liquid extraction*

Upon development of a suitable chromatographic method, optimisation of each of the PLE conditions was carried out systematically as per Section 2.1.6 in conjunction with the modified SPE method previously developed in the DCU laboratory (Bones *et al.*, 2006a). It was found that 5 min extraction times were insufficient and with increasing times (i.e. 10 and 15 min), recoveries seemed to decrease for some analytes, namely caffeine, naproxen, ketoprofen, flurbiprofen, trimethoprim and especially meclofenamic acid. A time of 10 min was chosen to allow sufficient extraction as well as to minimise the exposure of potentially unstable compounds to elevated temperatures or further extraction of matrix components. The option to divide extraction time into 5 min segments to enhance recoveries was investigated by introducing multiple cycles. It was found that, as before, a total of 10 min extraction time was optimum with two segments of 5 min. Lower extraction temperatures on average yielded higher recoveries with the exception of 40°C. The optimum was found to be 60°C. A significant decrease in recoveries was noticed for gemfibrozil, meclofenamic acid, clotrimazole, triclosan and nimesulide, with above the optimised temperature. This indicated either degradation due to elevated temperatures or a loss in method selectivity due to more efficient extraction of interfering matrix components. Similar studies have shown that a marked effect on the extraction of some pharmaceuticals from solid matrices may occur above 100°C, though no thermal degradation data using pressurised liquid extraction currently exists for all of the PPCPs investigated here (Göbel *et al.*, 2005). Another parameter considered due to the observed temperature effects was preheat time, in which the sample was heated to extraction temperature prior to solvent introduction. This step was removed and heated solvent was introduced directly with no preheating and, as before, recoveries improved. Percentage flush volume optimised at 100% of cell volume. All extraction solvents were investigated with both acetone/water and methanol/water offering the highest recoveries. Methanol/water displayed improved recoveries for more non-polar analytes and was chosen so as to minimise any unwanted elution during the SPE step. Pressure was merely used to keep hot organic solvents in the liquid phase and was not expected to have a pronounced effect on analyte recoveries and so was maintained at 1,500 psi.

An obvious means to boost detection limits for this study was to increase the quantity of solid sample to be extracted. However, in doing so it was necessary to fully investigate any potential loss in method sensitivity due to the sample matrix. Little or no discernible loss of signal intensity occurred in soil matrices within error limits for most of the PPCPs investigated with increasing sample weight. A notable reduction in method sensitivity was observed for paracetamol (log$P$ = 0.46), atenolol (log$P$ = 0.46), caffeine (log$P$ = –0.5), propranolol (log$P$ = 3.03), salbutamol (log$P$ = 1.44) and pravastatin (log$P$ not available) above a weight of 2–3 g. The signal-to-noise ratio plotted *versus* increasing sample weight for these selected PPCPs whilst maintaining the final PPCP concentration at 10 µg/ml showed generally more ion suppression of MS signals for polar analytes and may partly explain the marked reduction in sensitivity for these specific analytes with higher quantities of sample given their log$P$ value. However, given the complexity of the matrix, it was unsure whether this was solely due to MS signal suppression alone, or subject to exceeding either the sample/solvent solubility during pressurised liquid extraction or the SPE sorbent capacity. Nevertheless, a study of the effect of sample size was more useful for the complete method than just for its effect on MS intensities alone. An average of all optimum weights was taken and found to be 2.7 g. This study was not carried out for sludge and sample weight was maintained at 1 g. Also included in this extraction method development was oxytetracycline, which showed no recovery whatsoever and led to the conclusion that it was too heavily retained within the soil matrix to be extracted using this approach. Moreover, extracted ion chromatograms exhibited extensive peak distortion and broadening in standards with insufficient sensitivity for trace determinations. However, it was unsure how recovery for this analyte would be affected by variance in the sample matrix and

it was not excluded from purely qualitative investigations in all subsequent samples though no reliable data for method validation were gathered.

2.2.3 Solid-phase extraction

The already established SPE method in the DCU laboratory was now investigated and tailored further for routine analysis of soil and sludge samples following the PLE optimisation. An investigation into six different commercially available sorbent chemistries was carried out (Table 2.3). The original method utilised Phenomenex Strata-X surface-modified styrene-divinylbenzene which, according to the manufacturer, lends particular application to acidic, basic and neutral PPCP determinations through hydrophobic, hydrophilic and pi–pi retention mechanisms, as does the Waters Oasis HLB sorbent. The average pK_a for all analytes was 6.3 (excluding paracetamol, gemfibrozil, meclofenamic acid and clofibric acid for which no data

Table 2.3. Relative percentage recoveries for 21 pharmaceuticals and personal care products in spiked soil samples (compared with 10 µg/l standard in ultra-pure water).

Analyte[a]	Sorbent and relative percentage recovery[b] (barrel volume; sorbent mass)					
	LiChrolut EN (3 ml; 200 mg)	Varian Focus (6 ml; 50 mg)	Oasis HLB (6 ml; 200 mg)	Strata X (3 ml; 200 mg)	Strata X (6 ml; 200 mg)	Strata X-CW (6 ml; 200 mg)
Atenolol	18	3	8	11	8	0
Caffeine	105	5	111	108	96	99
Trimethoprim	46	45	85	92	85	1
Metoprolol	16	41	42	46	59	0
Propranolol	29	33	71	68	55	0
Carbamazepine	100	90	101	106	90	118
Clotrimazole	45	63	45	45	33	5
Salicylic acid	69	13	119	>130	124	56
Paracetamol	48	1	13	8	7	3
Clofibric acid	100	74	119	>130	114	42
Ketoprofen	78	82	91	92	77	57
Naproxen	102	128	125	>130	127	76
Warfarin	>130	>130	>130	>130	115	78
Flurbiprofen	121	105	94	100	69	75
Diclofenac	98	109	102	104	63	54
Indomethacin	90	108	88	100	>130	64
Meclofenamic acid	63	67	66	62	34	91
Gemfibrozil	84	98	90	93	54	42
Nimesulide	92	103	92	99	71	64
Sulfamethoxazole	122	83	>130	>130	128	101
Furosemide	>130	>130	>130	>130	>130	0
n = 70%	**12**	**12**	**15**	**14**	**10**	**7**

[a]Data not available for pravastatin, salbutamol, furosemide, triclosan, bezafibrate or ibuprofen.
[b]Based on peak heights in untreated soil compared with standard in ultra-pure water.

were available). Again, peak heights (now all extracted at a previously optimised pH of 5.5 on Strata X) were compared with a standard of all analytes in ultra-pure water to take into account any matrix affinity before any final sorbent choice was made. Upon comparison of all cartridge types, 6 ml Oasis HLB cartridges gave the highest overall recoveries with 15 target compounds out of 21 yielding relative percentage recoveries >70% (taken as acceptable recovery). In comparison with Strata X, Oasis HLB offered a 10% higher recovery on average than the 6 ml Strata X sorbent and performed similar to the 3 ml Strata X barrel format, albeit exhaustive loading times (>4 h for 1 l), indicating that lower flow rates may be required when using this sorbent. Varian Focus sorbents showed particular selectivity for gemfibrozil, nimesulide and clotrimazole. LiChrolut EN (ethylvinylbenzene-divinylbenzene) sorbents were comparable with the 6 ml barrel Strata X sorbent on average, with improved selectivity for paracetamol with a relative recovery of 48%. Recoveries were poor for the Strata X-CW sorbent though it was initially thought that its weakly acidic carboxylated functionality modified to a similar backbone to that of the Strata X sorbent would boost more polar and more basic analytes while maintaining some reversed-phase mechanisms for more non-polar analytes. In fact, when extractions were carried out over a pH range of 4–7, very little improvement or disimprovement in recovery was observed for any analyte. Coupling Strata X to Strata X-CW sorbents was not investigated. Taking into account loading times and overall recoveries, Oasis HLB cartridges were chosen as the sorbent for future studies. The extraction pH for both soil and sludge extract studies was maintained at 5.5.

An investigation into any requirement for SPE was also carried out. Soil spiked with 10 µg/g of each PPCP was extracted by pressurised liquid extraction with ethyl acetate/acetone as the extraction solvent (for its volatility) and dried down to microlitre volumes under nitrogen. This was then reconstituted in 1 ml of starting mobile phase as before. The more polar analytes showed increased recoveries (data not shown), though the general trend was towards a disimprovement in overall recovery. Moreover, the potential risk associated with no sample clean-up may have caused adverse damage to LC-MS instrumentation with increased use and, as such, it was decided to retain an SPE step. Final complete method conditions are shown in Table 2.2.

2.2.4 Method validation in soil and sludge

2.2.4.1 Linearity and upper range limit

Using both soil and digested sludge samples obtained from the university grounds and the Dublin satellite town waste-water facility, respectively, standard curves were plotted for each analyte as per Section 2.1.7. Experimental data fitting was carried out by linear regression analysis using peak height *versus* concentration. Acceptable correlation coefficients were obtained for most analytes with $R^2 \geq 0.98$ in most cases for a minimum of $n = 6$ data points. Improved linearity was observed for MS/MS ions in both matrices with $R^2 \geq 0.99$ in most cases. Obviously, sensitivity for all analytes varied and accounted for $n < 10$ data points being used for determination of linearity in some instances as the range of spiking concentrations was the same for nearly all analytes. For example, insufficient sensitivity for paracetamol may have accounted for poor linearity in either matrix over the range studied and it might have improved if studied over higher ranges. However, this range was selected to reflect actual expected residue concentrations in the sample types chosen for this study. On the other hand, in digested sludge matrices, upper concentration ranges were established for some PPCPs due to possible evidence of SPE sorbent capacity breakthrough above 7.5 µg/g spiking concentration. This was particularly true for carbamazepine, bezafibrate, metoprolol, ketoprofen, sulfamethazine and atenolol. As such, the upper concentration range limit for digested sludges was kept at 7.5 µg/g when determining all 27 compounds simultaneously. All correlation coefficients in both matrices are listed in Table 2.4 along with all other method validation quantities.

2.2.4.2 Limits of detection and quantification

It was clear from examination of method LODs and LOQs that MS/MS displayed enhanced performance in both matrices, with roughly between one- and tenfold improvement in sensitivity for all analytes combining the obvious added selectivity. There was no reduction in sensitivity for any analyte that displayed an MS/MS

Table 2.4. Complete method performance data in digested sludge and soil.

Analyte	t_r (min)/ (% RSD)[a]	Instrument precision (% RSD)[b]	R^2 (sludge)[c]	R^2 (soil)[c]	LOD in sludge[d] (ng/g)	LOD in soil[d] (ng/g)	Absolute % recovery in sludge[e]	Absolute % recovery in soil[e]	% MS ion suppression in sludge[f]	% MS ion suppression in soil[f]
Salbutamol*	4.5 (0.8)	6.3/16.5	0.9839	0.9999	90	65	40 ± 10	14 ± 4	<5	<5
Paracetamol	4.9 (0.2)	3.4/8.2	<0.9500	0.9743	n.c.	115	2 ± 1	6 ± 1	<5	16
Salicylic acid	4.9 (1.7)	2.6/5.6	0.9910	0.9986	30	30	45 ± 10	60 ± 15	<5	11
Sulfamethoxazole	5.4 (1.4)	8.8/12.9	n.c.	0.9650	n.c.	120	n.c.	110 ± 30	65	11
Atenolol*	5.6 (0.9)	6.8/6.3	0.9926	0.9955	30	15	60 ± 10	30 ± 5	13	<5
Caffeine*	9.6 (0.6)	4.6/7.1	0.9933	0.9923	350	50	65 ± 20	50 ± 5	45	<5
Sulfamethazine*	14.7 (0.3)	3.4/5.9	0.9913	0.9863	150	10	40 ± 10	70 ± 20	30	14
Clofibric acid*	19.0 (0.2)	4.5/5.0	0.9978	0.9887	6	2	95 ± 10	85 ± 10	<5	15
Trimethoprim*	20.7 (0.3)	4.2/5.6	0.9814	0.9877	50	10	120 ± 15	90 ± 25	52	<5
Naproxen*	21.3 (0.2)	2.2/5.7	0.9837	0.9941	8	2	105 ± 15	90 ± 5	42	26
Furosemide*	21.5 (<0.1)	11.4/4.7	0.9947	0.9916	20	9	120 ± 30	75 ± 5	49	<5
Ketoprofen*	22.0 (0.23)	4.2/5.6	0.9934	0.9981	7	2	110 ± 20	90 ± 15	41	<5
Metoprolol*	22.2 (<0.1)	5.1/8.1	0.9879	0.9822	15	2	70 ± 10	75 ± 15	47	<5
Pravastatin	22.9 (<0.1)	9.1/15.1	<0.9500	0.9708	270	225	50 ± 10	60 ± 10	70	<5
Warfarin	23.4 (<0.1)	4.1/5.2	0.9829	0.9972	7	1	110 ± 20	110 ± 10	61	<5
Bezafibrate*	23.8 (1.1)	4.8/6.2	0.9970	0.9935	8	1	130 ± 10	85 ± 20	45	<5
Flurbiprofen	25.7 (<0.1)	4.2/3.8	0.9851	0.9988	40	4	130 ± 20	85 ± 5	49	33
Ibuprofen	26.8 (<0.1)	3.0/4.8	0.9910	0.9918	30	5	110 ± 15	130 ± 10	47	33
Diclofenac*	27.4 (0.2)	2.2/8.9	0.9979	0.9890	7	3	120 ± 25	70 ± 10	56	37
Indomethacin	28.0 (0.4)	2.5/9.6	0.9901	0.9936	580	80	120 ± 30	110 ± 15	68	42
Carbamazepine*	29.3 (<0.1)	3.4/6.4	0.9939	0.9932	3	0.5	120 ± 15	95 ± 15	39	<5
Propranolol*	30.3 (0.3)	7.6/9.9	0.9995	0.9884	40	6	70 ± 10	50 ± 5	78	18
Meclofenamic acid	30.3 (0.2)	3.0/6.4	0.9771	0.9887	170	20	>130	>130 (±30)	–39	70

Table 2.4 *contd.*

Analyte	t_r (min)/ (% RSD)[a]	Instrument precision (% RSD)[b]	R^2 (sludge)[c]	R^2 (soil)[c]	LOD in sludge[d] (ng/g)	LOD in soil[d] (ng/g)	Absolute % recovery in sludge[e]	Absolute % recovery in soil[e]	% MS ion suppression in sludge[f]	% MS ion suppression in soil[f]
Nimesulide*	36.0 (0.1)	4.5/6.9	0.9879	0.9896	2	0.5	95 ± 20	75 ± 10	50	13
Gemfibrozil*	36.5 (0.1)	2.8/5.8	0.9863	0.9925	2	0.8	100 ± 20	60 ± 10	49	59
Clotrimazole*	40.0 (0.1)	6.5/8.2	0.9878	0.9762	50	7	50 ± 1	25 ± 5	63	43
Triclosan	41.4 (<0.1)	3.0/4.6	n.c.	0.9948	n.c.	3	n.c.	110 ± 10	n.c.	69

[a] $n = 11$ replicate injections of a 10 mg/l standard of all analytes.
[b] $n = 6$ replicates of a mid-range 1 mg/l standard of all analytes (peak height/peak area).
[c] Based on peak height.
[d] Based on signal-to-noise ratio of 3:1 for $n = 9$ injections of three low-level standards.
[e] For $n = 6$ replicate extractions of spiked digested sludge at a concentration of 1 µg/g.
[f] Comparison of spiked extract post-extraction with a standard in ultra-pure water.
n.c., non-calculable.
*Pharmaceuticals and personal care products measured in MS/MS mode; all others in MS.
LOD, limit of detection; MS, mass spectroscopy; RSD, relative standard deviation.

fragmentation pattern in direct infusion experiments. For soils, the method offered LODs of <20 ng/g for 20 analytes. The method displayed poorer sensitivity for paracetamol, salbutamol, caffeine, pravastatin, indomethacin and clotrimazole, but this could be explained due to poor overall percentage recoveries. With regard to LOQ data, 18 analytes were quantifiable below an overall concentration of 50 ng/g (the lower concentration range in linearity studies). Considering that the method was developed for a wide variety of compound chemistries displaying pK_a values ranging from 3.5 to 14.0 and log$P$ values ranging from –0.07 to 6.14, this method performed quite well in terms of application to a general screening of compounds in soils at trace levels. For digested sludges, the complexity of the matrix obviously played a larger role with respect to method sensitivity offering LODs <20 ng/g for only 10 analytes and LOQs <50 ng/g for eight analytes coupled with a smaller sample size. Complete loss in sensitivity was observed for sulfamethoxazole which could not be quantified at any of the concentration levels studied. Similarly, indomethacin and caffeine displayed markedly poorer method LOQs of >1 µg/g. It was uncertain how increased sample size would affect detection limits in sludges and whether trends would have been similar to those observed in soils. However, bearing in mind that breakthrough had been observed for some analytes at higher spiking concentrations even with 1 g sludge sample sizes, the effects may have been significant.

2.2.4.3 Percentage recovery and precision

The entire method was investigated for analyte recovery and it was found that percentage recoveries were generally comparable within error ranges for both matrices, though in soils recoveries were slightly lower for atenolol, salbutamol, propranolol, clotrimazole, bezafibrate and furosemide. Recoveries could not be reliably calculated for triclosan in sludges as concentrations present in the sample used for validation were at significantly high levels. However, high recoveries in soils showed that the method performed quite well with respect to triclosan. Method precision was acceptable with percentage recoveries varying less than 20% in most cases for $n = 6$ replicates. Upon investigation of instrument precision, retention times for this method varied <1% in most cases. Examination of replicate peak heights showed a variance of <5% in most cases.

2.2.4.4 Matrix effects

Any potential for MS signal suppression was semi-quantified here in both soils and sludges for all analytes by comparison of extracts spiked post-extraction (1 µg/g) to a standard in ultra-pure water in an experiment similar to that carried out by Díaz-Cruz *et al.* (2006). Hence, PPCPs in these samples were not subject to the entire extraction procedure and any deleterious signal effects could only be due to closely eluting analytes, or from other matrix components with similar retention times.

In soils, very little signal loss (<5%) occurred for the majority of analytes in most cases. However, ion suppression was more pronounced for analytes eluting in higher proportions of organic solvent in the mobile phase (>40%), which also coeluted with notably more matrix components. Most prominent was the suppression of gemfibrozil at 59–64% and meclofenamic acid at 70%, although its influence did not seem to negatively affect detection limits outside of a practical working low nanogram per gram range. It was found that, at this concentration level, the effect was observed more appreciably in digested sludges with average matrix-induced suppression of most analytes at 42 ± 20%, with a random distribution across the entire run showing a clear effect of matrix type on sensitivity. Good agreement between MS and MS/MS data was also observed. Loss in sensitivity for sulfamethoxazole in sludge matrices could be attributed in part to this suppression effect, though not entirely, as this investigation showed that suppression of sulfamethoxazole only occurred to 65% in this particular sludge sample and was clearly observed at *m/z* 254 in MS-extracted ion masses at this post-extraction spiking concentration. Signal effects due to the sample matrix were not confined to being negative however. An overall 39% enhancement in signal was observed for meclofenamic acid in digested sludge. However, it must be stated as with all work on PPCP residues that signal suppression or enhancement is heavily dependent on the sample–sample variance.

