Assessment of pharmaceutical residue levels in three Irish sewage treatment plants

Ph.D. Research Thesis by

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Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Ph.D. is entirely my own work, that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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Abstract

Pharmaceutical and metabolite residues have been reported in European aquatic matrices since the 1980s. Discharges from municipal sewage treatment facilities have been identified as the primary source of these residues in the environment. Reported removal rates from wastewater treatment plants are low and residues are found to be persistent in the environment.

The extent of pharmaceutical pollution in Irish waters is currently unknown. Therefore, the aims of this work were to develop an LC-MS/MS method for the simultaneous detection and identification of twenty pharmaceutical compounds commonly used in Ireland and to establish their influent and effluent concentrations in three wastewater treatment plants (WWTP) in the greater Dublin region. Results of a twelve month sampling programme, from the three plants, were then used to determine any seasonal variability in the occurrence of pharmaceutical contamination.

A combined SPE-LC-MS/MS method using Strata-X cartridges for sample preconcentration was developed to investigate the occurrence of the twenty pharmaceuticals in WWTP streams. Analytical separation was achieved using a reversed phase Sunfire column with gradient elution. Fourteen of the twenty analytes investigated were found in the wastewater treatment plants. Concentrations determined in the effluent streams were in the low μg/L range and consistent with those reported in previous studies. These concentrations are below known toxicity levels however the cumulative effect of discharged residues may impart a negative ecotoxicological effect. No correlation between flowrate, BOD, COD or suspended solids and WWTP effluent concentration was observed for the analytes. Seasonal variation of effluent concentration was investigated with respect to rainfall, temperature and sunlight hours. Again, no relationship was identified. Correlations were difficult to determine due to suppression of the analyte ions in influent samples and the potential for conjugated metabolites to deconjugated over the course of treatment. This work has established an inside into the level of pharmaceutical residues present in Irish wastewater treatment plant effluents and can be used as a baseline for future work in this area.

List of Figures

List of Tables

Abbreviations

ANN	- Artificial neural networks
BOD	- Biological oxygen demand
COD	- Chemical oxygen demand
D	- Dilution factor
DP	- Discharge point
EC_{50}	- The concentration that evokes 50% the maximal response of an agonist
ECOSAR	- Ecological structure activity relationships
ESI	- Electrospray ionisation
F_{pen}	- Percentage market penetration
GC	- Gas chromatography
HPLC	- High performance liquid chromatography
ICP-AES	- Inductively coupled plasma – atomic emission spectrometry
INAB	- Irish national accreditation board
K_d	- Solid/water distribution coefficient
K_{oc}	- The organic carbon coefficient
K_{ow}	- Octonal/water partition coefficient
LAS	- Linear alkyl benzene sulphonate
LC	- Liquid chromatography
LC_{50}	- The concentration that kills 50% of the test population
LOD	- Limit of detection
LOQ	- Limit of quantitation
logP	- Partition coefficient
MDD	- Maximum daily dose
MEC	- Measured environmental concentration
MS	- Mass spectrometry
n	- Sample size
n_h	- Number of neurons in the hidden layer
n_v	- Number of output variables
nd	- Not detected
NOEC	- No observed effect concentration
O_j	- The absolute value of the weight from the jth neuron
OSPAR	- Oslo Paris Commission
PEC	- Predicted environmental concentration
pK_a	- Acid dissociation constant
RMS	- Root mean squared error
PNEC	- Predicted no effect concentration
R^2	- Regression coefficient
Rt	- Retention time
SPE	- Solid phase extraction
V	- Wastewater (in litres) per person per day
v	- Relative effect of the input on the output of an ANN
w_{kj}	- the absolute value of the weight from the kth input to the jth neuron
WWTP	- Wastewater treatment plant

Publications, Posters and Presentations

Publications

Lacey, C., McMahon, G., Bones, J., Barron, L., Morrissey, A., Tobin, J.M. (2006) A Solid Phase Extraction and High Performance Liquid Chromatography Method for the Detection of Pharmaceutical Compounds. Environ 2006 Proceedings.

Lacey, C., McMahon, G., Bones, J., Barron, L., Morrissey, A., Tobin, J.M. (2008) An LC-MS method for the determination of pharmaceutical compounds in wastewater treatment plant influent and effluent samples. Talanta 75, 1089-1097.

Posters

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Presentations

Lacey, C., McMahon, G., Bones, J., Barron, L., Morrissey, A., Tobin, J. Analysis of pharmaceutical residues in wastewater using LC-MS techniques. Environ - 16th Irish Environmental Researchers Colloquium 2006, UCD, Dublin.

Lacey, C., Morrissey, A., Tobin, J. Assessment of pharmaceutical residue levels in receiving waters at Irish wastewater treatment plants. Environ - 17th Irish Environmental Researchers Colloquium 2007, Carlow Institute of Technology, Co. Carlow.

Lacey, C., Morrissey, A., Tobin, J. Determination of pharmaceutical compounds in wastewater treatment plants in the greater Dublin region Environ - 18th Irish Environmental Researchers Colloquium 2008, Dundalk Institute of Technology, Co. Louth.