2.2.5 *Application to sludge and soils from Ireland*

The developed method was applied to the identification and quantification of any PPCP residues in typical sludge samples from the primary Dublin waste-water treatment facility (Ringsend) in the form of the wet hydrolysed/digested sludge and the dried granular biosolid fertilisers. At the same time, the biosolid-enriched agricultural soil samples from the north Dublin area were screened in triplicate for the presence of any of the observed PPCPs in an attempt to establish any possible transport pathway from both biosolid types to soil and persistence over a 3- to 4-month period.

Digested sludge consistently displayed quantifiable levels of warfarin (163 ± 25 µg/kg) and carbamazepine (120 ± 10 µg/kg) for $n = 3$ replicate extractions. All fragment MS/MS ions of corresponding mass for both of these species were as a result of transitions from *m/z* 237 to 194 and from *m/z* 307 to 161, respectively, as can be seen in Figs 2.3 and 2.4 which display similar fragmentation patterns to injections of standards of both species. More interesting was the possible occurrence of relatively large amounts of triclosan (24.6 ± 0.9 mg/kg; Fig. 2.5a), being clearly observed at *m/z* 287, 289, 291 and 293. The theoretical intensity ratio for a standard isotopic pattern for triclosan as M, M+2, M+4 and M+6 species is ~100:96:31:3, based on standard binomial expansion algorithms and the experimentally determined ratio for a standard of triclosan in ultra-pure water was comparable at 100:95:36:4. The isotope pattern ratio obtained from possible triclosan contained within digested sludge was 100:102:35:3 and therefore closely matched both the standard and theoretical values. Initial work with MS/MS fragmentation yielded

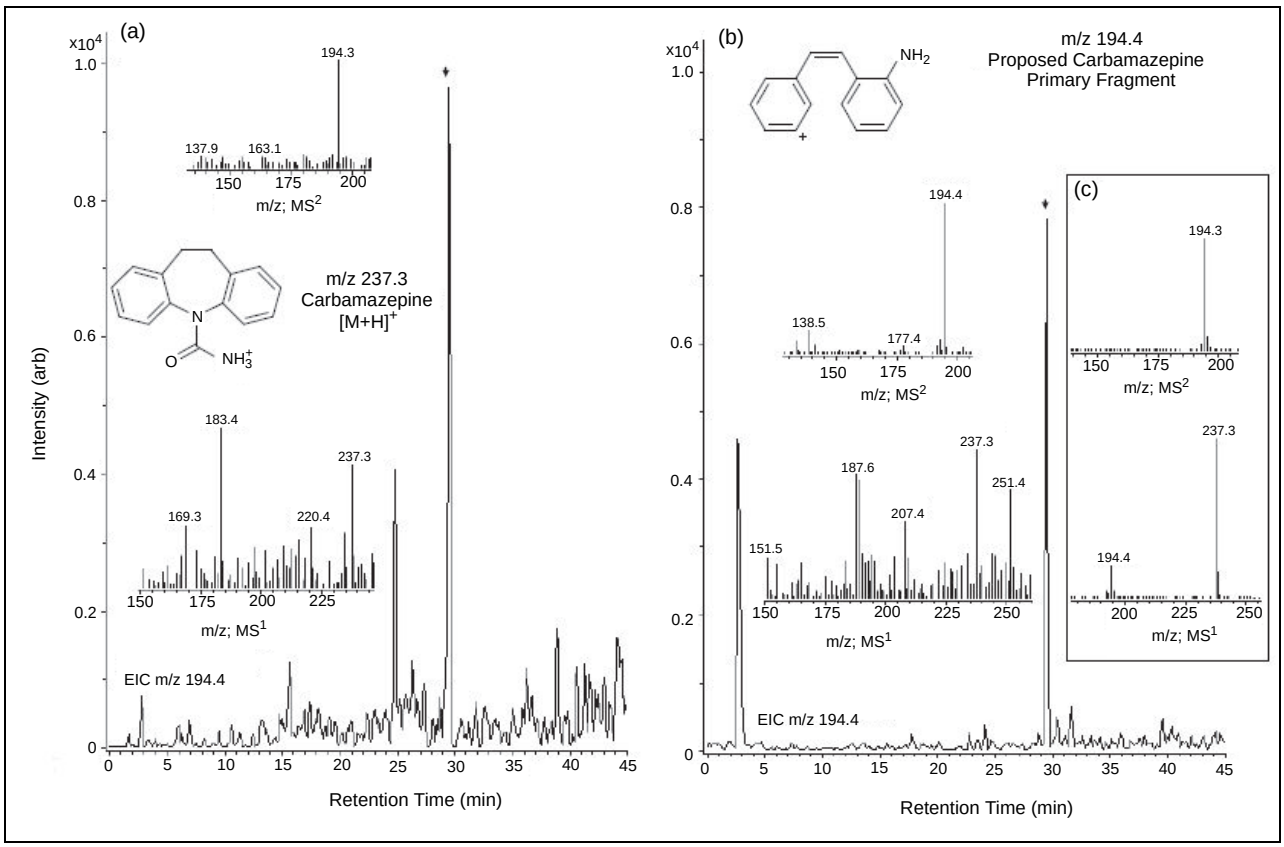


Figure 2.3. Tandem mass spectrometry (MS/MS, MS^2 above) spectra for the identification of carbamazepine in (a) digested sludge and (b) dried granular biosolid fertiliser (peaks indicated by ▼). (c) MS (MS^1 above) and MS/MS data for injection of a 10 mg/l standard of carbamazepine in ultra-pure water (spectra taken from peak apex). Offset for clarity on time and intensity axes. Reprinted with permission from the Royal Society of Chemistry (Barron *et al.*, 2008).

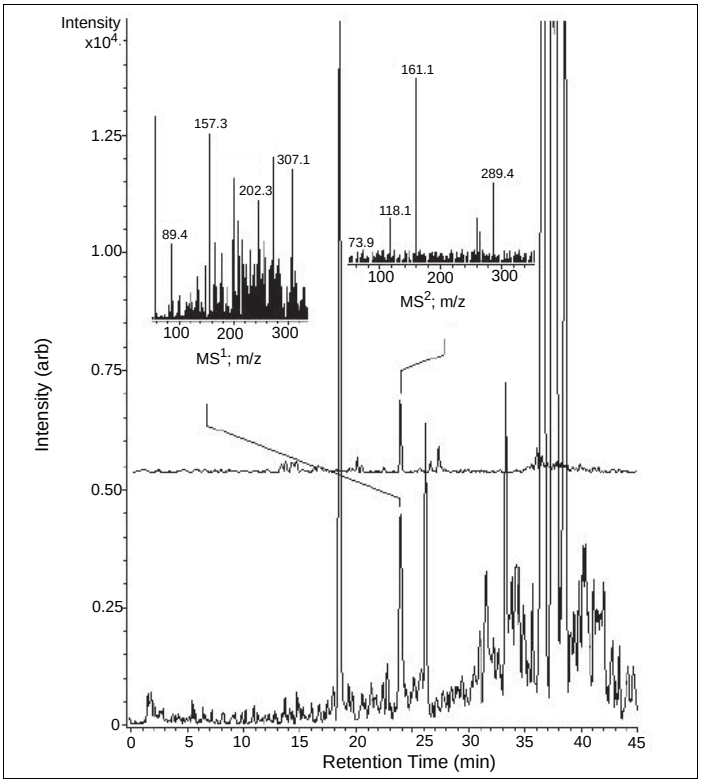


Figure 2.4. Extracted ion chromatograms and corresponding mass spectra for the identification of warfarin in hydrolysed and digested sludge from a major waste-water treatment facility in Dublin City. (MS^2, tandem mass spectrometry; MS^1, mass spectrometry.) Reprinted with permission from the Royal Society of Chemistry (Barron *et al.*, 2008).

no product ions of noteworthy intensity over a scan range of *m/z* 50–400, indicating a high stability of the $[M\text{-}H]^-$ and $[M+2\text{-}H]^-$ triclosan parent ions. Therefore, further indirect confirmation was possible by fragmentation of the isotope ions in the digested sludge sample, which also yielded no daughter ions of significant intensity. Taking this into account and coupled with matching retention times, it was deemed in all probability that this apparently abundant species was indeed triclosan. This antibacterial agent is available as an ingredient within a diverse selection of very common household personal care products, such as toothpastes, hand and face soaps, deodorants and perfumes, and represents one of the most widely used bioactive compounds. In the majority of cases, triclosan-containing products are used topically and washed directly into sewage systems and so it is not surprising that it may be present in waste-water or sludge samples with some profusion. Moreover, it was also present in the digested sludge from the satellite town waste-water facility used for analytical method validation, but at a lower concentration (~10 µg/g) after a semi-quantitative standard addition calibration (n = 2). Unfortunately as can be observed from Table 2.4, validation data for triclosan quantification were difficult to obtain in the sludge samples taken for this study given its degree of abundance. The correlation coefficients for replicate 5-point standard additions of triclosan in sludge cake and granular forms were >0.97 and >0.98, respectively, when analysed separately without any other spiked species present and good peak height agreement between separate extracts was observed (≤6% in both cases). Though the quantified levels seem to be relatively large in comparison to other residue concentrations, similarly elevated levels of triclosan have been reported in digested sludges at concentrations up to 30 mg/kg, showing that the molecule seems to readily concentrate onto biosolids (Heidler and Halden, 2007).

Analysis of the dried granular fertiliser showed an absence of warfarin. However, it was discovered that triclosan was still present, but at a significantly reduced concentration (5.8 ± 0.2 mg/kg), indicating that the heat treatment procedure at 450°C had a marked, though incomplete, effect on its molecular degradation (Fig. 2.5b). Further to this, concentrations determined for carbamazepine also reduced somewhat from 120 ± 10 µg/kg to 75 ± 16 µg/kg in the sludge cake and dried granular forms, respectively, again using analyte confirmation with MS-MS/MS transitions from *m/z* 237 to 194. Therefore, whilst only three of the selected pharmaceuticals could be determined in the original wet cake, apparently only one was completely degraded as a result of the thermal drying process. All determined PPCP residue concentrations were for $n = 2$ or $n = 3$ replicate analyses and 5-point standard addition. Other PPCP residues may have been present at lower than LOD concentrations, though this matrix type obviously hindered sensitive determinations at this level.

The ultimate goal of this study was to determine PPCP residues in soils as a result of biosolid enrichment. As the soil sampling window represented the longest possible time frame between routine sludge spreading, an idea as to the persistence of any PPCP residues identified in biosolid sludges could be obtained to some degree. However, it is important to note that PPCP exposure to these agricultural soils may not have been solely due to transport from biosolid materials and may have been introduced through a variety of other unknown routes. With this in mind, the determination of carbamazepine and triclosan were of particular concern, considering their concentration in both sludge fertiliser samples. Of the soil samples studied, all three agricultural soils and soil used for method validation yielded peaks matching the retention time of triclosan. Two of the agricultural soils

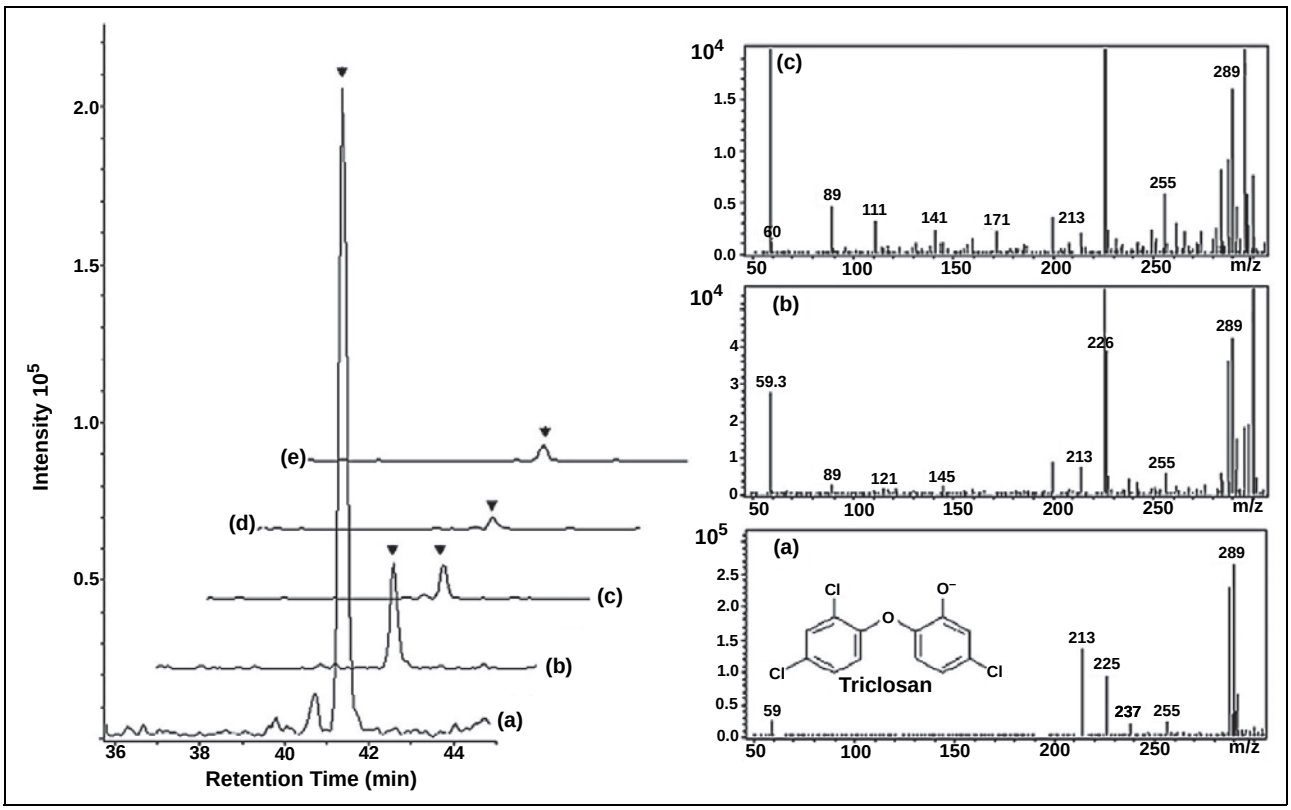


Figure 2.5. ***Left*****: extracted ion chromatograms and corresponding mass spectra for the identification of triclosan in (a) digested sludge, (b) dried granular biosolid fertiliser, and (c)–(e) sludge-enriched soils. Peaks corresponding to the retention time of triclosan are marked with a ▼.** ***Right*****: mass spectra for samples (a)–(c). Reprinted with permission from the Royal Society of Chemistry (Barron *et al.*, 2008).**

displayed peak heights at quantifiable levels. However, upon cross-examination of their mass spectra, confirmation of the presence of triclosan was not possible for two of these agricultural soil types and for the soil used in method validation. Figure 2.5 left (c)–(e) shows EICs at *m/z* 289 for all agricultural sampling sites. As can be observed, (c) was the only soil sample with an isotopic peak cluster somewhat similar to that in both sludge sample types and in the standards. Naturally, the question arose with respect to method carry-over, but upon examination of a blank extraction of analytical grade sand, neither triclosan nor any other PPCP was observed. As such, triclosan was determined at 178 ± 14 µg/kg dry weight of soil. Evidence has been proposed to support photochemical degradation of triclosan in aqueous media (Aranami and Readman, 2007), suggesting half-lives of approximately 4 and 8 days in laboratory-controlled experiments for sea water and fresh water, respectively. However, little is known of its photodegradation potential in biosolids or their potential effects on subsurface terrestrial biota while away from exposure to UV radiation. Some studies by Halden *et al.* have shown half-lives of between 120 and 540 days in soils and sediments, respectively (Halden and Paull, 2005), which could encompass quite easily the period of time between sludge-spreading events and resulting fresh exposure of triclosan to soils.

It was obvious that method detection and quantification limits were far lower in soils than in sludges and quantification of nimesulide at 11 ± 4 and 15 ± 1 µg/kg levels in two of the soil samples was possible, again using transitions from *m/z* 307 to 229 as species confirmation. Nimesulide, an NSAID, was prohibited for sale for a short time in Ireland by the IMB in 2007 due to occurrences of liver failure (IMB, 2007b). However, at this low concentration it is unknown what, if any, therapeutic effect trace nimesulide would have on biota. Contrary to the levels in both biosolid fertilisers, there was no trace of carbamazepine in any of the soil samples studied.

2.2.6 Analysis of Scandinavian digested sludges

In order to gain further insight into PPCPs in the Irish environment, further analytical method development was carried out at the Norwegian Institute for Water Research in Oslo, Norway, investigating the occurrence of an added selection of pharmaceuticals in sludges arising from across Scandinavia. As before, solid extracts were exposed to PLE, but clean-up and extraction steps and analysis involved mixed-mode SPE and ultra-HPLC (UHPLC) and allowed simultaneous determination of most residues in digested sludge in the mid to low nanogram per gram range. Selection of target compounds was based primarily on previously determined predicted environmental concentration (PEC)/predicted no-effect concentration (PNEC) ratios specific to Norway, as well as on a selection of ~25 of Ireland's top 100 most frequently prescribed and consumed pharmaceuticals. Ratios of >1 are deemed to require specific attention and the calculations of PECs are based on data specific for waste waters from Norway expressed in Eqn 1 below:

$$PEC_{water} = \frac{A \times (100 - R)}{365 \times P \times V \times D \times 100} \quad (1)$$

where A is the amount of the drug used in kg/year, R is the rate of removal (set to zero when no data were available to assess the worst-case scenario), P is the population, V is the volume of waste water *per capita* per day, and D is the dilution factor of the environment. In total, 43 pharmaceuticals were included in this study. This analyte set encompassed a diverse variety of further classifications such as antidepressants, macrolide antibiotics, anti-asthmatics, anti-cancer, illicit drugs, dihydropyridine calcium channel blockers and alpha blockers.

2.2.7 Mixed-mode SPE and UHPLC-MS/MS

Complex samples such as soils and sludges posed problems with ion suppression effects in MS. This could naturally be reduced by improving the resolving power of the chromatographic system and recent advances in separation science technology have seen the advent of UHPLC. This chromatographic technique uses sub-2 µm particles to achieve higher efficiency separations by increasing capacity. New pumping systems have been developed to combat the backpressure (BP) problem and the technique shows significant promise. The employment of UHPLC was considered here for the separation of a larger selection of PPCPs in such media. By studying van Deemter plots for four commercially available columns of

various particle sizes and lengths (using diclofenac as a test material), roughly a fourfold enhancement in peak efficiency was obtained over previously developed chromatographic methods using a 1.7 µm, 50 × 1 mm C_{18} column (Fig. 2.6). Once gradient elution was employed, separation power was dramatically improved (Fig. 2.7) with separation of the majority of analytes. Mass spectrometry involved polarity switching ESI-MS both in SIM and tandem multiple reaction monitoring (MRM) modes, allowing for extremely selective monitoring of each individual analyte. No mass spectral response was obtained for spiramycin or tetracycline. Determination of tetracyclines has been shown to be hindered by the presence of metals in the sample matrix and further modification of the method is required to include specific ligands such as ethylenediaminetetraacetic acid (EDTA) to reduce this effect.