1.0 Introduction

Pharmaceutical compounds are an indispensable element of both human and veterinary medicine. In recent years the presence and potential effects of such compounds in the environment have received increased attention. The first reported occurrence of pharmaceuticals in the environment was in 1976 in the USA. Clofibric acid, a primary metabolite of lipid regulators, was identified in treated wastewater at concentrations ranging from 0.8-2.0 μg/L (Garrison *et al.*, 1976; Fent *et al.*, 2006; Nikolaou *et al.*, 2007). Following this, pharmaceutical contamination in rivers was reported in the UK (Richardson and Bowron, 1985) and municipal wastewater in Canada (Rodgers *et al.*, 1986). However, pharmaceutical contamination in the environment was not researched in depth until the 1990s with the introduction and availability of methods capable of detecting trace contaminants in complex matrices. Since then numerous compounds have been identified in a variety of aquatic matrices including wastewater effluents (for example Ternes, 1998; Andreozzi *et al.*, 2003; Bendez *et al.*, 2005), surface waters (for example Thomas and Hilton, 2004; Zuccato *et al.*, 2005; Kasprzyk-Hordern *et al.*, 2008; Peng *et al.*, 2008) and drinking water (for example Jones *et al.*, 2005).

Although the toxicity of pharmaceutical compounds to both aquatic and terrestrial organisms is relatively unknown, a number of reported investigations have shown that pharmaceutical compounds pose a real threat to the environment (Oaks *et al.*, 2004; Fent *et al.*, 2006). For example diclofenac, which is frequently detected in aquatic matrices, has been found to have adverse effects in both rainbow trout and vulture populations. Diclofenac accumulates, with a concentration factor of up to 2732, in the liver of rainbow trout and causes histopathological alterations in both the kidneys and gills (Schwaiger *et al.*, 2004). In vulture populations this drug has been shown to cause renal failure and has resulted in a population decline in Pakistan (Oaks *et al.*, 2004). These studies highlight the potential danger to both terrestrial and aquatic life. Moreover, they underline the latent risk to humans.

Wastewater treatment plants (WWTPs) have been identified as the main point source of pharmaceuticals and personal care products in the environment. These facilities receive a continuous input of compounds, either in the parent form or as an array of metabolites, as a result of usage. Reported removal efficiencies in WWTPs have often been low - for example a WWTP in Germany had a removal rate of 7% for carbamazepine while the average removal rate for the fourteen investigated compounds was 65% (Ternes, 1998). Compounds not removed were released to receiving water bodies in effluent streams.

Both the number of pharmaceutical compounds licensed for human use and their annual consumption have increased dramatically over the past number of years. In Ireland, the number of compounds licensed for human use by the Irish Medicines Board increased by 942 to approximately 6000 from 2004 to 2005 (IMB, 2005). These compounds and their metabolites may potentially enter the environment. Despite this there is no monitoring of the level of contamination caused by such pollutants in most Irish waters to date.

The following sections aim to review the current knowledge available on the occurrence, fate and toxicity of pharmaceutical compounds in the environment.

1.1 Entry of Pharmaceuticals to the Environment

The routes of entry and dispersal of human pharmaceuticals into the environment are outlined in Figure 1.1.1. Pharmaceuticals may be divided according to their use for examining their routes to the environment. For this investigation veterinary and aquaculture use of pharmaceuticals will not be considered although for completion are included in Figure 1.1.1. Pharmaceuticals used for human medicinal purposes are not completely metabolised in the body. Once ingested, compounds can be metabolised by phase I and phase II reactions (Gibson and Skett, 1986), both of which take place principally in the liver (Rang and Dale, 1987). Phase I metabolism serves to functionalise the drug. This consists of oxidation,

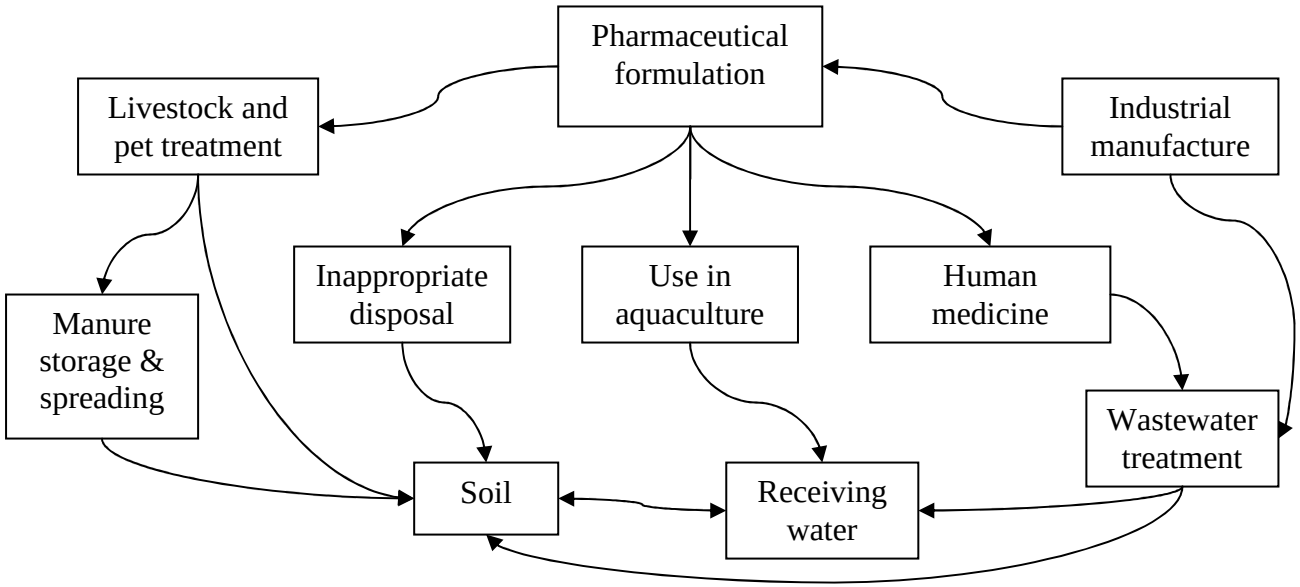


Figure 1.1.1 Pharmaceutical routes of entry into the environment (adapted from Halling-Sorensen *et al.*, 1998)

reduction, hydrolysis, dethioacetylation or isomerisation of the parent compound to produce a chemically reactive group. Products of phase I metabolism may be more toxic than the parent compound. Phase II metabolism results in conjugation making a compound hydrophilic and easier to excrete. Both phases of metabolism change the physical and chemical behaviour of the parent compound and produce a metabolite with decreased lipid solubility thereby increasing renal excretion (Gibson and Skett, 1986; Rang and Dale, 1987). An overview of the metabolisation of a parent compound to phase I and II metabolites is shown in Figure 1.1.2.

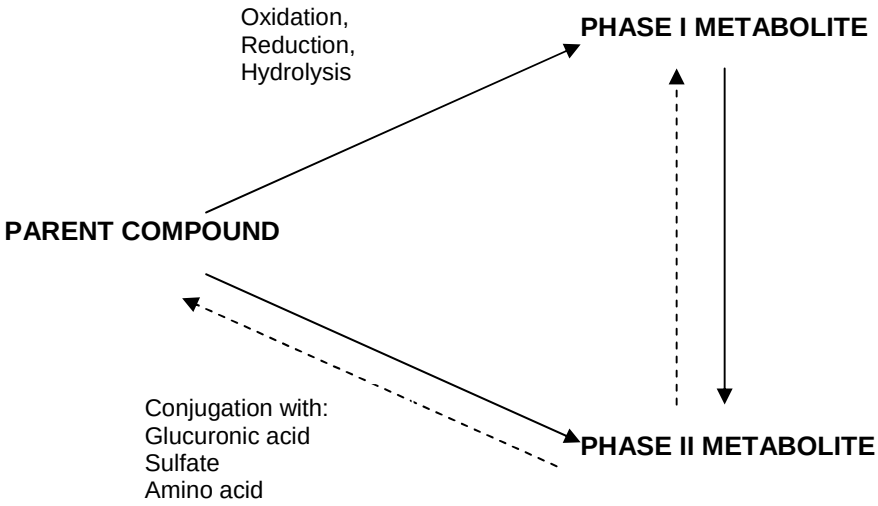


Figure 1.1.2 Metabolisation of parent compound. Solid lines represent transformation into more water-soluble compounds, dotted lines indicate a reactivation of phase II metabolites (Gibson and Skett, 1986; Halling-Sorensen *et al.*, 1998; Daughton and Ternes, 1999).

Pharmaceuticals are excreted from the body primarily in the urine (approximately 70%) and 30% in the faeces either as the parent compound or as metabolites. The percentage of metabolites formed varies with each compound, resulting in a cocktail of pharmaceutical compounds and metabolites being released to sewage (Ashton *et al.*, 2004; Heberer, 2002; Steur-Lauridsen *et al.*, 2000). Conjugated forms of a compound may be cleaved during wastewater treatment to produce the parent compound thereby increasing the concentration of a compound released to the

environment (Ternes and Joss, 2006). Inappropriate disposal of expired or unused drugs to sewage waters or in domestic waste is another potential source of contamination (Gros *et al.*, 2006).

Recent studies have reported that the efficiency of wastewater treatment plants for the removal of pharmaceutical compounds is low (Ternes, 1998). Pharmaceutical residues that pass through wastewater treatment plants are then released to receiving waters. Reported concentrations of selected compounds found in effluents and receiving water bodies are presented in Section 1.2. Organic hydrophobic compounds within the sewage may be sorbed onto sludge particles during wastewater treatment. Sorption onto sludge allows for the bioaccumulation of compounds (Diaz-Cruz *et al.*, 2003). The subsequent use of this sludge as soil fertiliser may potentially introduce pharmaceutical residues to ground water (Halling-Sorensen, 1998).

The reuse and recycling of water contaminated with wastewater effluent introduces a reduction in water quality. Pharmaceuticals have been detected in drinking water in Germany, the UK, Italy and the USA indicating that water reuse does occur (Jones *et al.*, 2005). Contamination of drinking water supplies may occur due to drinking water plants being located downstream of WWTP discharge points or contamination of ground water by leaching from WWTP sludge spread as fertiliser.

1.2 Environmental Occurrence

The knowledge of pharmaceutical contamination in the environment has increased in recent years with the development of analytical techniques capable of detecting trace quantities of these compounds. The most commonly detected compounds in environmental matrices are outlined in Figure 1.2.1 and include the following therapeutic classes: antibiotics, lipid regulators, anti-inflammatories, steroids and hormones, β-blockers, tranquilisers, antiepileptics, diuretics and cancer therapeutics. An overview of the concentration of pharmaceutical compounds, selected for this study (Section 2.3), detected in sewage influent and effluent streams, surface water, ground water and drinking water is presented in Table 1.2.1.