Using the previously developed PLE method, target compounds were extracted from sludges at 60°C and 1,500 psi for $n = 2$ cycles into solutions of methanol/water. With sample adjustment to pH 4, a mixed-mode SPE sample clean-up/concentration technique allowed for selective extraction of acidic, basic and neutral species using a combination of reversed-phase and strong cation exchange sorption mechanisms (Strata XC). Additionally, both sorbents consisted of hydrophobic–hydrophilic balanced (Oasis HLB) materials to allow for a wider range of compound polarities to be extracted. This was particularly evident for compounds such as atenolol. Recoveries for PPCPs in ultra-pure water using only the developed SPE method as well as complete method recoveries in sludges are listed in Table 2.5. The recoveries ranged from <5% to >75%. It was clear that mixed-mode approaches were not suitable for all analytes.

The developed method was applied to the determination of those analytes that showed adequate recovery (>50–60%) and method sensitivity at nanogram per gram levels. Five WWTP locations from Sweden and Norway were identified and samples of digested and thermally dried sludge were taken and results shown in Table 2.6. Of particular interest was the presence of sertraline, citalopram and triclosan at relatively high milligram per kilogram levels in every sample and similar to Irish sludges (Fig. 2.8). This may be related to one waste-water facility receiving effluent from an upstream cosmetics factory. All identified compounds were quantified using an internal standard for $n = 2$ replicates.

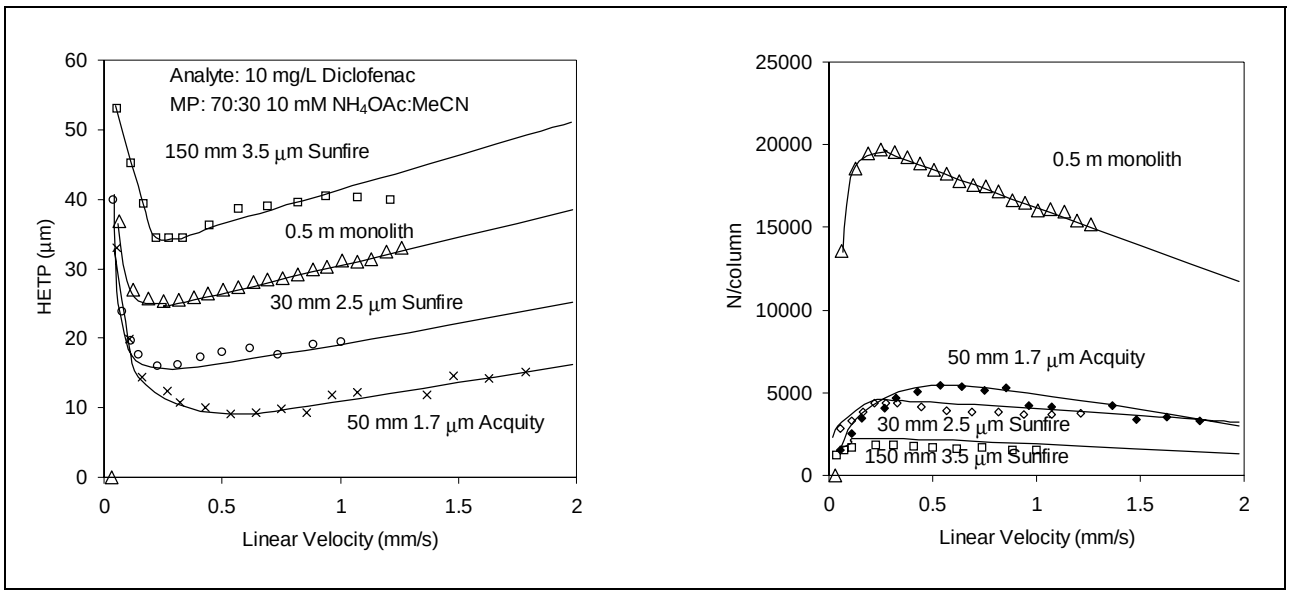


Figure 2.6. Comparison of 3.5 µm, 2.5 µm and 1.7 µm particulate columns of various lengths, with a 0.5 m monolith with respect to (a) height equivalent to a theoretical plate (HETP) and (b) N/column. Test compound: 10 mg/l diclofenac. (NH_4OAc/MeCN, ammonium acetate/acetonitrile).

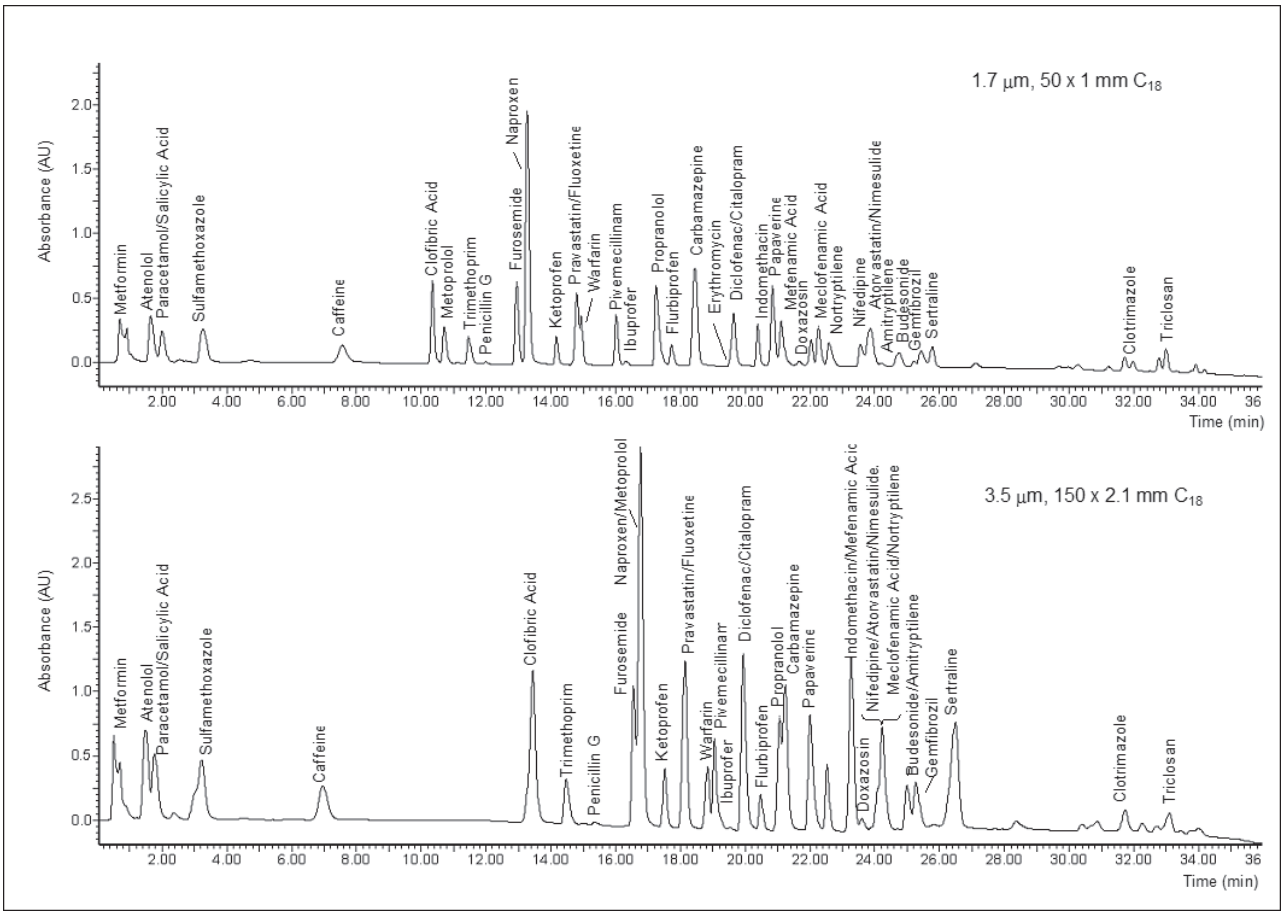


Figure 2.7. Gradient elution of all analytes and detection with UV at 220–230 nm to highlight improved separation power with 1.7 µm particulate columns.

2.2.8 Characterisation of a 0.5 m monolithic column

The advantage of UHPLC is clear from Section 2.2.7. However, drawbacks to UHPLC obviously lie in the generation of excessive BPs (often reaching >10,000 psi), adding unnecessary cost as well as other associated phenomena such as frictional heating. Alternatively, a simpler means to improve efficiency is to increase column length. With the BP constraints of all particulate phases studied thus far, this highlights the suitability of monolithic columns, given their highly dual porous structure. The efficiency of the 3.5 µm Sunfire column and the coupled monolith were characterised using van Deemter curves on an Agilent 1200 LC system. Diclofenac (NSAID) was chosen as a test analyte at 10 mg/l concentration and was run isocratically (70:30 ammonium acetate/acetonitrile) with UV detection only at 220 nm for $n = 2$ replicates. The separation efficiency of a single monolith was also compared with that of five monoliths coupled in series. Flow rates up to only 0.26 ml/min were possible with the 3.5 µm particulate column (herein referred to as Sunfire) due to excessive BPs >3,200 psi. The monolithic columns were investigated up to 0.4 ml/min (BP: 525 psi on one monolith; 1,710 psi on the coupled monoliths at 0.4 ml/min).

2.2.8.1 Comparison of HETP and number of theoretical plates (N)

Figure 2.6 shows that the Sunfire column was less efficient as expected. The five coupled monoliths performed slightly better with a height equivalent to a theoretical plate (HETP) = 25 µm, while a single monolith gave the most efficient result with HETP = 20 µm. This further illustrates the advantage monoliths have over particle-packed columns. The dual porosity structure of monoliths allows for faster mass transfer rates and therefore provides excellent efficiency even at higher flow rates. The 0.5 m monolith produced

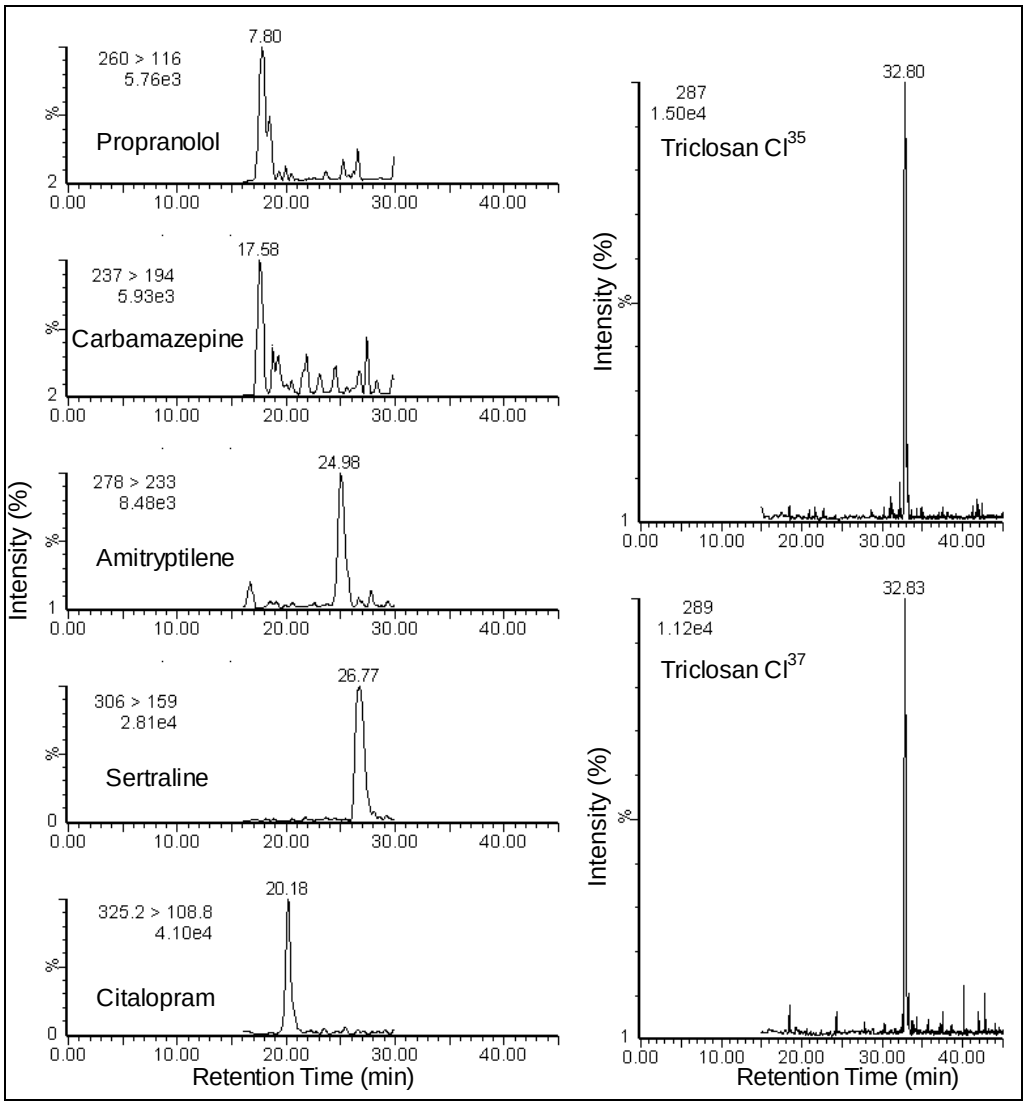


Figure 2.8. Digested sludge from Swedish waste-water treatment plant (No. 2) showing levels of pharmaceutical and personal care product residues.

considerably higher N (~20,000 plates) than both a single monolith (5,100 plates) and the Sunfire column (5,200 plates). At ~1.25 mm/s, the coupled monoliths generated ~1,500 psi in comparison with 2,000 psi for the Sunfire column at the same flow rate. Therefore, the long monolith produced more than three times the number of theoretical plates per column than the Sunfire at half the operating pressure. The single monolith, as expected, exhibited the lowest BPs but was comparable with the Sunfire in the number of plates provided.

2.2.8.2 Effect of temperature on efficiency

Along with the high mass transfer characteristics of monolithic phases, the use of increased temperatures could result in even higher efficiency through reductions in mobile phase viscosity. Isocratic separations using diclofenac were performed at 10, 15, 20 and 45°C (operating maximum temperature). The number of theoretical plates for the 0.5 m monolithic column increased from 14,500 at 10°C to 23,500 at 45°C, with a decrease in plate height of approximately 13 µm and a reduction in BP of over 1,000 psi. The slopes of the van Deemter plots indicated only a marginal efficiency loss at higher linear velocities (Fig. 2.9). Therefore, this column shows promise for application to very complex sample types such as soil or sludge. When a comparison was made of the monolith data at 45°C against the Sunfire data at ambient temperatures, the advantages of coupled

Table 2.5. Pharmaceuticals and personal care products selection and performance of the mixed-mode solid-phase extraction approach, final method recoveries and ion suppression data.

Analyte	PEC_{Norway}/PNEC	Mixed-mode SPE % recovery in DIW[a]	Total mixed-mode method % recovery in sludge matrix[b]	% ion suppression[c]
		% Recovery	% Recovery	
Atenolol		47 ± 13	59 ± 6	34
Paracetamol	4.78	13 ± 2	6 ± 3	8
Sulfamethoxazole	1.85	30 ± 13	16 ± 2	<5
Tetracycline	3.71	–	<5	–
Metoprolol	0.20	61 ± 2	44 ± 5	33
Penicillin G		–	<5	–
Ketoprofen		59 ± 1	63 ± 7	23
Pravastatin	7.64	48 ± 4	<5	–
Fluoxetine	4.87	36 ± 15	49 ± 25	11
Pivmecillinam	0.74	35 ± 40	<5	92
Propranolol	12.51	74 ± 15	22 ± 2	59
Carbamazepine	0.23	69 ± 11	60 ± 2	6
Erythromycin	0.01	125 ± 24	60 ± 23	67
Spiramycin	1.36	–	<5	–
Doxazosin	3.77	>130	52 ± 12	40
Amitryptilene	0.78	–	23 ± 3	72
Nortryptilene		17 ± 3	<5	70
Nifedipine	0.55	43 ± 21	18 ± 5	18
Budesonide		65 ± 8	<5	66
Clotrimazole		7 ± 2	16 ± 6	<5
Simvastatin	7.58	–	65 ± 9	–27
Trimethoprim	1.86	55 ± 5	37 ± 6	36
Tamoxifen		43 ± 21	23 ± 15	17
Clofibric acid		64 ± 19	71 ± 9	<5
Furosemide		59 ± 31	<5	10
Naproxen	0.03	61 ± 20	48 ± 5	13
Warfarin		34 ± 14	55 ± 12	43
Flurbiprofen		58 ± 26	43 ± 12	20
Diclofenac	0.02	33 ± 24	49 ± 13	12
Nimesulide		41 ± 20	43 ± 7	17
Gemfibrozil		30 ± 18	49 ± 9	35
Papaverine		57 ± 11	54 ± 13	46
Mefenamic acid		26 ± 23	59 ± 9	29
Sertraline	2.53	29 ± 11	7 ± 1	38

Table 2.5 ***contd.***

Analyte	PEC_{Norway}/PNEC	Mixed-mode SPE % recovery in DIW[a]	Total mixed-mode method % recovery in sludge matrix[b]	% ion suppression[c]
		% Recovery	% Recovery	
Citalopram	0.17	94 ± 6	35 ± 1	52
Salicylic acid		>130	86 ± 5	<5
Fluoxetine		75 ± 20	53 ± 1	41
Caffeine		3 ± 4	32 ± 1	<5
Phenazone	3.06	>130	77 ± 3	<5
Metformin	0.10	2 ± 1	<5	64
Meclofenamic acid		67 ± 8	51 ± 13	29
Triclosan (Cl^{35} and Cl^{37} isotopes)		17 ± 3	NC (40)[d]	NC (–285)[d]
Ibuprofen	1.19	33 ± 15	55 ± 7	19
Indomethacin		40 ± 20	50 ± 7	23

[a]Using Oasis HLB and Strata XC cartridges in series in this order for $n = 3 \times 3$ replicates.
[b]Using digested sludge from Bekkelaget WWTP in Norway ($n = 3 \times 3$) and absolute recovery.
[c]Sludge extract reconstituted in 0.5 ml of 5 mg/l solution of all analytes in 90:10 10 mM ammonium acetate/acetonitrile compared with identical solution without sludge extract.
[d]High concentrations of triclosan in sludge prevented accurate determination of recovery values. Calculated recoveries are in parentheses and represent a guideline only.
DIW, deionised water; PNEC, predicted no-effect concentration; SPE, solid-phase extraction.

monoliths were further obvious. The Sunfire column reached its optimum efficiency at a plate height of 26 µm and then steadily lost efficiency as linear velocity increased. This optimum plate height was at a flow rate of only 0.04 ml/min which was impractical for this application due to excessive analysis times. Therefore, to reduce analysis time, efficiency had to be sacrificed with this phase more than with the coupled monolith. It reached a minimum plate height of 21 µm at a flow rate of 0.16 ml/min, four times that of the Sunfire. This illustrates that higher flow rates can be used with monoliths without a significant loss of efficiency.

2.2.8.3 Peak capacity

Peak capacity (P_c) has previously been used to determine the performance of monolithic columns over particle columns (Eqn 2):

$$P_c = 1 + \frac{t_g}{W} \quad (2)$$

where t_g is the time of the gradient and W is the peak width at baseline (units for both were decimal mins). A working standard containing ~50 PPCPs in ultra-pure water was injected (20 µl) onto both columns using an identical gradient. The gradient run time was 90 min, over which the percentage acetonitrile increased linearly from 0 to 100% (MP_A = 10 mM ammonium acetate). Twenty peaks were selected and widths divided into the gradient run time, the average of these figures was then taken and the peak capacity per hour was also reported. Table 2.7 contains the data obtained from the peak capacity study.

Previous work in the DCU laboratory investigated ten coupled monolithic columns (100.0 × 3.0 mm ID) for the separation of protein digests. The 1 m monolith reported excellent peak capacity values per hour.

In this study peak capacity values per run and per hour were compared for the 0.5 m monolith and the 3.5 µm particulate Sunfire columns. Four experiments were carried out, each under a different set of chromatographic conditions. At identical flow rates, both columns displayed identical peak capacities. When BP was matched, the monolith operated at a much higher flow rate, which allowed almost twice the number of peaks to be separated within the run time. A

Table 2.6. Identified pharmaceuticals and personal care products and quantities in sludge (using internal standard method for *n* = 2 replicates).

Analyte	Site					
	No. 1	No. 2	No. 3	Bekkelaget[a]	Bekkelaget[b]	Arendal
	Digested sludge	Digested sludge	Digested sludge	Dried biosolids	Dried biosolids	Dried biosolids
Atenolol	7.5 ± 0.3	11 ± 1	<LOQ	<LOQ	<LOQ	<LOQ
Paracetamol	–	<LOQ	–	–	<LOQ	–
Metoprolol	<LOQ	–	–	35 ± 7	108 ± 85	–
Propranolol	573 ± 73	194 ± 55	201 ± 15	90 ± 13	101 ± 3	229 ± 45
Carbamazepine	712 ± 45	392 ± 39	294 ± 21	714 ± 46	732 ± 34	1247 ± 101
Erythromycin	–	<LOQ	–	–	–	–
Amitriptyline	647 ± 200	623 ± 25	829 ± 33	239 ± 48	378 ± 51	617 ± 197
Clotrimazole	<LOQ	–	–	264 ± 45	–	529 ± 93
Simvastatin	300 ± 82	–	325 ± 27	458 ± 48	<LOQ	–
Diclofenac	<LOQ	<LOQ	–	–	–	–
Nimesulide	<LOQ	–	–	<LOQ	–	–
Gemfibrozil	<LOQ	<LOQ	–	<LOQ	–	–
Papaverine	<LOQ	194 ± 28	–	<LOQ	–	–
Sertraline	5686 ± 143	22439 ± 1504	9488 ± 3384	2514 ± 1498	4627 ± 1981	20736 ± 5217
Citalopram	32823 ± 1114	21255 ± 2741	15289 ± 418	2480 ± 2167	6265 ± 342	19145 ± 396
Metformin	2496 ± 810	–	–	–	–	–
Triclosan	<LOQ	32009 ± 1167	<LOQ	<LOQ	<LOQ	<LOQ

[a]Sampled on 29/30 September 2006.
[b] Sampled on 7 September 2006.
LOQ, limit of quantification.

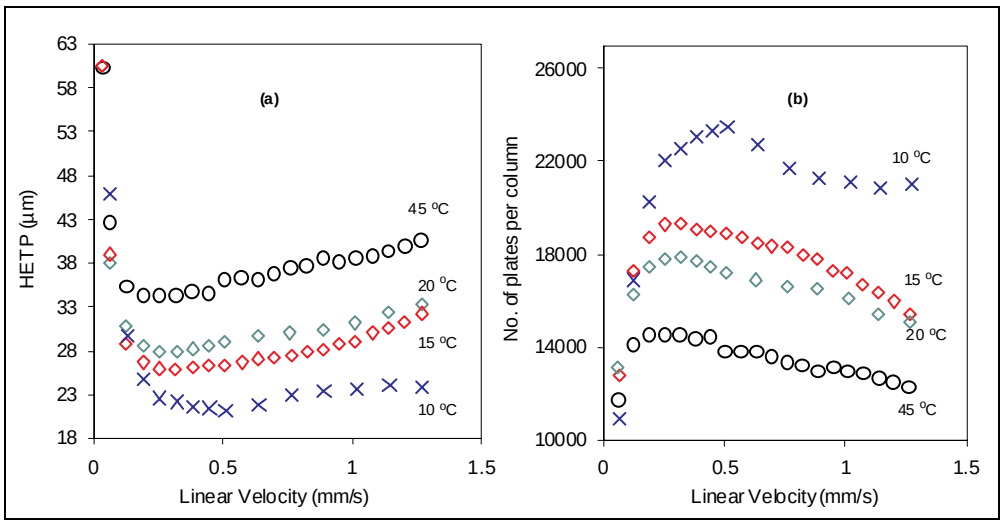


Figure 2.9. (a) Height equivalent to a theoretical plate (HETP), and (b) number of plates per column *versus* linear velocity generated on five coupled monoliths at incremental temperatures. Test compound: 10 mg/l diclofenac.

Table 2.7. Peak capacity values calculated for the 0.5 m monolith and the 3.5 µm particulate Sunfire columns.

	F[a]		BP[b]		t_0[c]		υ[d]	
	P_c	P_c/h	P_c	P_c/h	P_c	P_c/h	P_c	P_c/h
Monolith	104	69	86	57	108	72	132	88
Sunfire	103	69	50	33	50	33	93	62

[a]Flow rate was 0.2 ml/min for both columns with backpressures (BPs) of 34 bar for the monolith and 146 bar for the Sunfire.
[b]Flow rates were 0.18 ml/min and 0.04 ml/min for the monolith and Sunfire columns, respectively, resulting in BPs of ~ 30–31 bar (570 psi).
[c]Flow rates were 0.28 ml/min and 0.04 ml/min for the monolith and Sunfire columns, respectively, resulting in dead times of 9.94 and 9.73 min, respectively.
[d]Flow rates were 0.36 ml/min and 0.16 ml/min for the monolith and Sunfire columns, respectively, resulting in linear velocities of 1.07 and 1.04 mm/s, respectively.

similar result was obtained when the void volumes of the columns were correlated. For the t_0 value of the 3.5 µm particulate Sunfire column to match that of the monolith, the flow rate had to be lowered to 0.04 ml/min. This resulted in only half the number of peaks being separated in 90 min than those generated on the monolithic column. Finally, linear velocities of the mobile phase were matched and the peak capacities were again evaluated. Once more, the monolithic column reported higher peak capacity at lower BPs of ~60 bar. By calculating a ratio of P_c/h to BP as an indication of the column providing highest efficiency separation (per hour at the lowest pressure drop), the resulting values were 1.44 and 0.42, respectively. The higher value for the monolithic column indicated that it was an excellent choice for high-efficiency separations at BPs that can be achieved using standard HPLC instrumentation and offers a realistic alternative to UHPLC.

2.2.8.4 Investigation of loading capacity

Due to the increased length and increased surface area of the monolith available for separation of analytes, it was expected that the long column would have an increased capacity for large sample volumes. Three test compounds (metoprolol, cocaine and diclofenac) were chosen (10 mg/l) and were injected at volumes of 10–900 µl. The separations were carried out isocratically, with the same mobile phase composition as in Section 2.2.8.1 over 30 min. Peak height, width at half height ($W_{1/2}$) and tailing asymmetry (A_s) were examined for each injection volume. Peak height increased linearly with injection volume up to 900 µl for all three test compounds (R^2 = 1.000, 0.9989 and 0.999 for metoprolol, diclofenac and cocaine, respectively) (Fig. 2.10). The 0.5 m monolith was capable of accepting almost 1 ml of sample. $W_{1/2}$ increased up to 900 µl injection volumes as expected. Although metoprolol and cocaine exhibited some deviations at low injection volumes of 10, 20 and 30 µl, the increase in width throughout the study was not significant. Peak width increased only minimally for metoprolol and diclofenac and remained constant or even slightly decreased for cocaine. Overall, $W_{1/2}$ increased for metoprolol and diclofenac by ~4 s. $W_{1/2}$ for cocaine only increased by ~2.5 s over the injection volumes studied. Peak tailing asymmetry is often caused by simultaneous retention mechanisms. Tailing results in an A_s >1. Peak fronting (A_s < 1) is mainly caused by overloading of the column. Diclofenac maintained a high level of symmetry up to 100 µl with a fronting value of 0.883, after which the symmetry of the peaks decreased with increasing injection volume. Metoprolol and cocaine both exhibited signs of asymmetry at low injection volumes and these values decreased rapidly until after 100 µl, when they began to even out.

The effect of increased sample loading on H demonstrated that only about a 10% loss in efficiency was observed for each analyte at an injection volume of 200 µl. $W_{1/2}$ for metoprolol and diclofenac increased by approximately 6% between sample volumes of 10 and 200 µl, while the peak widths for cocaine only increased by approximately 3% over the same range. For cocaine, H decreased initially and levelled off at higher injection volumes.

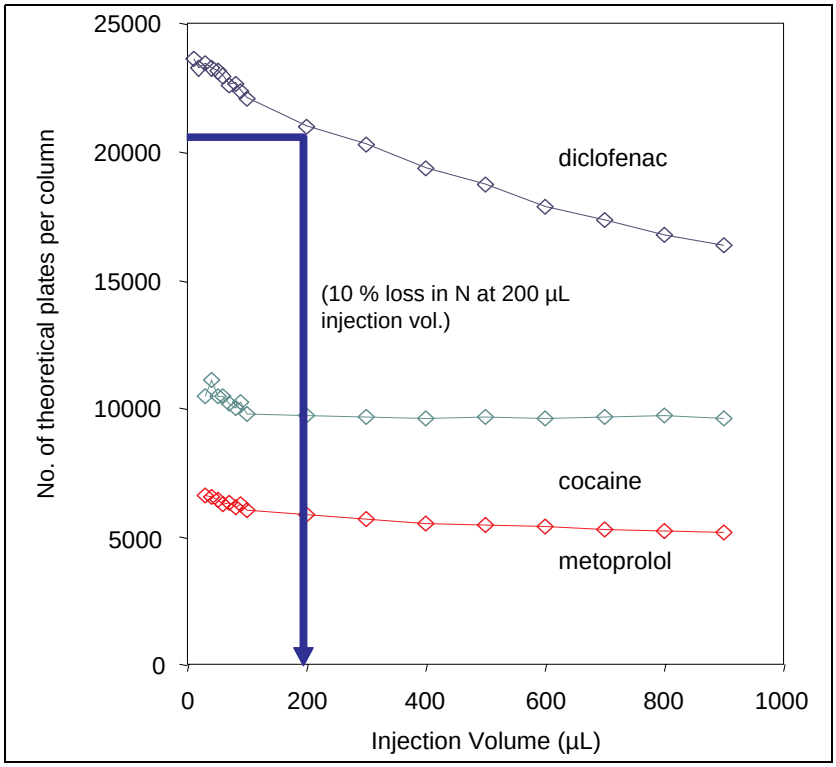


Figure 2.10. Plot of number of theoretical plates (*N*) *versus* injection volume.

2.2.8.5 Application to multi-residue PPCP mixtures

The long monolith was applied to the separation of increasing volumes of a mixed standard containing up to 50 pharmaceuticals and illicit drugs in ultra-pure water. This study was carried out to demonstrate the superior loading capacity of the 0.5 m monolith when separating a complex mixture of analytes and also to optimise gradient conditions for the separation of numerous target analytes. Injections of the 0.33 mg/l standard were made at volumes of 10, 20, 50 and 100 µl and separated over a 110 min run time at 0.2 ml/min and a column temperature of 45°C, and detected at a wavelength of 230 nm. A gradient was employed, mobile phase B (20:80 10 mM ammonium acetate/acetonitrile) was increased from 0 to 100% over 90 min and held for 20 min and a re-equilibration time back to 100% mobile phase A (90:10) for 30 min.

A soil sample where PLE extracts had a final spiking concentration of 0.2 mg/kg was analysed by LC-MS/MS using the 0.5 m monolithic column. The extraction procedure was very simple with no SPE clean-up or pre-concentration step required. A number of the pharmaceuticals were not detected in the spiked soil sample, e.g. amphetamine, doxazosin, budesonide, citalopram, sertraline, papaverine and ranitidine. This was most likely due to ion suppression effects or incomplete extraction. Not all pharmaceuticals were completely resolved from each other in this gradient profile (particularly caffeine/atenolol, morphine/benzoylecgonine and cocaine/heroin). However, the majority of the target analytes were adequately resolved with R_S>>1 in some cases. Due to the length of the monolithic bed, even highly polar PPCPs were retained above 24 min and were, in many cases, well resolved from each other. Later eluting compounds were well retained by the monolithic bed but not so much so as to overextend retention times and broaden peaks. See Fig. 2.11 for an overlay of incremental sample volumes on a multi-residue separation using a 0.5 m monolith.

2.2.8.6 Matrix effects in soil and sludge extracts

The degree of analyte signal suppression was determined by comparing matrix-matched standards (soil and sludge) with a 1 mg/l standard in ultra-high-purity water (samples subjected to the entire PLE/SPE procedure in Section 2.1.6). Any reduction in signal

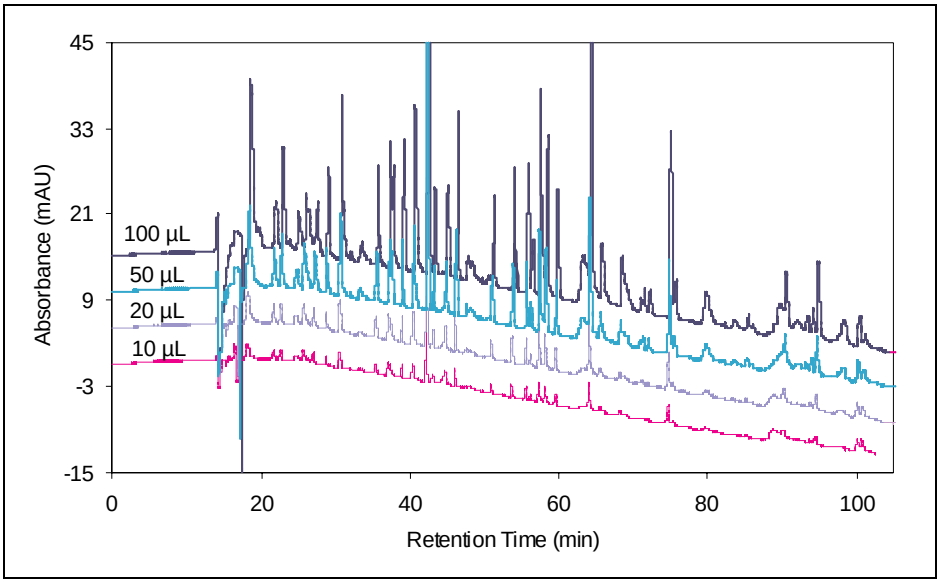


Figure 2.11. Overlaid chromatograms obtained after 10–100 µl injections of a 0.33 mg/l standard of 51 pharmaceuticals and illicit drugs in ultra-pure water.

intensity must therefore be due to matrix components within the sample or coeluting PPCPs. Results from the 0.5 m monolith were also compared with the 3.5 µm particulate Sunfire column under the same chromatographic conditions. Suppression is calculated as the percentage loss of analyte recovery. Relative suppression of all analytes on both columns was calculated as a ratio of their respective absolute suppression percentages with a relative suppression <1 indicating lower levels of suppression on the monolithic column. An example of comparative suppression studies in sludges is presented in Table 2.8.

Overall the monolith outperformed the particulate phase in separating soil components. In general, the suppression effect was quite low for the monolithic column with percentage suppression ≤10% for roughly half the target analytes. This was true for only 9/41 PPCPs when separated on the Sunfire column. Of the 41 drugs compared in the study, suppression levels were lower for 32 drugs on the monolith, six analytes exhibited lower levels of ion suppression on the Sunfire column and three of the analytes were equally affected in both experiments. High levels of suppression were observed mostly for less polar analytes, e.g. amitriptyline, sertraline, clotrimazole, tamoxifen and simvastatin. This phenomenon could be explained by the high levels of simultaneously eluting organic matrix components. Suppression values reported as negatives indicate some enhancement of signals. Chloramphenicol exhibited enhancement on the Sunfire, while low levels of suppression were reported by the monolith. Conversely, many results were quite consistent between columns with suppression values for the monolith considerably lower, e.g. sulfapyridine, cimetidine, phenazone, trimethoprim, ketoprofen.

Ion suppression was much more significant for pharmaceuticals in sludge extracts. The average level of matrix-induced ion suppression for the target analytes on the monolith and Sunfire columns were 43 ± 14% and 50 ± 19%, respectively. Only 37 pharmaceuticals were compared in the sludge suppression study as chloramphenicol and nifedipine were undetected by both columns whilst budesonide and sertraline were undetected. Moreover, signals corresponding to chloramphenicol and nifedipine were low-intensity peaks. Overall, the monolith reported lower levels of ion suppression for a total of 25/37 PPCPs, with two drugs being equally quenched by matrix components on both columns. As with soil, ion suppression in sludge increased for less polar sludge components. However, high levels of suppression were present throughout the separation, with metformin, sulfamethazine and sulfamethoxazole

Table 2.8. Percentage and relative suppression for 37 pharmaceuticals in digested sludge. Calculated by comparing a digested sludge extract spiked post-extraction with a 1 mg/l standard solution in Milli-Q water. Cells marked n/a (not applicable) indicate that values could not be calculated. Those marked with an asterisk indicate *n* = 1 replicate. Shaded cells indicate those that exhibited less suppression on the monolithic column (Sunfire refers to the 3.5 µm particulate Sunfire column).