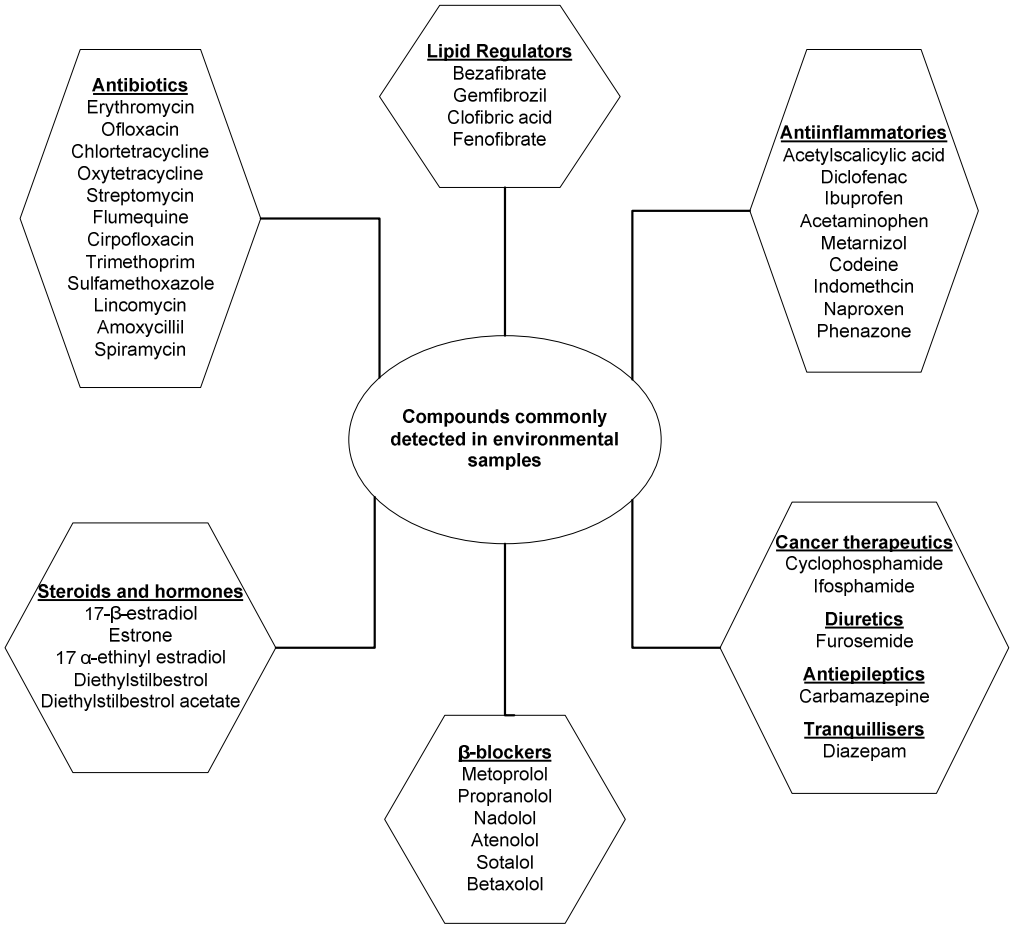


Figure 1.2.1 Compounds most commonly detected in environmental matrices (Nikolaou *et al.*, 2007).

Pharmaceutical	Environmental concentration (ng/L)	Matrix	Reference
Bezafibrate	27	Drinking water (Germany)	Jones *et al*., 2005
	1550-7600	Sewage influent (Austria)	Clara *et al.*, 2005
	nd-4800	Sewage effluent (Austria)	Clara *et al.*, 2005
	70	Sewage effluent (Canada)	Gagné *et al.*, 2006
	134.3-202.7	River Lambro (Italy)	Zuccato *et al.*, 2000
	15.1-22.4	River Po (Italy)	Zuccato *et al.*, 2000
	<10-66	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<10-76	River Ely (Wales)	Kasprzyk-Hordern *et al.,* 2008
Caffeine	11160	Sewage effluent (Canada)	Gagné *et al.*, 2006
	230000	Sewage influent (Germany)	Heberer, 2002
	180	Sewage effluent (Germany)	Heberer, 2002
	8100	Sewage Effluent (Canada)	Verenithch *et al.*, 2006
	1600	Receiving water (Canada)	Verenithch *et al.*, 2006
Carbamazepine	900	Ground water (Germany)	Sacher *et al*., 2001
	24 & 258	Drinking water (Canada & US)	Jones *et al*., 2005
	1780	Sewage influent (Germany)	Heberer 2002
	85	Sewage effluent (Canada)	Gagné *et al.*, 2006
	325-1850	Sewage influent (Austria)	Clara *et al.*, 2005
	465-1594	Sewage effluent (Austria)	Clara *et al.*, 2005
	900-1200	Sewage effluent (France)	Andreozzi *et al.*, 2003
	3700	Sewage effluent (Germany)	Ternes, 1998
	1780	Sewage influent (Germany)	Heberer, 2002
	1630	Sewage effluent (Germany)	Heberer, 2002
	1680	Sewage influent (Sweden)	Bendez *et al.*, 2005
	1180	Sewage effluent (Sweden)	Bendez *et al.*, 2005
	290	Sewage effluent (Italy)	Zuccato *et al.*, 2005
	180	River Lambro (Italy)	Zuccato *et al.*, 2005
	30	River Po (Italy)	Zuccato *et al.*, 2005
	360	Sewage influent (Canada)	Miao *et al.*, 2005
	250	Sewage effluent (Canada)	Miao *et al.*, 2005
	<0.5-356	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<0.5-684	River Ely (Wales)	Kasprzyk-Hordern *et al.,* 2008

Table 1.2.1 Occurrence of pharmaceuticals in the environment. nd = not detected.