Name	% MS suppression		Relative suppression	Name	% MS suppression		Relative suppression
	Sunfire	Monolith			Sunfire	Monolith	
Metformin	73 ± 4	81 ± 3	1.1	**Propranolol**	58 ± 1	47 ± 3	0.8
Salbutamol	8 ± 4	19 ± 2	2.3	**LSD**	67 ± 1	52 ± 2	0.8
Sulfamethoxazole	47 ± 1	53 ± 2	1.1	**Indomethacin**	60*	60 ± 13	1
Atenolol	12 ± 2	19 ± 1	1.6	**Carbamazepine**	59 ± 8	54 ± 14	0.9
Caffeine	7 ± 1	21 ± 18	2.9	**Cocaethylene**	50 ± 2	51 ± 3	1
Sulfapyridine	55 ± 2	54 ± 3	1	**Ketamine**	33 ± 3	33 ± 3	1
Cimetidine	42 ± 1	40 ± 2	1	**Citalopram**	73 ± 1	53 ± 1	0.7
Sulfamethazine	70 ± 1	54 ± 2	0.8	**Papaverine**	54 ± 1	49 ± 2	0.9
Morphine	68 ± 2	27 ± 10	0.4	**EDDP**	58 ± 1	50 ± 1	0.9
Benzoylecgonine	19 ± 4	24 ± 5	1.3	**Doxazosin**	46 ± 2	42 ± 3	0.9
Ranitidine	n/a	n/a	n/a	**Temazepam**	58 ± 5	54 ± 7	0.9
Phenazone	16 ± 3	15 ± 7	1	**Nortriptyline**	60 ± <1	47 ± 2	0.8
MDMA	63 ± 3	37 ± 7	0.6	**Nifedipine**	n/a	n/a	n/a
Trimethoprim	45 ± 2	44 ± 5	1	**Budesonide**	23 ± 15	n/a	n/a
Metoprolol	63 ± 1	53 ± 7	0.8	**Methadone**	68 ± <1	42 ± 1	0.6
Ketoprofen	63 ± 3	44 ± 0	0.7	**Amitriptyline**	56 ± 1	47 ± 13	0.8
Tramadol	42 ± 1	31 ± 2	0.7	**Diazepam**	50 ± 4	49 ± 3	1
Chloramphenicol	n/a	n/a	n/a	**Sertraline**	n/a	13 ± 4	n/a
Bezafibrate	57 ± 4	33 ± 15	0.6	**Clotrimazole**	78 ± 1	57 ± 8	0.7
Cocaine	47 ± 1	53 ± 1	1.1	**Tamoxifen**	39 ± <1	46 ± 2	1.2
Heroin	39 ± 1	56 ± 6	1.4	**Simvastatin**	69 ± 2	45 ± 20	0.7

EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; MDMA, 3,4-methylenedioxymethamphetamine; MS, mass spectrometry; LSD, lysergic acid diethylamide.

reporting very low recoveries. The sludge matrix obviously contained a wide range of organic compounds, ranging from polar to non-polar, eluting throughout the run and affecting all target analytes.

2.3 Conclusions

Analytical methods were developed, optimised, validated and applied to the determination of a large selection of bioactive PPCP residues in digested sludges and biosolid-enriched soils. Ion suppression effects in mass spectrometry arising from matrix components were more pronounced in digested sludge samples and remain a common concern for development of highly sensitive analytical methods.

Residues identified in soils and sludges originating from Ireland were carbamazepine, warfarin, nimesulide and triclosan for a validated method to determine 27 PPCPs. It was found that triclosan concentrations were reduced from the wet sludge cake

to the thermally dried biofertiliser and was present, though at significantly lower levels, in biosolid-enriched soils after a 3- to 4-month settling period. Once the method was extended further to a selection of 43 PPCPs and applied to Scandinavian sewage sludge it could be observed that a larger selection of compounds were identified. Of particular concern was the presence of high milligram per kilogram levels of the antidepressants sertraline and citalopram.

2.4 Publications

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3 Sorption and Fate in Soil

One of the main concerns associated with PPCPs in the environment is the potential for pathogens to become resistant to antibiotics (Ciniglia *et al.*, 2005). However, the majority of reported work deals with PPCP residues in aquatic matrices. In general, PPCPs are small- to medium-sized organic molecules, with varying degrees of hydrophobicity and may be neutral or charged. Therefore, considering the amount of biosolids recycled as fertilisers, an investigation into the sorption behaviour of pharmaceuticals to sludge and soil is critical in assessing any environmental risk posed by the approximate 7,000 human and 1,000 veterinary pharmaceutical products now licensed for sale in Ireland (IMB, 2006) and internationally. Simple sorption behaviour is often adequately described using the solid–water distribution coefficient (K_d). However, sorption mechanisms onto complex matrices such as soils and activated/deactivated sludges are not simply defined. They may not only depend on the physico–chemical properties of the individual molecules, but also on conditions of temperature, pH and ionic strength. This, in turn, may greatly influence degrees of biotransformation and photolysis (Carballa *et al.*, 2008). Several methods exist for categorising sorption of many chemically diverse compounds, predominantly centred on studies of octanol–water partition coefficients (log*P*) and normalised organic carbon sorption coefficients (K_{oc}). However, these quantities can only be used as an approximate guide to help interpret sorption behaviour and do not take into account other factors such as cation bridging, cation exchange, hydrogen bonding and the polarity of functional groups (Goss and Schwarzenbach, 2001).

Non-linear correlation modelling techniques, such as artificial neural networks (ANNs), can be used to find correlations in complex data sets, such as those required to predict water–solid distribution coefficients (K_d) in soil or sludge mixtures, and the successful use of ANNs with such complex data sets has been demonstrated in other areas of environmental chemistry (Pastor-Bárcenas *et al.*, 2005; Sahoo *et al.*, 2005). In training the network, the aim is to map the area between the groups of input objects and the regions that are eventually formed in the plane of the output neurons. What happens inside the network is usually ignored and is considered as a black box with *i* inputs and *j* outputs. Among the various learning methods in neural network computing, by far the most popular is the feed-forward, back-propagation method and it is often used in analytical applications. This type of network learns by adjusting statistical weight on connections between its layers in a direction that minimises the overall or absolute error. However, it should be noted that the empirical nature of such networks implies that output prediction is based only on data sets used for training. Nevertheless, ANNs can act as a useful tool to reduce the time and cost of obtaining experimentally determined data sets.

The aim of this work was to study the sorption behaviour of a large selection of pharmaceutical compounds in water–sludge and water–soil through calculation of K_d values from their associated Freundlich sorption isotherms over a selected concentration range. The compounds were chosen to cover a wide variety of diverse physico–chemical properties, as well as therapeutic classes, including antibiotics, beta blockers, analgesics, antidepressants, anti-cancer and illicit drugs. The study applied the above experimental data to investigate the suitability of ANNs, trained using a significant data set of molecular descriptors for common pharmaceutical compounds, to firstly identify which molecular descriptors were significant in the use of ANNs to predict K_d onto both soil and sludges and secondly to then use ANNs, trained using such data, to accurately predict values for K_d for PPCPs.

3.1 Experimental

3.1.1 Reagents

A selection of 54 analytical grade pharmaceuticals (≥97% purity in all cases) was made to stock concentrations of 1,000 mg/l and stored in the dark at 4°C. See Tables 2.1 and 3.1 for the full list. All other chemicals as in Section 2.1.1.

Table 3.1. Experimentally determined K_d values for pharmaceuticals in soil and sludge.

Compound	K_d (soil)[a] (l/kg)	n[b]	logK_f[b]	R^2	logK_{oc}	K_d (sludge)[a] (l/kg)	n[b]	logK_f[b]	R^2	logK_{oc}	Literature logK_{oc} (Ref.)
Amitriptyline	138	0.904 ± 0.052	2.104 ± 0.031	0.9888	3.56	1049	3.341 ± 0.655	6.123 ± 0.864	<0.900	3.53	–
Amphetamine	29	0.601 ± 0.087	1.653 ± 0.031	0.9398	2.90	–	–	–	–	–	–
Atenolol	15	1.177 ± 0.024	1.081 ± 0.053	0.9612	2.60	11	2.235 ± 0.159	0.830 ± 0.043	0.9751	1.53	–
Benzoylecgonine	28	1.151 ± 0.069	1.424 ± 0.018	0.9783	2.89	–	–	–	–	–	–
Bezafibrate	14	1.409 ± 0.130	1.080 ± 0.217	0.9669	2.60	–	–	–	–	–	–
Budesonide	12	1.770 ± 0.180	1.032 ± 0.053	0.9684	2.50	449	1.426 ± 0.165	2.868 ± 0.099	0.9491	3.16	–
Caffeine	25	0.797 ± 0.097	1.449 ± 0.053	0.9660	2.84	14	1.113 ± 0.089	1.115 ± 0.031	0.9695	1.66	–
Carbamazepine	13	1.587 ± 0.128	0.981 ± 0.038	0.9808	2.56	43	1.008 ± 0.033	1.631 ± 0.009	0.9938	2.14	**2.12** (Drillia *et al.*, 2005) **2.40–2.50** (Stein *et al.*, 2008) **2.26–3.48** (Carballa *et al.*, 2008)
Chloramphenicol	42	1.025 ± 0.058	1.619 ± 0.022	0.9915	3.06	–	–	–	–	–	–
Cimetidine	11	1.511 ± 0.241	0.854 ± 0.075	0.9074	2.48	–	–	–	–	–	–
Citalopram	250	0.630 ± 0.038	2.198 ± 0.036	0.9813	3.84	282	0.706 ± 0.012	2.243 ± 0.011	0.9981	2.96	–
Clofibric acid	9	0.212 ± 0.044	1.090 ± 0.020	0.9230	2.40	5	1.014 ± 0.087	0.722 ± 0.039	0.9854	1.23	**1.16** (Ternes *et al.*, 2004) **1.41** (Loffler *et al.*, 2005)
Clotrimazole	1,029	1.117 ± 0.186	3.157 ± 0.269	0.9231	4.45	8128	1.350 ± 0.353	4.647 ± 0.776	<0.900	4.42	–
Cocaethylene	26	0.952 ± 0.036	1.422 ± 0.011	0.9927	2.85	–	–	–	–	–	–
Cocaine	22	0.977 ± 0.096	1.349 ± 0.024	0.9622	2.78	–	–	–	–	–	–
Diazepam	30	1.159 ± 0.083	1.444 ± 0.023	0.9746	2.91	–	–	–	–	–	**2.40–2.80** (Stein *et al.*, 2008) **2.28** (Loffler *et al.*, 2005)
Diclofenac	9	0.579 ± 0.130	1.012 ± 0.044	<0.900	2.39	105	1.214 ± 0.062	2.124 ± 0.033	0.9923	2.53	**2.20–3.420** (Carballa *et al.*, 2008) **1.79–2.22** (Chefetz *et al.*, 2008)
Doxazosin	2,469	0.672 ± 0.081	2.865 ± 0.155	0.9189	4.83	8163	0.479 ± 0.093	2.689 ± 0.184	<0.900	4.42	–
EDDP	198	0.883 ± 0.027	2.233 ± 0.155	0.9961	3.74	–	–	–	–	–	–
Erythromycin	68	0.528 ± 0.096	1.607 ± 0.062	0.9095	3.27	190	1.365 ± 0.131	2.634 ± 0.184	0.9474	2.79	–
Flurbiprofen	11	0.451 ± 0.066	1.146 ± 0.024	0.9384	2.47	65	0.874 ± 0.068	1.801 ± 0.023	0.9649	2.32	–
Indomethacin	32	0.941 ± 0.035	1.508 ± 0.013	0.9945	2.95	214	0.799 ± 0.055	2.200 ± 0.044	0.9766	2.84	–
Ketoprofen	9	1.589 ± 0.099	0.718 ± 0.032	0.9807	2.38	–	–	–	–	–	–
MDMA	17	0.929 ± 0.052	1.271 ± 0.018	0.9878	2.68	–	–	–	–	–	–
Methadone	82	0.733 ± 0.033	1.890 ± 0.019	0.9915	3.35	–	–	–	–	–	–

Table 3.1 *contd.*

Compound	K_d (soil)[a] (l/kg)	n[b]	logK_f[b]	R^2	logK_{oc}	K_d (sludge)[a] (l/kg)	n[b]	logK_f[b]	R^2	logK_{oc}	Literature logK_{oc} (Ref.)
Metoprolol	20	1.253 ± 0.078	1.261 ± 0.021	0.9846	2.75	18	1.019 ± 0.062	1.262 ± 0.018	0.9784	1.78	–
Naproxen	11	1.105 ± 0.178	1.018 ± 0.054	0.9052	2.48	36	0.942 ± 0.110	1.556 ± 0.032	0.9237	2.06	**2.00–3.00** (Carballa *et al.*, 2008)
Nifedipine	11	2.938 ± 0.900	0.818 ± 0.167	<0.900	2.47	27	0.428 ± 0.077	1.407 ± 0.020	<0.900	1.94	–
Nimesulide	15	1.279 ± 0.078	1.144 ± 0.021	0.9082	2.60	66	1.018 ± 0.123	1.823 ± 0.049	0.9447	2.33	–
Nortriptyline	117	0.976 ± 0.054	2.064 ± 0.025	0.9815	3.51	600	1.905 ± 0.399	3.571 ± 0.357	<0.900	3.29	–
Papaverine	5,442	0.642 ± 0.213	3.059 ± 0.476	<0.900	5.18	–	–	–	–	–	–
Paracetamol	32	0.693 ± 0.071	1.588 ± 0.030	0.9508	2.94	19	0.574 ± 0.105	1.429 ± 0.046	<0.900	1.79	–
Phenazone	8	2.483 ± 0.558	0.325 ± 0.234	<0.900	2.36	8	2.269 ± 0.312	0.484 ± 0.102	0.9297	1.41	–
Propranolol	58	0.871 ± 0.012	1.753 ± 0.004	0.9988	3.21	331	0.815 ± 0.048	2.394 ± 0.038	0.9793	3.03	**3.64** (Drillia *et al.*, 2005)
Ranitidine	50	0.691 ± 0.027	1.708 ± 0.013	0.9935	3.13	–	–	–	–	–	–
Salbutamol	26	1.136 ± 0.069	1.403 ± 0.021	0.9854	2.86	11	2.903 ± 0.213	0.627 ± 0.062	0.9840	1.54	–
Salicylic acid	82	0.845 ± 0.209	1.235 ± 0.124	<0.900	3.35	23	0.847 ± 0.232	1.416 ± 0.128	<0.900	1.88	–
Sertraline	139	0.685 ± 0.072	2.051 ± 0.037	0.9572	3.58	1883	0.732 ± 0.111	2.898 ± 0.170	0.9347	3.79	–
Simvastatin	85	0.623 ± 0.089	1.816 ± 0.067	0.9416	3.37	1347	1.769 ± 0.119	4.223 ± 0.181	0.9821	3.64	–
Sulfamethazine	9	3.145 ± 0.710	0.562 ± 0.125	0.9351	2.39	15	1.206 ± 0.069	1.153 ± 0.020	0.9836	1.69	–
Sulfamethoxazole	8	1.110 ± 0.067	0.915 ± 0.056	0.9770	2.34	11	1.467 ± 0.183	0.941 ± 0.062	0.9697	1.54	**1.79** (Drillia *et al.*, 2005) **1.30–1.40** (Stein *et al.*, 2008) **2.06–3.47** (Carballa *et al.*, 2008)
Sulfapyridine	8	2.626 ± 0.141	0.376 ± 0.048	0.9886	2.34	–	–	–	–	–	–
Tamoxifen	1,626	0.666 ± 0.058	2.763 ± 0.091	0.9693	4.65	2533	1.265 ± 0.259	3.894 ± 0.446	<0.900	3.92	–
Temazepam	25	1.241 ± 0.056	1.349 ± 0.014	0.9878	2.84	–	–	–	–	–	**2.80–2.90** (Stein *et al.*, 2008)
Tramadol	22	1.035 ± 0.097	1.336 ± 0.026	0.9574	2.79	–	–	–	–	–	–
Triclocarban	438	0.496 ± 0.132	2.251 ± 0.129	<0.900	4.08	R	–	–	–	–	–
Triclosan	127	1.571 ± 1.202	2.440 ± 0.666	0.2991	3.54	R	–	–	–	–	–
Trimethoprim	26	1.152 ± 0.048	1.432 ± 0.013	0.9913	2.86	68	0.874 ± 0.051	1.811 ± 0.019	0.9799	2.35	–
Warfarin	8	2.428 ± 0.359	0.828 ± 0.096	0.9192	2.36	27	0.617 ± 0.051	1.434 ± 0.174	0.9671	1.95	–

[a] K_d values calculated as the mean of three C_w data points from low, medium and high concentrations within the range studied except in the case of nifedipine (medium C_W only).
[b] Values reported as the mean ± standard error in slope (n) and intercept (logK_f). Data points on isotherm plotted as the mean of triplicate batch experiments. R, retained completely by sludge and no K_d calculable.
EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; MDMA, 3,4-methylenedioxymethamphetamine.

3.1.2 Instrumentation

Liquid chromatography was performed on an Agilent HP1100 high-performance liquid chromatograph. Detection was carried out using a Bruker Daltonics Esquire LC ESI ion trap MS in single and tandem MS modes in positive or negative ionisation mode. All separations were carried out on a Waters Sunfire 3.5 µm, 150 × 2.1 mm analytical column at a flow rate of 0.2 ml/min. Mobile phases were 10:90 (A) and 80:20 (B) acetonitrile/10 mM ammonium acetate with a gradient profile as follows: 0–5 min was 100% A; 5–28 min acetonitrile raised to 45%; 28–35 min acetonitrile raised to 80% and kept constant for a further 10 min. Re-equilibration time was 15 min and injection volumes were 10 µl. Batch experiments were carried out in 25 ml glass vials with PTFE-lined caps and placed in a Heidolph Vibramax 100 platform shaker. All glassware was silanised prior to use and any losses due to further adsorption to glassware were noted and final results corrected. Particle size distribution was performed using a Malvern Mastersizer S (Malvern Instruments Ltd., Malvern, UK) instrument laser diffraction-based granulometer. For PLE of solids, a Dionex ASE-200 (Dionex Corporation, Sunnyvale, CA, USA) was employed using the described procedure in Chapter 2.

3.1.3 Molecular descriptors and ANN software

Generation of molecular descriptors was carried out using Parameter Client Software (Virtual Computational Chemistry Laboratory, Munich, Germany) (Tetko *et al.*, 2005). Descriptors were generated from canonical simplified molecular input line entry specifications, as well as literature reported log*P* and pK_a values. Those generated from Parameter Client software are abbreviated as follows: empirical formula (EF), molecular weight (MW), Moriguchi log*P* (Mlog*P*), Ghose–Crippen log*P* (Alog*P*), Ghose–Crippen molar refractivity (AMR), topological surface area (TPSA) with polar contributions from nitrogen, oxygen, phosphorus and sulfur atoms, unsaturation Index (UI), hydrophilic factor (HF), aromatic ratio (AR), the number of rotatable bonds (RB), the sum of van der Waals volumes based on the carbon atom (Σ VWV), the sum of atomic polarisabilities based on the carbon atom (Σ AP), the sum of Sanderson atomic electronegativities (Σ SAEN) based on the carbon atom, and the sum of the Kier–Hall electrotopological states (Σ KHES). For prediction of K_d, Trajan neural network software version 3.0 was used (Trajan Software Ltd, Durham, UK). A selection of the 37 inputs was investigated for a feed-forward, back-propagation-type neural network. Several additional input entries statistically derived from the empirical formula were also included, such as the total number of carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, fluorine and chlorine atoms, the number of multiple bonds (MB), double bonds (DB) and triple bonds (TB), the number of 3- to 7-membered rings (3-7 MR), the total number of rings, and the number of benzene-like rings (BR).