Pharmaceutical	Environmental concentration (ng/L)	Matrix	Reference
Clofibric Acid	5.3	Drinking water (Italy)	Jones *et al.*, 2005
	70, 165, 170 & 270	Drinking water (Germany)	Jones *et al*., 2005
	111	Belfast Lough (N. Ireland)	Thomas and Hilton, 2004
	5.77	River Po (Italy)	Calamari *et al*., 2003
	3.2-5.3	Drinking water (Italy)	Zuccato *et al.*, 2000
	720	Sewage effluent (Germany)	Ternes, 1998
	460	Sewage influent (Germany)	Heberer, 2002
	480	Sewage effluent (Germany)	Heberer, 2002
	<0.3-164	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<0.3-6	River Ely (Wales)	Kasprzyk-Hordern *et al.,* 2008
	22-248	Surface water (China)	Peng *et al.*, 2008
Clotrimazole	6-34	River Tyne (UK)	Roberts and Thomas, 2005
Diclofenac	590	Ground water (Germany)	Sacher *et al*., 2001
	6	Drinking water (Germany)	Jones *et al*., 2005
	195	River Mersey (UK)	Thomas and Hilton, 2004
	905-4114	Sewage influent (Austria)	Clara *et al.,* 2005
	780-1680	Sewage effluent (Austria)	Clara *et al.*, 2005
	460	Sewage effluent (Canada)	Verenithch *et al.*, 2006
	194	Sewage effluent (Canada)	Lishman *et al.*, 2006
	1600	Sewage effluent (Germany)	Ternes, 1998
	3020	Sewage influent (Germany)	Heberer, 2002
	2510	Sewage effluent (Germany)	Heberer, 2002
	<0.5-85	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<0.5-261	River Ely (Wales)	Kasprzyk-Hordern *et al.,* 2008
Furosemide	<6-267	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<6-630	River Ely (Wales)	Kasprzyk-Hordern *et al.*, 2008

Table 1.2.1 (continued) Occurrence of pharmaceuticals in the environment. nd = not detected.

Pharmaceutical	Environmental concentration (ng/L)	Matrix	Reference
Gemfibrozil	260	Sewage Influent (Canada)	Lee *et al*., 2005
	820	Sewage Effluent (Canada)	Lee *et al.*, 2005
	70	Drinking water (Canada)	Jones *et al*., 2005
	2100	Sewage influent (Canada)	Metcalfe *et al.*, 2003
	1300	Sewage effluent (Canada)	Metcalfe *et al.*, 2003
	110	Detroit river (Canada)	Metcalfe *et al.*, 2003
	70	Hamilton Harbour (Canada)	Metcalfe *et al.*, 2003
	840-4760	Sewage effluent (France)	Andreozzi *et al*., 2003
	710	Sewage influent (Sweden)	Bendez *et al.*, 2005
	180	Sewage effluent (Sweden)	Bendez *et al.*, 2005
	71	Sewage effluent (Canada)	Gagné *et al.*, 2006
	246	Sewage effluent (Canada)	Lishman *et al.*, 2006
	480	Sewage effluent (Canada)	Verenithch *et al.*, 2006
	40	Surface receiving water (Canada)	Verenithch *et al.*, 2006
Ibuprofen	6770	Sewage influent (Canada)	Lee *et al.*, 2005
	310	Sewage effluent (Canada)	Lee *et al.*, 2005
	<5-41	River Rhine (Germany)	Halling-Sorensen, 1998
	144-2370	River Tyne (UK)	Roberts and Thomas, 2006
	3	Drinking water (Germany)	Jones *et al.*, 2005
	928	River Thames (UK)	Thomas and Hilton, 2004
	1200-2679	Sewage influent (Austria)	Clara *et al.*, 2005
	22-2400	Sewage effluent (Austria)	Clara *et al.*, 2005
	6700	Sewage effluent (Canada)	Verenithch *et al.*, 2006
	9.5	Receiving water (Canada)	Verenithch *et al.*, 2006
	786	Sewage effluent (Canada)	Gagné *et al.*, 2006
	90.6-92.4	River Lambo (Italy)	Zuccato *et al.*, 2000
	nd-4.0	River Po (Italy)	Zuccato *et al.*, 2000
	380	Sewage effluent (Canada)	Lishman *et al.*, 2006
	<0.3-100	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<0.3-93	River Ely (Wales)	Kasprzyk-Hordern *et al.,* 2008

Table 1.2.1 (continued) Occurrence of pharmaceuticals in the environment. nd = not detected.

Pharmaceutical	Environmental concentration (ng/L)	Matrix	Reference
Indomethcin	280	Sewage influent (Canada)	Lee *et al*., 2005
	180	Sewage effluent (Canada)	Lee *et al*., 2005
	190	Sewage effluent (Canada)	Lishman *et al.*, 2006
Mefenamic Acid	34 and 104	River Mersey and Thames (UK)	Thomas and Hilton, 2004
	196	Belfast Lough (N. Ireland)	Thomas and Hilton, 2004
	<0.3-169	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<0.3-33	River Ely (Wales)	Kasprzyk-Hordern *et al.,* 2008
Metoprolol	<0.5-11	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<0.5-12	River Ely (Wales)	Kasprzyk-Hordern *et al.,* 2008
	509-1774	Sewage effluent (France)	Miège *et al.*, 2006
Pravastatin	117	Sewage influent (Canada)	Miao and Metcalfe, 2003
	59	Sewage effluent (Canada)	Miao and Metcalfe, 2003
	<60	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<60	River Ely (Wales)	Kasprzyk-Hordern *et al.,* 2008
Propranolol	35-107	River Tyne (UK)	Roberts and Thomas, 2005
	56	Belfast Lough (N. Ireland)	Thomas and Hilton, 2004
	20	River Thames (UK)	Thomas and Hilton, 2004
	230	Sewage effluent (Germany)	Ternes, 1998
	<0.5-40	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<0.5-91	River Ely (Wales)	Kasprzyk-Hordern *et al.,* 2008
	416-1111	Sewage effluent (France)	Miège *et al.*, 2006
Salbutamol	nd-3.1	River Lambo (Italy)	Zuccato *et al.*, 2000
	nd-4.6	River Po (Italy)	Zuccato *et al.*, 2000
	<0.5-4	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<0.5-8	River Ely (Wales)	Kasprzyk-Hordern *et al.,* 2008

Table 1.2.1 (continued) Occurrence of pharmaceuticals in the environment. nd = not detected.