3.1.4 Sampling sites, pretreatment and procedures

Agricultural soil was taken from Corrstown, Co. Dublin, and was air-dried for 1 week, ground and sieved to a particle size of ≤100 µm. Digested sludge was sampled from a major WWTP in Dublin City (population equivalent 1.7 million), freeze dried and ground. Weights of 0.5 g soil or sludge were transferred to silanised vials (ESI) to which 20 ml of a spiked solution of PPCPs were added (125–1,000 µg/l in 10 mM $CaCl_2$, $n \geq 5$) in three sets (maximum total of ~0.3 mg of total PPCPs per flask) and shaken for 24 h to allow equilibration with both phases as recommended by OECD test guidelines and Drillia *et al.* (2005). The measured pH of both soil and sludge mixtures was 6.3 ± 0.1. The unsettled aqueous phases of each flask were centrifuged at 7,500 rev./min for 10 min. Triplicate aliquots of all supernatants were analysed by LC-MS/MS and the mean of each measurement used for plotted data. In parallel, three soil/sludge samples were shaken in 10 mM $CaCl_2$ only and exactly 1 ml of the unsettled aqueous portion centrifuged. For matrix-matched standards, two of these supernatant aliquots were dried at 37°C under nitrogen and reconstituted in 1 ml of a 1 mg/l standard of all analytes. The final solution was used as an analytical blank to correct for trace PPCPs present in the original samples. All analyses were run along with standards in ultra-pure water. Where quantification limits were reached, linearity was based on greater than five out of a total of eight concentrations studied.

3.1.5 Soil transport studies – procedures

A glass fibre filter paper was placed at the bottom end of a silanised glass column fitted with a stopcock tap. This column was filled with ~43 g of the agricultural soil studied in this chapter to a depth of 10 cm. The average spreading load range of biosolids has been quoted as ~3–11 t/ha. The diameter of the soil column was 2.5 cm. By applying exactly 1.5 g of biosolid to the top of the column, an intermediate biosolid load was maintained to emulate practical environmental conditions. Artificial rainfall (leaching solution) was prepared by dissolving the appropriate amount of $CaCl_2$ in ultra-pure water to give a final concentration of 10 mM. Approximately 20–30 ml of this solution were percolated through the amended soil column and leachates collected for preparation of matrix-matched standards. Following this, a weight of 10 µg of each of 12 selected PPCPs was spiked on top of the sludge. According to Met Éireann statistics, the average 6-monthly rainfall recorded at Dublin Airport for 2008 was ~400–500 mm (Met Éireann, 2009). Back-calculation of this figure leads to a volume of ~200–250 ml for this soil column. Leaching solution was subsequently added dropwise to the soil column to allow percolation of PPCPs for a total of 210 ml. Leachates were collected in ~5 ml intervals (volume was subsequently recorded) and run neat on LC-MS/MS. After the percolation study, the sludge was removed from the column and the soil column sectioned into eight discrete depths and stored in a silanised glass vial until analysis. The validated PLE, SPE, LC-MS/MS procedure for 27 PPCPs in Irish sludges/soils was used to analyse solids. A range of concentrations of the 12 PPCPs was added to soil and extracted simultaneously for quantitation.

3.2 Results and Discussion

3.2.1 Batch sorption experiments

Solid–water distribution coefficients, K_d, were determined by analysis of liquid phases after a 24 h equilibration time similar to that of Chefetz and co-workers (Chefetz *et al.*, 2008) and by correlating the logarithm of PPCP analytical concentrations in the subsequently determined solid (C_s) and aqueous phases (C_w) in µmol/kg and µmol/l, respectively, according to the Freundlich model (Eqn 3):

$$C_s = K_f . C_w^n \quad (3)$$

where K_f (in $\mu mol^{1-n} l^n/kg$) and n represent both the Freundlich affinity constant and exponent, respectively, calculated from the intercepts and slopes of generated isotherms. Particle size analysis of the agricultural soil indicated that it consisted of 20% clay, 69% silt and 11% sand, classifying it as a sandy mud. Lower concentration range limits studied here for all PPCPs lay slightly above the method quantification limits and reported residues in such media have shown to be present at comparable concentration ranges to some of those studied here (Beausse, 2004).

The majority of generated isotherms displayed correlation coefficients of $R^2 \geq 0.95$. Inspection of n obtained for some pharmaceuticals further suggested some degree of non-linearity, in particular in the cases of nifedipine, phenazone, sulfamethazine and papaverine. Exponents of $n >> 1$ may imply that the isotherm is upward convex, indicating that the presence of more pharmaceutical compound in the sorbent enhances the free energies of further sorption. The opposite may also be true for $n << 1$. This seemed particularly evident only in the case of nifedipine with all other compounds showing varying degrees of non-linearity based on random distribution. Therefore, data in Table 3.1 represent $K_d^{nifedipine}$ only for a single median C_w concentration of the range studied, even though K_d varied from ~1 to 11 l/kg across all concentrations. However, nifedipine sorption potential was lower in comparison with some other PPCPs. Where isotherm linearity was observed ($R^2 > 0.90$; $n \approx 1$), K_d was calculated from three selected C_w values at low, medium and high concentrations over the entire range studied.

Generally, soil–water experiments revealed that few compounds exhibited strong affinity for soil particles with $K_d^{soil} \leq 50$ l/kg for 33 out of the 49 selected compounds (see Table 3.1). On the other hand, some PPCPs showed apparent raised affinities, in particular, papaverine, doxazosin and tamoxifen. However, possible concurrent processes, such as chemical or biological transformation, cannot be ignored when evaluating these data. Such effects may alter the degree of persistence and/or concentration in soils, even over relatively short periods of time. In an attempt

to observe any such effects during the experimental determination of K_d, 48 h sorption and dissipation kinetics were investigated in soil–water suspensions. Although accurate mass balancing was not possible here for such a wide selection of chemically diverse compounds in these complex matrices, it was observed that when examining the trends in relative PPCP concentrations in liquid and solid phases per unit time that the majority of compounds reached equilibrium with the solid phase after 24 h. Some of the more heavily sorbed compounds (K_d > 100 l/kg) within extracted solid portions decayed somewhat after 24 h, possibly suggesting some transformation within the solid phase. Semi-quantitative studies indicated in particular that the sorbed quantities of heroin and morphine were markedly lower before 24 h in contrast to data obtained from liquid phase analysis. Therefore, K_d values determined from the aqueous phases were indeed exaggerated. Similar transformations of psychoactive drugs in solid phases were observed recently by Stein *et al*. (2008). In line with their work, morphine levels also decreased within liquid phases in our experiments and sorbed quantities were visibly lower than expected. Catalytic transformation of morphine in aqueous solutions under conditions of oxygen, air, sunlight, UV irradiation and inorganic impurities has been well documented (Vermeire and Remon, 1999), yielding products mainly of pseudo-morphine and morphine-*N*-oxide in a 9:1 ratio. No emergence of chromatographic peaks corresponding to these compounds (at *m/z* 573 and 301, respectively) was evident over t = 0–48 h in either matrix. Furthermore, as morphine is a metabolite of heroin, both of these compounds were omitted from further study to ensure the reliability of data generated and until such rapid transformation pathways are elucidated.

Exposure, and hence sorption, of pharmaceuticals to digested sludges is far less likely than that for activated sludges within the waste-water treatment process. Therefore for convenience, K_d was determined in digested sludge–water mixtures simply to investigate a matrix with a higher degree of organic matter than soil (measured total organic carbon (TOC) was 3.77% and 30.78% for soil and digested sludge, respectively) and to relate determined K_d values to any occurrence data reported previously in such matrices. Over the concentration range studied, triclosan and triclocarban were completely retained by the sludge and, as a result, K_d^{sludge} could not be calculated reliably. The apparent elevated sorption behaviour of these two PPCPs observed here is reflected in previous sorption studies (Ying *et al*., 2007), quantitative chemical analysis of digested sludges and biosolid-enriched soils (Barron *et al*., 2008) and studies of their release into aqueous run-offs within the soil environment (Topp *et al*., 2008). Carballa *et al*. investigated the sorption behaviour of some of the other selected pharmaceuticals studied here in raw sludge and showed that K_d data generated for carbamazepine (~35 l/kg [43 l/kg, this study]), naproxen (~11 l/kg [36 l/kg]), diclofenac (~66 l/kg [105 l/kg]) and sulfamethoxazole (~23 l/kg [11 l/kg]) were reasonably similar, even though the degree of sample variance would be significant (Carballa *et al*., 2008). Further comparisons of these PPCPs with the data from this study for sludge showed similarities, with $\log K_{oc} \approx 2–3$ in all cases.

To take into account all possible charged or neutral forms of compounds at pH 6.3 and by using associated pK_a and hydrophobicity data, the octanol–water distribution ratio, D_{ow}, was correlated with experimentally determined K_d values for both matrix types using only compounds showing higher degrees of linearity from Freundlich isotherms (i.e. $R^2 \geq 0.95$). In general, those compounds displaying a higher D_{ow} had higher K_d values and *vice versa*. However, no defined correlation was observed in either matrix, highlighting that D_{ow} alone was insufficient to model sorption behaviour to soil or sludge (Fig. 3.1). Indeed, as suggested earlier, sorption onto environmental matrices can depend on several hydrophobicity-independent variables. This was further apparent when examining K_{oc}. Compounds such as warfarin, clofibric acid and the sulfonamides showed considerable variance in K_{oc} between matrices (Table 3.1). Others showed good correlation, such as indomethacin, propranolol and amitriptyline. Comparison of measured K_{oc} values in both matrices lay within any literature-reported ranges for a small selection of PPCPs even with a relatively large variance in sample organic carbon content, highlighting that K_d data were not over-exaggerated in comparison with other studies. Taking all of this into

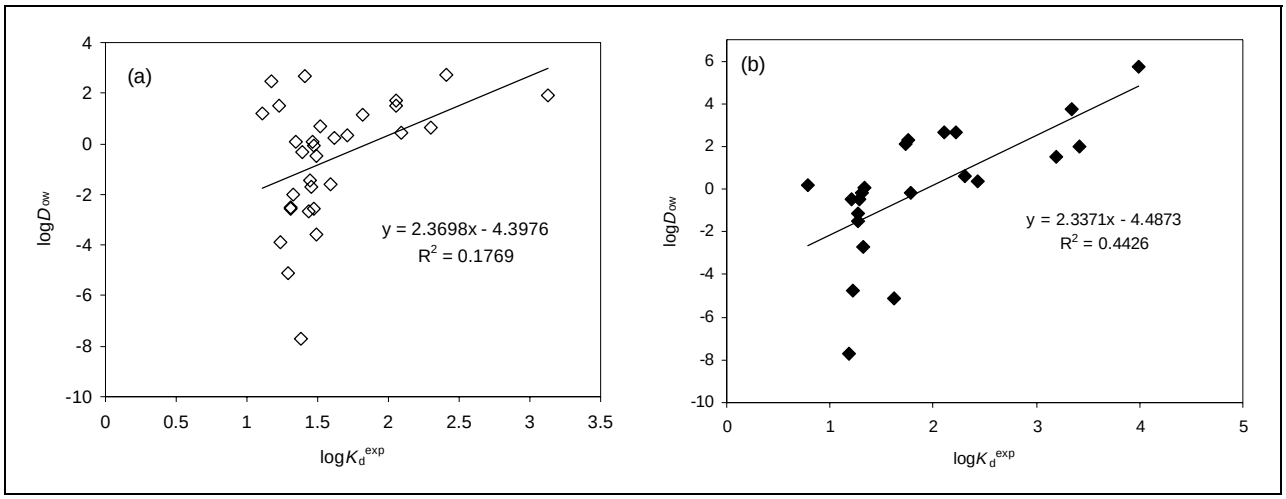

Figure 3.1. Correlation of logD_{ow} *versus* logK_d^{exp} using literature-reported log$P$ and pK_a in (a) soil and (b) sludge (at pH 6.3). Only experimentally generated K_d data from isotherms with $R^2 \geq 0.95$ were included in both correlations.

account, it is likely that highly complex sorption behaviour exists and, as a result, alternative modelling techniques were required to encompass a larger set of variables of unknown interdependence.

3.2.2 *Prediction of K_d using ANN computing*

Using the previously described molecular descriptors as inputs, an ANN was investigated. Optimisation of the network architecture was carried out for a network defined as (37, *N*, 1), where 37 is the number of inputs, *N*, the number of the nodes in the hidden layer and 1 is one output (moment = 0.6; learning rate = 0.3). It was found that the minimum of root-mean-square error was achieved for $N = 3$ or $N = 4$. Increasing the number of nodes up to $N = 8$ showed no significant improvement and therefore $N = 4$ was chosen as optimum. Several architectures with two hidden layers were examined and although satisfactory agreement was obtained during the training phase, the simplicity of a three-layer ANN was preferred. Using (37, 4, 1) and the number of training epochs at ~100,000, excellent correlation of predicted ($K_d^{predicted}$) *versus* experimentally determined distribution coefficients (K_d^{exp}) was possible for the majority of the compounds studied with correlation coefficients of $R^2 \geq 0.9997$ from both water–soil and water–sludge experiments (insets Fig. 3.2a and Fig. 3.2b, respectively). Upon examination of those compounds with $K_d^{exp} \leq 70$ l/kg, some degree of residual error existed, which might be expected at this level due to small reductions in aqueous phase concentrations. Using log scales to more equally represent all values of K_d, correlation coefficients decreased to $R^2 = 0.8866$ and $R^2 = 0.9367$ for soil–water and sludge–water experiments, respectively (Fig. 3.2), but, overall, K_d could be predicted quite accurately with slopes close to 1 in both cases. In the case of salicylic acid and papaverine, where linearities and detection limits were poor, the ANN showed the most absolute variance in accuracy from the predicted value. Residual errors in water–soil revealed that K_d could be predicted for 39 out of 49 analytes within a 10 l/kg proximity to the experimentally determined value (Fig. 3.3). Hence, this network was most applicable to the prediction of K_d for those compounds that may have increased potential to concentrate in this soil type.

3.2.3 *Individual molecular descriptor contributions to $K_d^{predicted}$ in soil*

Using the optimal architecture, the training data set for water–soil was fed back into the neural network. In a somewhat similar approach to that described by Zheng *et al.* (2006), the fractional contribution (Eqn 4) of each molecular descriptor to the overall variance, V_x, from $K_d^{predicted}$ was determined for a single compound, *x*. This was achieved using the absolute deviation, D_x, observed by systematically removing one input category (or molecular descriptor), *i*, from the network.

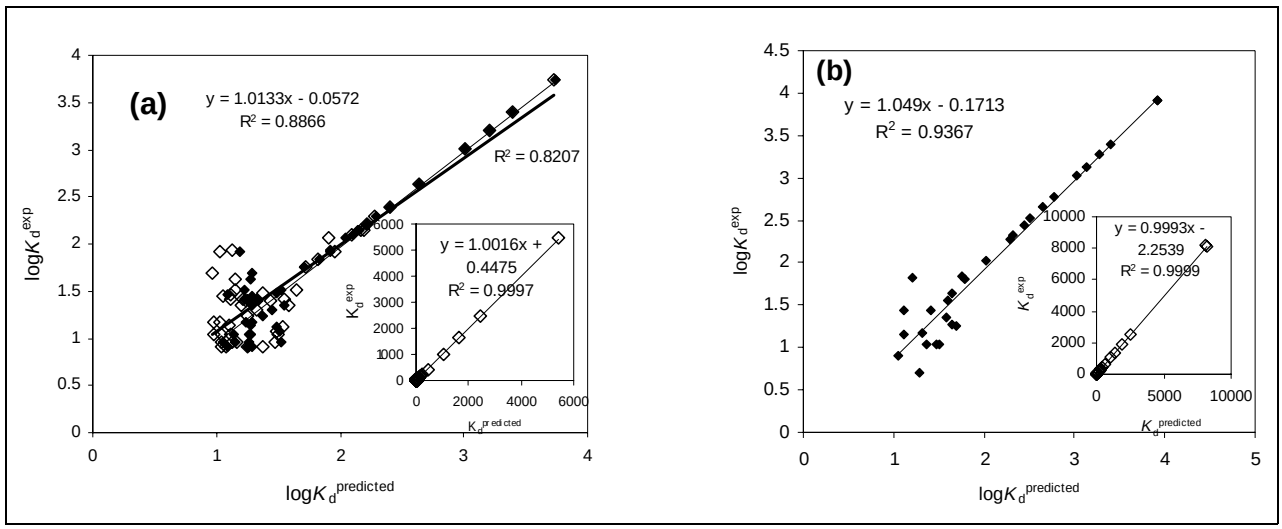

Figure 3.2. Comparison of logK_d^{exp} and log$K_d^{predicted}$ using the optimised tri-layer neural network for (a) water–soil, and (b) water–sludge experiments. Insets: K_d^{exp} *versus* $K_d^{predicted}$ showing improved correlation at high values of K_d. Data point markers: (♦) prediction inclusive of experimentally determined log*P* and pK_a; (◊) prediction using only quantitative structure–activity relationship generated molecular descriptors.