Pharmaceutical	Environmental concentration (ng/L)	Matrix	Reference
Salicylic Acid	54000	Sewage influent (Germany)	Ternes, 1998.
	6860	Sewage influent (Canada)	Lee *et al*., 2005
	140	Sewage effluent (Canada)	Lee *et al*., 2005
	2200	Sewage effluent (Canada)	Verenithch *et al.*, 2006
	400	Receiving water (Canada)	Verenithch *et al.*, 2006
	106	Sewage effluent (Canada)	Lishman *et al.*, 2006
	<0.3-302	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<0.3-234	River Ely (Wales)	Kasprzyk-Hordern *et al.,* 2008
	9-2098	Surface water (China)	Peng *et al.*, 2008
Sulfamethoxazole	410	Ground Water (Germany)	Sacher *et al*., 2001
	49	Sewage effluent (Canada)	Gagné *et al.*, 2006
	nd-145	Sewage influent (Austria)	Clara *et al.*, 2005
	nd-91	Sewage effluent (Austria)	Clara *et al.*, 2005
	900	Sewage effluent (Germany)	Hirsch *et al.*, 1999
	<0.5-2	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<0.5-4	River Ely (Wales)	Kasprzyk-Hordern *et al.,* 2008
Trimethoprim	164	Sewage effluent (UK)	Hilton and Thomas, 2003
	200	Downstream of WWTP effluent (UK)	Hilton and Thomas, 2003
	12	Streams (UK)	Ashton *et al.*, 2004
	28.6	Belfast Lough (N. Ireland)	Thomas and Hilton, 2004
	46	Tyne estuary (UK)	Thomas and Hilton, 2004
	134	Mersey estuary (UK)	Thomas and Hilton, 2004
	4-19	River Tyne (UK)	Roberts and Thomas, 2005
	440	Sewage influent (Switzerland)	Göbel *et al.*, 2005
	400	Sewage effluent (Switzerland)	Göbel *et al.*, 2005
	65	Sewage effluent (Canada)	Gagné *et al.*, 2006
	5000	Hospital effluent (New Mexico)	Brown *et al.*, 2006
	4-19	River Tyne (UK)	Roberts and Thomas, 2005
	<1.5-126	River Taff (Wales)	Kasprzyk-Hordern *et al.,* 2008
	<1.5-183	River Ely (Wales)	Kasprzyk-Hordern *et al.,* 2008

Table 1.2.1 (continued) Occurrence of pharmaceuticals in the environment. nd = not detected.

Numerous pharmaceuticals are ubiquitous in the environment with concentrations in the ng/L and low μg/L detected in wastewater streams, surface water and in some cases drinking water. However, the majority of studies on the occurrence of pharmaceuticals in the environment is focused on wastewater treatment plant streams, as they have been identified as the principal source of environmental contamination (Daughton and Ternes, 1999). A wide array of compounds has been identified in both influent and effluent streams. Bezafibrate was detected in effluent streams at concentrations ranging from 70 – 4800 ng/L (Clara *et al.*, 2005; Gagné *et al.*, 2006). Surface water contamination has been identified in four rivers in both Italy and Wales at low μg/L concentrations. Contamination of the drinking water supply with bezafibrate has also been identified in Germany (Jones *et al.*, 2005). Carbamazepine has been reported in numerous studies in influent and effluent streams from wastewater treatment plants (Heberer, 2002; Bendez *et al.*, 2005; Clara *et al.*, 2005; Miao *et al.*, 2005). Minimal degradation or removal was observed in the investigated treatment facilities. Contamination of surface and drinking water supplies with carbamazepine has also been identified in Italy, Wales, Canada and the US at low μg/L concentrations (Sacher *et al.*, 2001; Jones *et al.*, 2005; Zuccato *et al.*, 2005; Kasprzyk-Horden *et al.,* 2008). Clofibric acid is also frequently detected in environmental matrices at low mg/L concentrations. Removal of clofibric acid from wastewater streams was inefficient in an investigation in Berlin (Heberer, 2002). Although clotrimazole has not been monitored extensively, low ng/L concentrations were observed in the River Tyne in the UK (Roberts and Thomas, 2005). Diclofenac has been identified in influent and effluent streams from wastewater treatment facilities. Incomplete removal was observed in studies where both streams were monitored (Clara *et al.*, 2005; Heberer, 2002). Gemfibrozil has been identified in numerous investigations of influent and effluent streams, surface waters and drinking water in several countries including Canada and Sweden at low μg/L concentrations (Metcalfe *et al.*, 2003; Bendez *et al.*, 2005). Ibuprofen has

also been shown to be ubiquitous in the environment with contamination of all aquatic matrices including drinking water (Zucatto *et al.*, 2000; Jones *et al.*, 2005; Lee *et al.*, 2005). Published removal rates of ibuprofen from wastewater streams are inconsistent with >90% removal reported in one investigation (Lee *et al.*, 2005) while ~10% removal was observed in another (Clara *et al.*, 2005). Indomethcin, mefenamic acid, metoprolol, pravastatin and salbutamol have been reported less frequently in the environment. Most reported occurrences are in surface water indicating that there is incomplete removal of these compounds during wastewater treatment (Thomas and Hilton, 2004; Lee *et al.*, 2005; Kasprzyk-Hordern *et al.*, 2008). Indomethcin was detected in both influent and effluent streams from eight municipal treatment plants in southern Ontario in Canada and removal rates of ~46% were observed (Lee *et al.*, 2005). Removal rates for pravastatin were 50% in a municipal treatment plant in Peterborough Canada (Miao and Metcalfe, 2003).