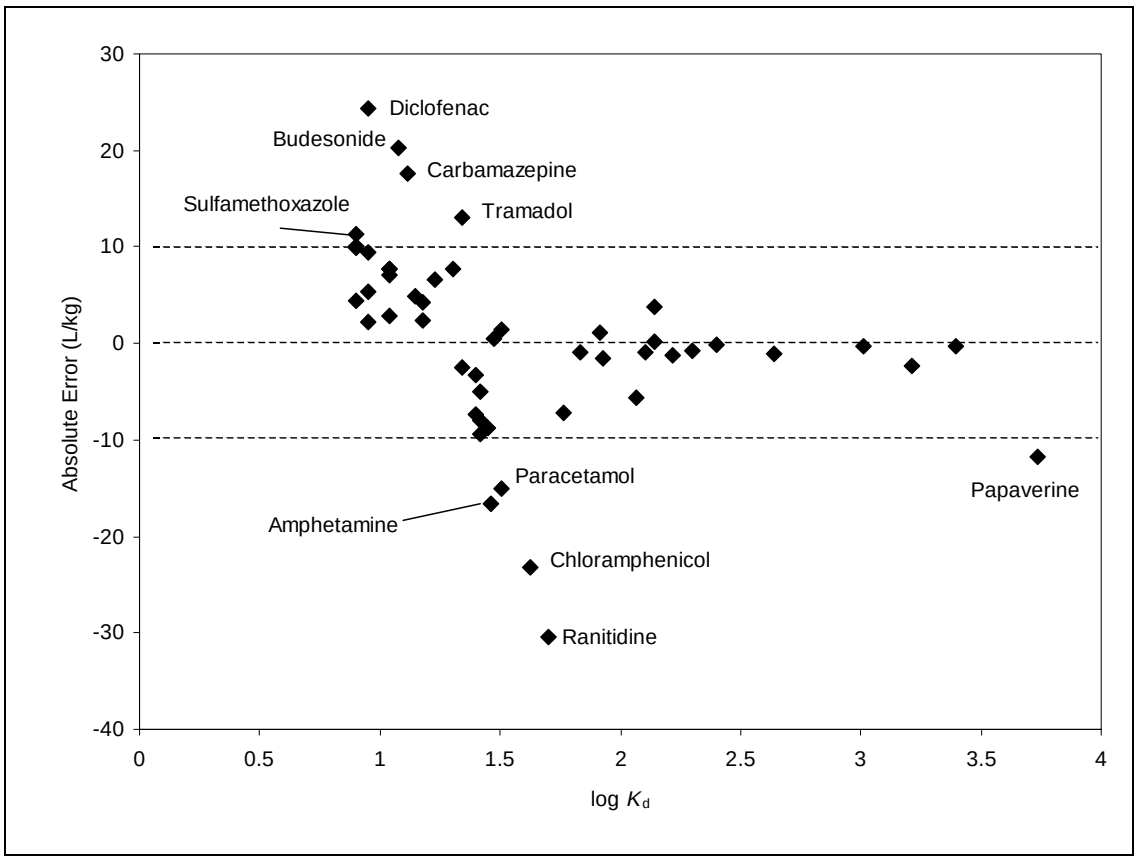

Figure 3.3. Residual error of $K_d^{predicted}$ using neural networks in order of increasing K_d^{exp} (left to right). Error for salicylic acid omitted here for clarity (error = 82 l/kg).

It was then possible to describe the overall precision of the network (% $\Delta K_d^{predicted}$) for all compounds and all molecular descriptors as in Eqn 5.

$$V_x = \frac{(D_x)_i}{\sum_{i=1-37}(D_x)_i} \quad (4)$$

$$\Delta K_d^{predicted}(\%) = \frac{100 \cdot V_x}{\sum_{x=1-51} V_x} \quad (5)$$

It was found in general that $K_d^{predicted}$ for each compound depended on each descriptor to different degrees as can be seen in Fig. 3.4. Mlog*P* contributed most to $K_d^{predicted}$ when omitted from the network with ~11% deviation. This statistically derived quantity uses 13 algorithm input parameters including the sum of hydrophobic atoms, sum of hydrophilic atoms, proximity effects, unsaturated bonds, amphoteric properties as well as the presence of a quaternary nitrogen and the number of nitro groups to calculate a theoretical log*P* based on 1,230 test compounds. The order of contribution of the next four molecular descriptors (highest to lowest) was pK_a, the number of sulfur atoms (S), the number of double bonds (DB), and the hydrophilic factor (HF) with a loss of $K_d^{predicted}$ precision of ~6–9% when omitted from the network. It should be noted however, that molecular descriptors in ANN are merely numerical inputs in a complex algorithm to achieve the lowest error in $K_d^{predicted}$ and may not necessarily highlight preferred sorption mechanisms within the environment. Upon investigation of the removal of both sets of experimentally determined descriptors, it was found that although some correlation was still observed (R^2 = 0.8207), generally there existed a slight trend towards a reduction of confidence in $K_d^{predicted}$ and indicated that predicted values using only quantitative structure–activity relationship (QSAR)-based descriptors could not be relied on completely in this instance (Fig. 3.2a). For descriptors of molecules containing 3 MR and 4 MR, of which there were none, there was no change in $K_d^{predicted}$ indicating that the network was not influenced by superfluous null or missing input data. Overlaid in Fig. 3.4 is the percentage $\Delta K_d^{predicted}$ relative to K_d^{exp}, which shows a more even distribution of all descriptors in

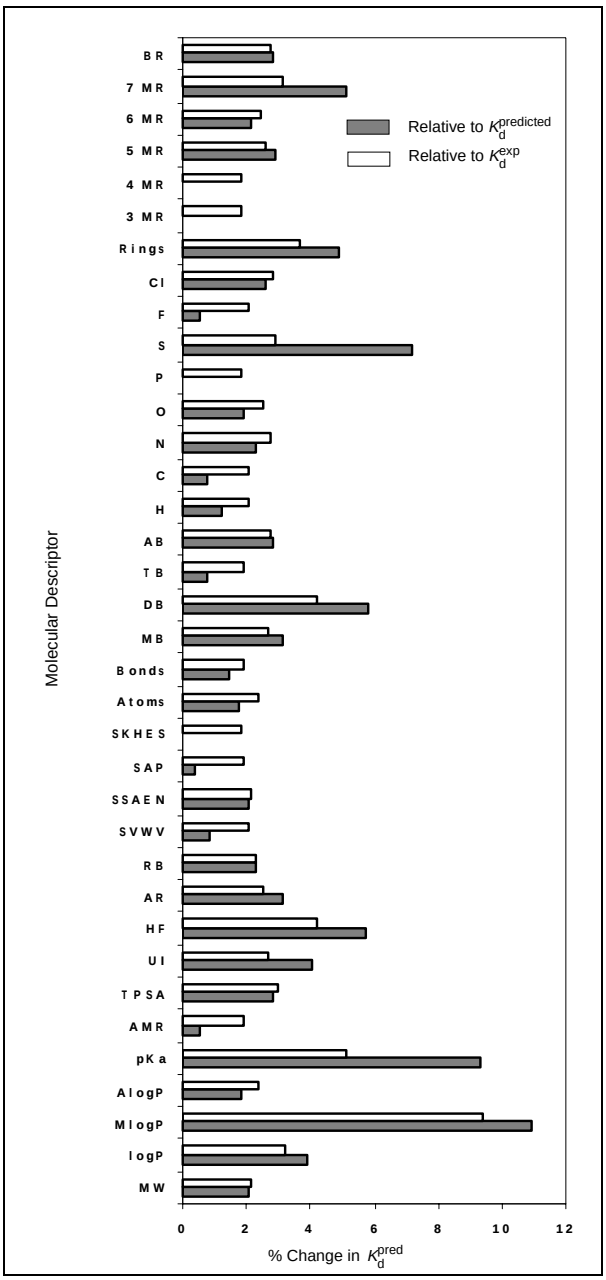

Figure 3.4. Overall percentage $\Delta K_d^{predicted}$ relative to $K_d^{predicted}$ for removal of one molecular descriptor overlaid with error relative to K_d^{exp}. Abbreviations as defined in text.

contribution to accuracy, obviously arising from the deviation of $K_d^{predicted}$ from K_d^{exp}. Similarities were still evident in the fact that Mlog*P* was the largest contributor.

It was also observed that predictions for some molecules were affected more than others with removal of specific molecular descriptor data. For

instance, the antidepressants amitriptyline and nortriptyline, which are structurally very similar, recorded ~30 and 57% changes in $K_d^{predicted}$, respectively, with the removal of MlogP (MlogP for both ≥4.53), whereas the beta blockers atenolol and propranolol were left relatively unchanged (≤2%, MlogP ≤ 2.53). Similarly, the antibacterial agents triclocarban and triclosan relied heavily both on literature-reported pK_a and on MlogP out of all descriptors. The contribution to $\Delta K_d^{predicted}$ between all compounds for the removal of logP, MlogP, pK_a, HF and DB from the optimised network is shown in Fig. 3.5.

3.2.4 *Prediction of K_d for additional pharmaceuticals*

For validation purposes, the network was applied to predictions of K_d for four additional pharmaceutical compounds in soil, which had previously not been included in the training set. Literature-reported data for furosemide, ibuprofen, mefenamic acid (Bones *et al.*, 2006b) and meclofenamic acid (Pedraza *et al.*, 1983; Ruckebusch and Toutain, 1983) were log*P* = 2.0, 4.0, 4.2 and 5.9, and and pK_a = 3.9, 4.2, 4.0 and 3.7, respectively. Sorption isotherms were constructed for $n \geq 5$ data points and revealed K_d^{exp} of 109, 17, 21 and 27 l/kg. Upon application of the ANN, $K_d^{predicted}$ values of 109, 17, 12 and 17 l/kg for meclofenamic acid, mefenamic acid, ibuprofen and furosemide were obtained, with ratios of experimental/predicted K_d values equal to 1.00, 1.00, 1.75 and 1.65, respectively, an average of 1.35 for the four structurally diverse compounds, and were all within 10 l/kg of the experimentally determined value.

3.2.5 *Transport of 12 PPCPs in biosolid-enriched soils*

The main aim of this project was to assess the potential threat of PPCPs to the soil environment. The term 'threat' can have many different interpretations. Firstly, it was important to ascertain by chemical analysis which PPCPs were present in biosolids to begin with. As these biosolids may be spread on land either as landfill, or as an agricultural fertiliser, the main 'threat' therefore lies within the retention of such PPCPs within the soil compartment where they may have an ecotoxicological effect. Complex cocktails of PPCPs can have altered modes of action, which may augment or diminish their single exposure efficacy (Quinn *et al.*, 2008a,b, 2009). Therefore, if antibiotics, for instance,

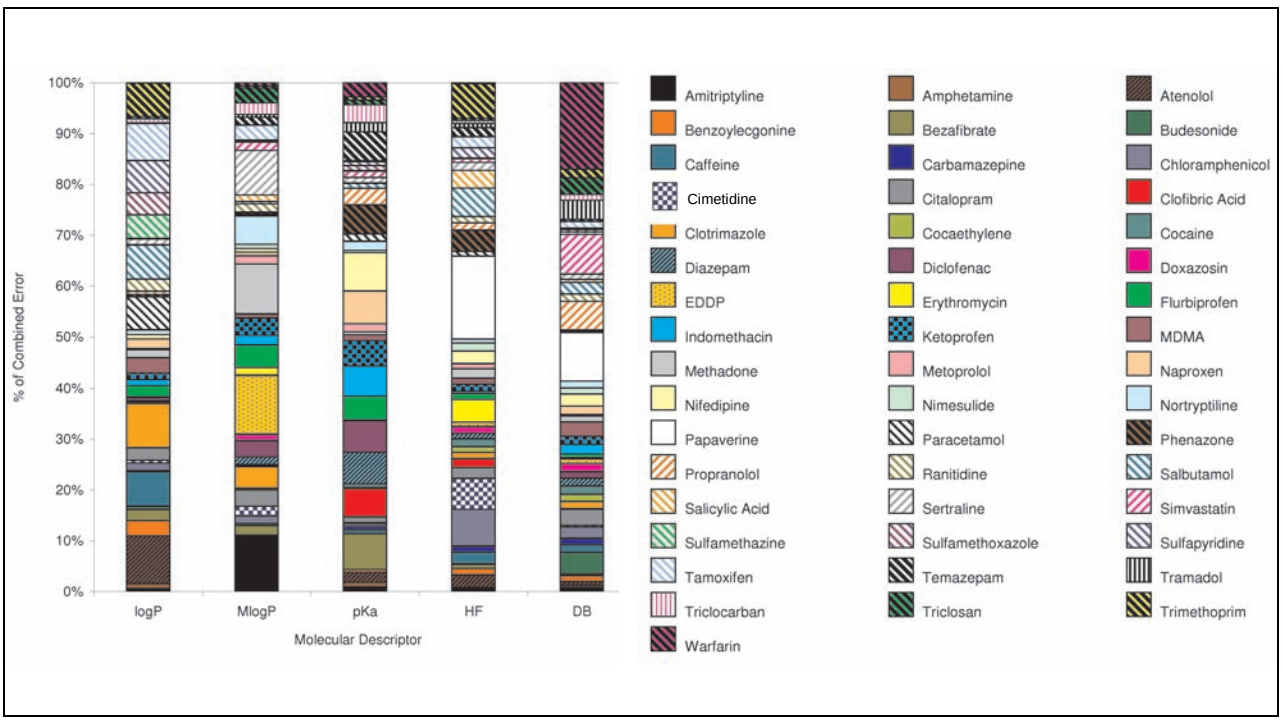

Figure 3.5. Contribution of each molecule to percentage error for the top five selected $K_d^{predicted}$-dependent descriptors. Compounds listed alphabetically from bottom to top for clarity.

were present and stable in the soil for sufficient periods of time after application of contaminated biosolids, the potential (in the presence of other drugs) for pathogens to become resistant may increase. Naturally, within the agricultural sector and in a worst-case scenario, such resistant food-borne pathogens (such as *Clostridium botulinum* and *Listeria monocytogenes*) could have a disastrous effect on the human population. Therefore, the potential threat may not be directly to humans, but may manifest itself as a result of several intermediate knock-on effects.

It was observed, as with sorption studies, that some PPCPs had a stronger affinity for the soil matrix than others. For example, sulfamethoxazole was among the first to elute from the soil column. In line with partitioning data, is the elution of salbutamol ($\log P = 0.11$; $K_d = 26$ l/kg) after sulfamethoxazole ($\log P = 0.89$; $K_d = 8$ l/kg). This further highlights the complexity of the sorption/desorption process, which in this case did not rely solely on hydrophobic interactions alone. Similarly, carbamazepine displayed a lower K_d (13 l/kg) than ketoprofen or bezafibrate, but only began to elute at high volumes of leaching solution. Therefore, in assessing transport behaviour, it is important to bear in mind K_d within sludge. Carbamazepine displayed the highest K_d of all compounds in sludges (43 l/kg) and its relatively high transport potential through soil was obviously hindered by its affinity for the biosolid layer. By examining the cumulative weight of PPCP leached over the course of the percolation test, it can be observed that only sulfamethoxazole was completely eluted from the soil column after an accelerated and substantial simulated rain event. All elution profiles are shown in Fig. 3.6.

It is extremely important to note that, in this case, the percolation test lasted approximately 14 days, considerably shorter than a 6-month time frame for this level of rainfall. Therefore, other factors may emerge to alter the persistence of these compounds such as biological or chemical transformation. In an attempt to quantify the occurrence of PPCP in all compartments, the soil and sludge layers were extracted using the described procedure. As can be observed from Fig. 3.7, those compounds not entirely eluted in leachates were observed in varying concentrations per unit depth. For example, propranolol and metoprolol ($K_d^{soil} = 58$ and 20 l/kg and $K_d^{sludge} = 331$ and 18 l/kg, respectively) were retained as expected towards the top of the soil column, with propranolol remaining to a large degree within the sludge layer. Similarly, trimethoprim (bacteriostatic antibiotic; $K_d = 68$ and 26 l/kg for sludge and soil, respectively) was retained

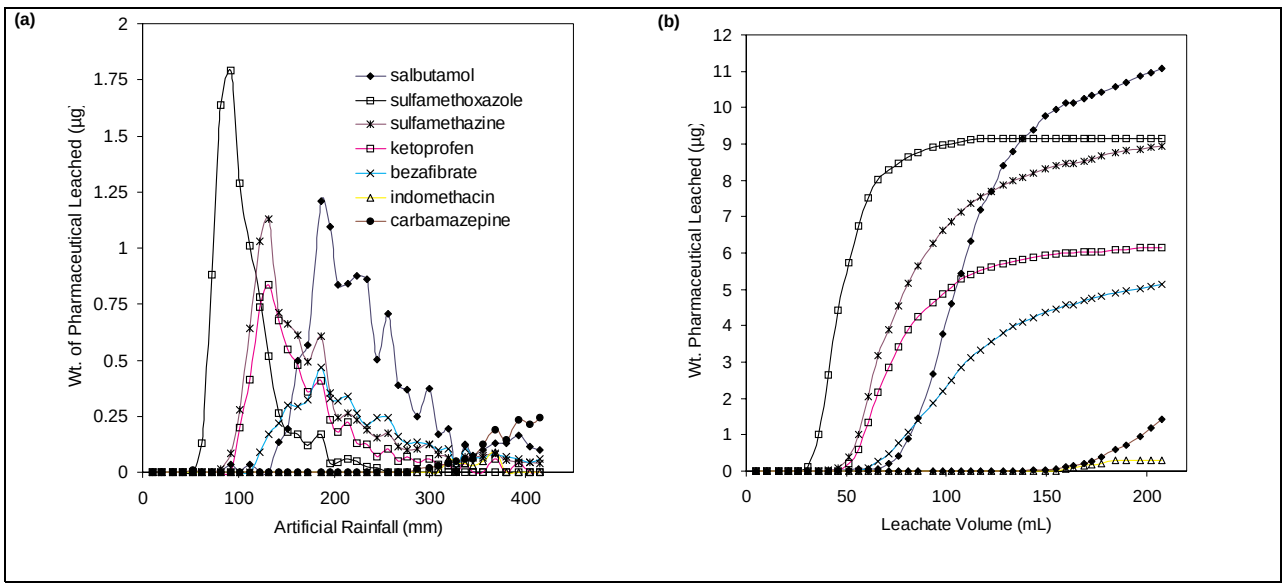

Figure 3.6. Elution of 12 pharmaceutical and personal care products (PPCPs) from a 10 cm sludge-amended soil column represented as (a) concentration within leachate fraction, and (b) cumulative mass of PPCP eluted.