Sulfamethoxazole and trimethoprim have also been determined in influent and effluent streams, ground and surface water at ng/L and μg/L concentrations (Table 1.2.1). Trimethoprim was detected in hospital effluent at 5μg/L (Brown *et al.*, 2006) which is more than five times the concentration of that detected in effluent streams from municipal plants (Gobel *et al.*, 2005; Gagné *et al.*, 2006). This highlights the importance of hospital effluents in final environmental loading of pharmaceuticals.

Drinking water contamination by pharmaceuticals is not deemed to be a general problem. Rather, it is considered a concern associated with water reuse or contaminated ground water used for drinking water. The majority of drinking water samples analysed contains no contamination. Only a small number of references may be found in literature concerning the detection of pharmaceutical residues in drinking water, because the concentrations present are below the detection limits of current analytical techniques (Daughton and Ternes, 1999). Most reported drinking water contamination in Europe has been identified in Berlin, Germany. This is due to the high percentage (~75%) of bank filtrate (from bank filtration) and

contaminated groundwater used in drinking water production in Berlin (Heberer, 2002). Limited reports have identified contamination of Italian supplies with clofibric acid (Zuccato *et al.*, 2000; Jones *et al.*, 2005). Contamination of Canadian drinking water supplies with carbamazepine and gemfibrozil has also been reported at levels of 24ng/L and 70ng/L respectively (Jones *et al.*, 2005).

1.3 Wastewater Treatment

Traditionally wastewater treatment plants were designed to treat domestic waste through sedimentation processes followed by microbial degradation and flocculation of solids and occasionally tertiary treatment (Daughton and Ternes, 1999). The extent to which each phase is used greatly affects the efficiency of a WWTP in the removal of pharmaceutical compounds. For example microbial degradation plays a key role in the removal of polar compounds like acidic pharmaceuticals in activated sludge (Quintana *et al.*, 2005).

Treatment of municipal wastewater is a sequential process of mechanical, biological and chemical processes (Figure 1.3.1). Preliminary and primary treatments involve the mechanical removal of large debris and the sedimentation of suspended solids usually by gravity settling. Organic matter, oils, fats and greases float to the top and are also removed in the clarifier during primary treatment.

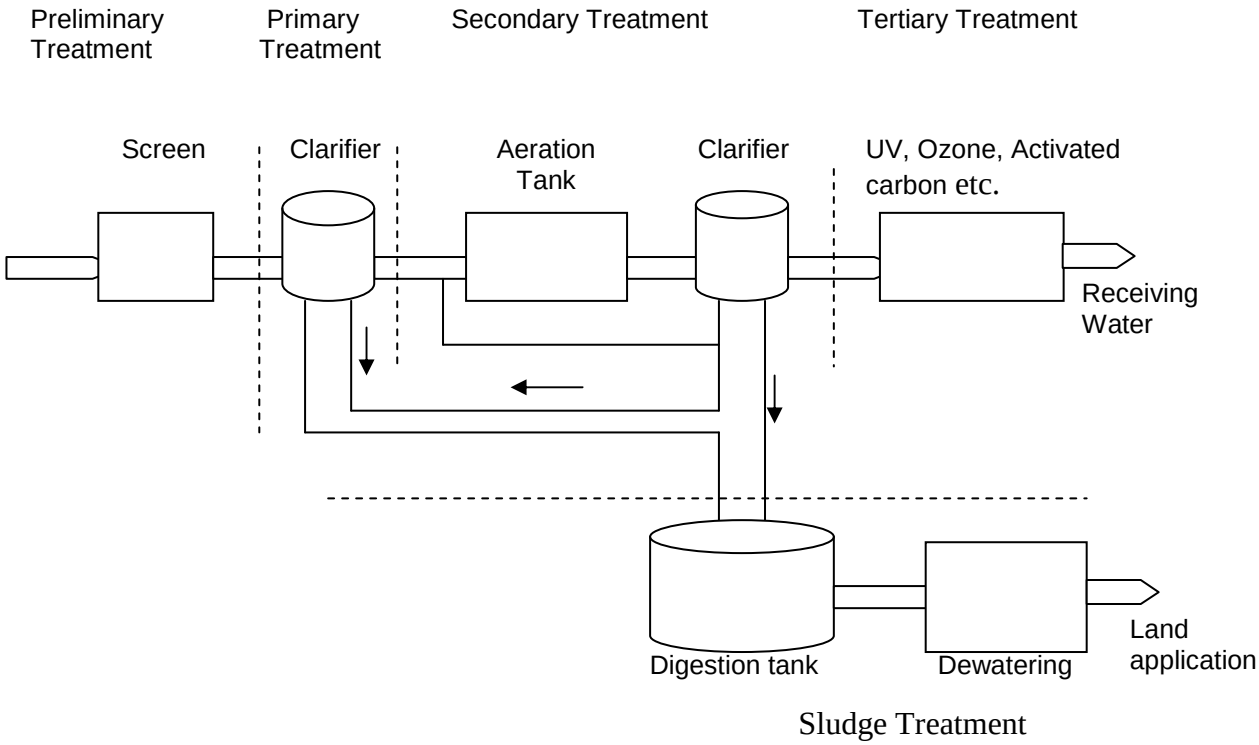


Figure 1.3.1 Schematic of wastewater treatment (Adapted from Swords WWTP schematic).