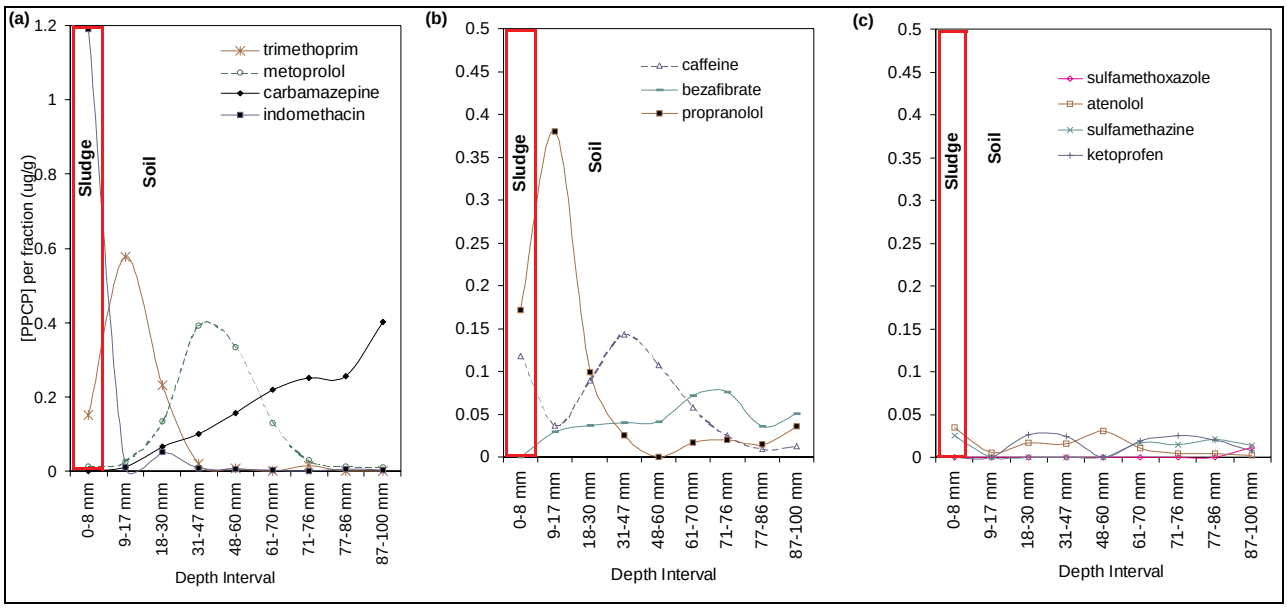

Figure 3.7. Pharmaceutical and personal care product (PPCP) concentrations per unit depth after leaching with ~200 ml of artificial rainfall. Analysis by (a) pressurised liquid extraction, (b) solid-phase extraction, and (c) liquid chromatography–tandem mass spectrometry.

somewhat in the sludge layer and was not present in leachates. The most interesting profile is that of carbamazepine, which displayed a broad concentration gradient over the entire depth range studied. This may further support the hypothesis that retention was hindered (and hence zones broadened) somewhat by high affinity for the sludge layer. By combining all of the above concentrations per unit depth with those found in leachates, a mass balance was constructed (Fig. 3.8). Most interestingly, only

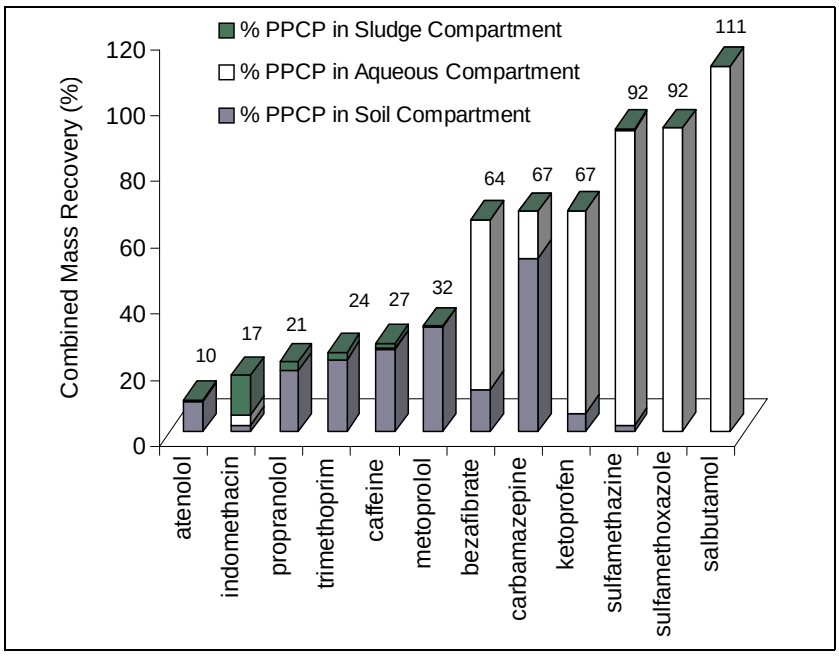

Figure 3.8. Mass balance of 12 pharmaceuticals and personal care products (PPCPs) in soil, sludge and leachate after the percolation experiment.

three out of 12 analytes could be recovered fully from the soil system. The others showed significant levels of possible degradation, either due to chemical or biological means. Particularly evident was the loss of compounds that showed a high affinity for solid media such as metoprolol, carbamazepine and propranolol. Others may have been subject to losses due to the insufficient analytical method recoveries (such as atenolol; see Table 2.3).

3.3 Conclusions

It is evident that PPCP sorption and mobility behaviour in environmental matrices is especially complex and those correlations using only hydrophobic interactions may be insufficient. Moreover, considerable further work is required to establish any possible chemical or biological transformation pathways for these compounds in both solid and aqueous matrices to determine sorption behaviour and persistence more accurately. That said, the benefits of computational methods such as artificial neural networks in modelling such data are clear, especially for PPCPs showing indications of elevated sorption behaviour. Therefore, such modelling techniques could provide useful information to those investigating the potential risks associated with concentration of pharmaceuticals in soil with a lesser dependence on expensive chemical analysis.

3.4 Reprints from Publications

A large proportion of this chapter has been reprinted with permission from The Royal Society of Chemistry from the article by Barron *et al.* (2009) in *Analyst*.

4 Project Conclusions

It is clear from the results generated during this project that PPCPs are indeed present in the solid environment and that their ecotoxicological effects need to be substantially further addressed. Principally, the main conclusion is that, depending on molecular affinity for the solid compartments, certain PPCPs may persist beyond the waste-water treatment process and may even concentrate in biosolids. Therefore, a new set of selection criteria for these PPCPs is critical in assessing any risk to the environment and for implementation of any monitoring strategies. Certainly, sales and usage data may assist in identification of those compounds warranting further study, but sales and occurrence data are obviously not proportionally related. Obviously, if a PPCP is not consumed in a country, then it could be considered of minimal or no risk by process of elimination. That said, compounds that are consumed in lesser yearly amounts may still display concentration ability in solids over those products sold in considerably higher quantities. Therefore, an evaluation of K_d for all PPCPs sold in Ireland is indeed desirable, however impractical.

Chemical analysis in this area still proves to be very challenging, primarily due to the complexity of the sample matrices involved. In particular, ion suppression effects in mass spectrometry are observed and this further affects the reliable quantitation of PPCPs due to the inherent inhomogeneity of solid samples. Radio-labelled standards can be used to circumvent this problem, but need to be used at significantly higher quantities to address the problem adequately. This can add considerable cost to the analysis, coupled with their extremely limited availability. The use of new separation technologies such as UHPLC can somewhat tackle this problem by providing better resolution from interfering compounds. However, it has been shown here that, arguably, a more cost-effective and practical method to separate multi-component mixtures in complex samples may lie in the further development of long LC columns based on monolithic chemistries. Finally, the most inhibiting factor in the development of chemical analysis methods for trace PPCPs in soils and sludges is the performance of 'broad-stroke' extraction techniques suitable for large analyte mixtures with high degrees of chemical diversity. This can certainly be done by developing several separate extraction methods coupled to LC-MS/MS (using different PLE solvents and/or SPE sorbents), but with considerable cost with respect to analysis time and complexity. For optimal performance, each extraction method must be tailored for compounds with similar physico–chemical properties (mostly depending on molecular/sorbent pK_a and log$P$). Approaches in this project to mixed-mode SPE extraction proved difficult and the recovery gain was minimal for the considerable extra effort involved. It seems that the best option is again to target those analytes that may concentrate in solids and focus the analysis on these only.

The assessment of PPCP affinity for soils revealed that those compounds showing high K_d values should be considered more as a possible threat to the soil environment. This must be coupled with in-depth persistence studies for these compounds to assess their transport and stability in the treatment process. Of particular note is the concentration ability of some antimicrobial agents such as triclosan and triclocarban in soils and sludges. Triclosan was found in several cases to be present after treatment at the 20–30 mg/kg level. Other compounds of interest are the antidepressants sertraline and citalopram, again showing persistence and concentration ability. That said, those compounds showing lower K_d values (<20 l/kg) have been reported as occurring more frequently in waste-water effluents in the Republic of Ireland (Lacey *et al.*, 2008).

Soil percolation tests revealed that K_d data were further supported, with those compounds expressing low partition coefficients tending to be present more in leachates than being retained in the soil compartment. Examples of this were the sulfonamides, which are of particular interest to the soil environment given their use as veterinary antibiotics. Also, the higher affinity of

some compounds for sludges retarded their elution significantly (e.g. carbamazepine). Overall, significant losses in recoveries were observed for the majority of compounds showing long residence times in the soil column. It could be concluded that some compounds, despite their affinity for solid media, were chemically or biologically transformed in soils and therefore reduced their apparent persistence. This needs substantial further study despite their apparent degradation. For example, identification of any pathogens involved in the degradation process may highlight increased resistance to antibiotics as a result.

Overall, this work has highlighted that emerging 'novel' pollutants such as PPCPs warrant much further study, particularly with relation to increased or decreased efficacy in complex mixtures. Chemical analysis still constitutes the only unambiguous means to measure bioavailability of such compounds in the environment. There still is no concrete conclusion as to the threat of pharmaceuticals to the environment, but they are present and more focus should be certainly placed on the analysis of solid materials.

5 References

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Acronyms

3-7 MR	The number of 3- to 7-membered rings
Alog*P*	Ghose–Crippen log*P*
AMR	Ghose–Crippen molar refractivity
ANN	Artificial neural network
AP	The sum of atomic polarisabilities based on the carbon atom
API	Active pharmaceutical ingredient
AR	Aromatic ratio
A_s	Peak asymmetry
BOD	Biochemical oxygen demand
BP	Backpressure
BR	The number of benzene-like rings
C_s	Concentration in the solid phase
C_w	Concentration in the aqueous phase
DB	The number of double bonds
DBP	Dibutylphthalate
DCU	Dublin City University
DEHP	Diethylhexylphthalate
$\Delta K_d^{predicted}$	The change in predicted solid–water partition coefficient
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EDTA	Ethylenediaminetetraacetic acid
EF	Empirical formula
EIC	Extracted ion chromatogram
ESI	Electrospray ionisation
EtOAc	Ethyl acetate
EU	European Union
GC	Gas chromatography
HETP	Height equivalent to a theoretical plate
HF	Hydrophilic factor
HLB	Hydrophilic–lipophilic balanced
HPLC	High-performance liquid chromatography
IDA	Industrial Development Agency
IMB	Irish Medicines Board
K_d	Solid–water partition coefficient

K_f	Freundlich affinity constant
KHES	The sum of Kier–Hall electrotopological states
K_{oc}	Organic-carbon normalised solid–water partition coefficient
K_{ow}	Octanol–water partition coefficient
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantitation
LSD	Lysergic acid diethylamide
m/z	Mass-to-charge ratio
MB	The number of multiple bonds
MBR	Membrane bioreactor technology
MDMA	3,4-methylenedioxymethamphetamine
MeCN	acetonitrile
MlogP	Moriguchi log*P*
MP	Mobile phase
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry (also MS^2)
MW	Molecular weight
N	Chapter 2: number of theoretical plates
N	Chapter 3: number of nodes in the hidden layer
NH_4OAc	Ammonium acetate
NIVA	Norwegian Institute for Water Research
NSAID	Non-steroidal anti-inflammatory drug
OSPAR	The Oslo–Paris Convention
PBT	Persistence, bioaccumulation, toxicity
P_c	Peak capacity
PE	Population equivalent
PEC	Predicted environmental concentration
PLE	Pressurised liquid extraction
PNEC	Predicted no-effect concentration
PPCPs	Pharmaceuticals and personal care products
PTFE	Polytetrafluoroethylene
RB	The number of rotatable bonds
RSD	Relative standard deviation
SAEN	The sum of Sanderson atomic electronegativities
SIM	Single ion monitoring

SPE	Solid-phase extraction
SRM	Selected reaction monitoring
SRT	Solids retention time
SS	Suspended solids
t_0	Column void time
TB	The number of triple bonds
TOC	Total organic carbon
TPSA	Topological surface area (with polar contributions from N, O, P and S atoms)
UHPLC	Ultra-high pressure liquid chromatography
UI	Unsaturation index
UK	United Kingdom
USE	Ultrasonication extraction
UV	Ultraviolet
VWV	The sum of van der Waals volumes based on the carbon atom
W	Peak width at baseline
$W_{1/2}$	Peak width at half height
WWTP	Waste-water treatment plant

An Ghníomhaireacht um Chaomhnú Comhshaoil

Is í an Gníomhaireacht um Chaomhnú Comhshaoil (EPA) comhlachta reachtúil a chosnaíonn an comhshaol do mhuintir na tíre go léir. Rialaímid agus déanaimid maoirsiú ar ghníomhaíochtaí a d'fhéadfadh truailliú a chruthú murach sin. Cinntímid go bhfuil eolas cruinn ann ar threochtaí comhshaoil ionas go nglactar aon chéim is gá. Is iad na príomh-nithe a bhfuilimid gníomhach leo ná comhshaol na hÉireann a chosaint agus cinntiú go bhfuil forbairt inbhuanaithe.

Is comhlacht poiblí neamhspleách í an Ghníomhaireacht um Chaomhnú Comhshaoil (EPA) a bunaíodh i mí Iúil 1993 faoin Acht fán nGníomhaireacht um Chaomhnú Comhshaoil 1992. Ó thaobh an Rialtais, is í an Roinn Comhshaoil agus Rialtais Áitiúil a dhéanann urraíocht uirthi.

ÁR bhFREAGRACHTAÍ

CEADÚNÚ

Bíonn ceadúnais á n-eisiúint againn i gcomhair na nithe seo a leanas chun a chinntiú nach mbíonn astuithe uathu ag cur sláinte an phobail ná an comhshaol i mbaol:

- áiseanna dramhaíola (m.sh., líonadh talún, loisceoirí, stáisiúin aistrithe dramhaíola);
- gníomhaíochtaí tionsclaíocha ar scála mór (m.sh., déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta);
- diantalmhaíocht;
- úsáid faoi shrian agus scaoileadh smachtaithe Orgánach Géinathraithe (GMO);
- mór-áiseanna stórais peitreail.
- Scardadh dramhuisce

FEIDHMIÚ COMHSHAOIL NÁISIÚNTA

- Stiúradh os cionn 2,000 iniúchadh agus cigireacht de áiseanna a fuair ceadúnas ón nGníomhaireacht gach bliain.
- Maoirsiú freagrachtaí cosanta comhshaoil údarás áitiúla thar sé earnáil - aer, fuaim, dramhaíl, dramhuisce agus caighdeán uisce.
- Obair le húdaráis áitiúla agus leis na Gardaí chun stop a chur le gníomhaíocht mhídhleathach dramhaíola trí comhordú a dhéanamh ar líonra forfheidhmithe náisiúnta, díriú isteach ar chiontóirí, stiúradh fiosrúcháin agus maoirsiú leigheas na bhfadhbanna.
- An dlí a chur orthu siúd a bhriseann dlí comhshaoil agus a dhéanann dochar don chomhshaol mar thoradh ar a ngníomhaíochtaí.

MONATÓIREACHT, ANAILÍS AGUS TUAIRISCIÚ AR AN GCOMHSHAOL

- Monatóireacht ar chaighdeán aeir agus caighdeáin aibhneacha, locha, uiscí taoide agus uiscí talaimh; leibhéil agus sruth aibhneacha a thomhas.
- Tuairisciú neamhspleách chun cabhrú le rialtais náisiúnta agus áitiúla cinntí a dhéanamh.

RIALÚ ASTUITHE GÁIS CEAPTHA TEASA NA HÉIREANN

- Cainníochtú astuithe gáis ceaptha teasa na hÉireann i gcomhthéacs ár dtiomantas Kyoto.
- Cur i bhfeidhm na Treorach um Thrádáil Astuithe, a bhfuil baint aige le hos cionn 100 cuideachta atá ina mór-ghineadóirí dé-ocsaíd charbóin in Éirinn.

TAIGHDE AGUS FORBAIRT COMHSHAOIL

- Taighde ar shaincheisteanna comhshaoil a chomhordú (cosúil le caighdéan aeir agus uisce, athrú aeráide, bithéagsúlacht, teicneolaíochtaí comhshaoil).

MEASÚNÚ STRAITÉISEACH COMHSHAOIL

- Ag déanamh measúnú ar thionchar phleananna agus chláracha ar chomhshaol na hÉireann (cosúil le pleananna bainistíochta dramhaíola agus forbartha).

PLEANÁIL, OIDEACHAS AGUS TREOIR CHOMHSHAOIL

- Treoir a thabhairt don phobal agus do thionscal ar cheisteanna comhshaoil éagsúla (m.sh., iarratais ar cheadúnais, seachaint dramhaíola agus rialacháin chomhshaoil).
- Eolas níos fearr ar an gcomhshaol a scaipeadh (trí cláracha teilifíse comhshaoil agus pacáistí acmhainne do bhunscoileanna agus do mheánscoileanna).

BAINISTÍOCHT DRAMHAÍOLA FHORGHNÍOMHACH

- Cur chun cinn seachaint agus laghdú dramhaíola trí chomhordú An Chláir Náisiúnta um Chosc Dramhaíola, lena n-áirítear cur i bhfeidhm na dTionscnamh Freagrachta Táirgeoirí.
- Cur i bhfeidhm Rialachán ar nós na treoracha maidir le Trealamh Leictreach agus Leictreonach Caite agus le Srianadh Substaintí Guaiseacha agus substaintí a dhéanann ídiú ar an gcrios ózóin.
- Plean Náisiúnta Bainistíochta um Dramhaíl Ghuaiseach a fhorbairt chun dramhaíl ghuaiseach a sheachaint agus a bhainistiú.

STRUCHTÚR NA GNÍOMHAIREACHTA

Bunaíodh an Ghníomhaireacht i 1993 chun comhshaol na hÉireann a chosaint. Tá an eagraíocht á bhainistiú ag Bord lánaimseartha, ar a bhfuil Príomhstiúrthóir agus ceithre Stiúrthóir.

Tá obair na Gníomhaireachta ar siúl trí ceithre Oifig:

- An Oifig Aeráide, Ceadúnaithe agus Úsáide Acmhainní
- An Oifig um Fhorfheidhmiúchán Comhshaoil
- An Oifig um Measúnacht Comhshaoil
- An Oifig Cumarsáide agus Seirbhísí Corparáide

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag ball air agus tagann siad le chéile cúpla uair in aghaidh na bliana le plé a dhéanamh ar cheisteanna ar ábhar imní iad agus le comhairle a thabhairt don Bhord.

NDP
National Development Plan 2007 - 2013

Science, Technology, Research and Innovation for the Environment (STRIVE) 2007-2013

The Science, Technology, Research and Innovation for the Environment (STRIVE) programme covers the period 2007 to 2013.

The programme comprises three key measures: Sustainable Development, Cleaner Production and Environmental Technologies, and A Healthy Environment; together with two supporting measures: EPA Environmental Research Centre (ERC) and Capacity & Capability Building. The seven principal thematic areas for the programme are Climate Change; Waste, Resource Management and Chemicals; Water Quality and the Aquatic Environment; Air Quality, Atmospheric Deposition and Noise; Impacts on Biodiversity; Soils and Land-use; and Socio-economic Considerations. In addition, other emerging issues will be addressed as the need arises.

The funding for the programme (approximately €100 million) comes from the Environmental Research Sub-Programme of the National Development Plan (NDP), the Inter-Departmental Committee for the Strategy for Science, Technology and Innovation (IDC-SSTI); and EPA core funding and co-funding by economic sectors.

The EPA has a statutory role to co-ordinate environmental research in Ireland and is organising and administering the STRIVE programme on behalf of the Department of the Environment, Heritage and Local Government.

epa
Environmental Protection Agency
An Ghníomhaireacht um Chaomhnú Comhshaoil

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SSTI
Strategy for Science, Technology and Innovation

Comhshaol, Oidhreacht agus Rialtas Áitiúil
Environment, Heritage and Local Government