Secondary treatment refers to biological treatment to reduce the biological oxygen demand of the wastewater. This biological treatment, known as activated sludge treatment, uses a diversified group of microorganisms ranging from bacterial genera found primarily in flocculated agglomerations of microbes (flocs): *Zooglea, Pseudomoas, Bacillus, Achromobacter and Nitrosomonas* to protoza *Spirostomum* and *Amoeba proteus* and rotifers: *Philodina* spp. and *Notommata* spp.. The make up of activated sludge systems is variable and often plant specific as there is internal selection within each system. Factors influencing organism selection within a system are outlined in Figure 1.3.2. The final stage of secondary treatment is clarification of the sludge. Biomass is allowed to settle out of solution to yield a clarified effluent. The final effluent is then discharged into a receiving water body or subjected to tertiary treatment.

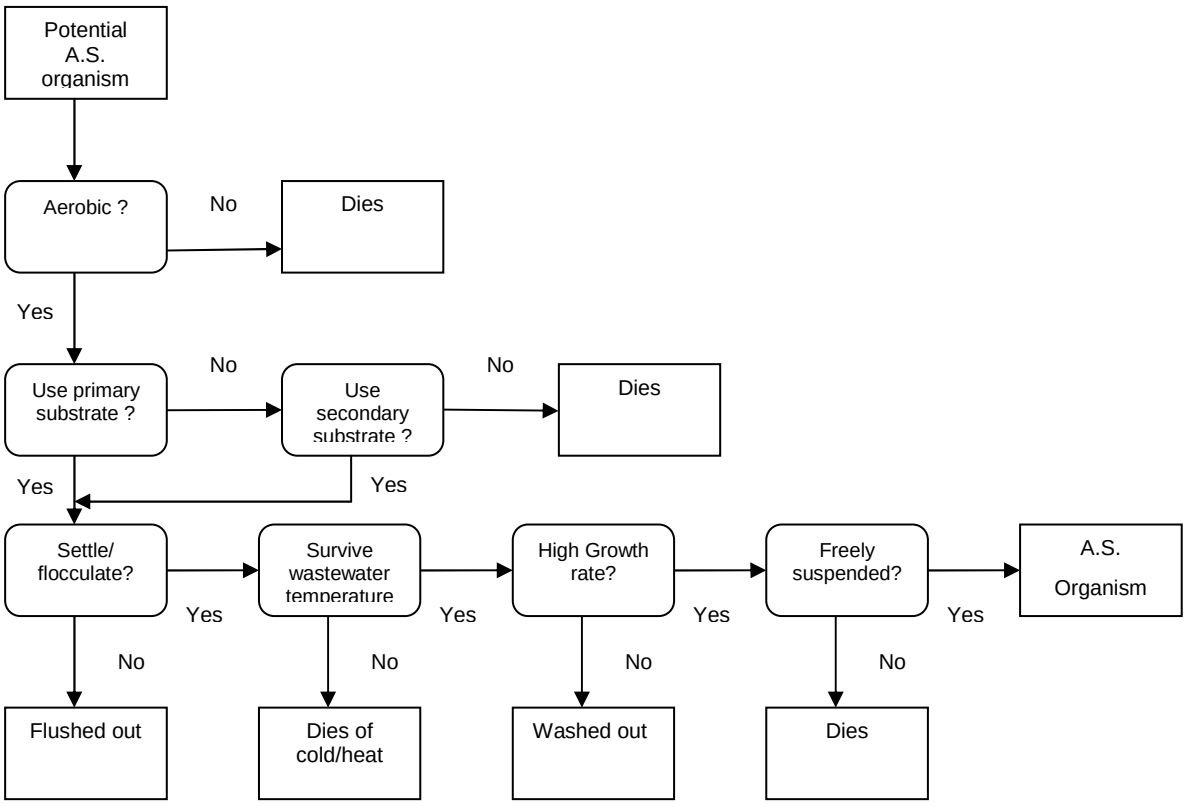


Figure 1.3.2 Organism selection for activated sludge treatment (Henze, 2002).

The purpose of tertiary treatment is to improve the quality of effluent from wastewater facilities. Tertiary treatments include both physical and chemical methods such as precipitation, ozonation and ultraviolet exposure for the removal of excess nutrients and suspended material and disinfection. Ultraviolet exposure may also assist the removal of residual organics from effluent streams (Gebhardt and Schröder, 2007; Canonica *et al.*, 2008).

1.4 Pharmaceuticals in Wastewater Treatment Plants

Pharmaceutical substances designed for human medicinal purposes are predominantly hydrophilic in nature in order to improve uptake by the human body. Sorption to activated sludge, as discussed in Section 1.3, is a method of micropollutant removal within wastewater treatment plants. Compounds with a low solid-liquid partitioning coefficient exhibit low or negligible sorption within a WWTP. This implies that a high proportion of hydrophilic pharmaceutical compounds remain in the liquid phase in WWTP systems (Ternes and Joss, 2006). Increasing instances of pharmaceutical compounds in aquatic matrices confirms the incomplete removal of these pollutants in many WWTPs (Giger, *et al.*, 2003).

Limited data on the behaviour and removal of pharmaceuticals as they pass through a WWTP are available. Consequently, it is difficult to determine degradation and removal efficiencies of WWTPs. Removal efficiencies are determined by measuring and comparing the influent and effluent concentrations. These vary significantly between WWTPs due to differences in the treatment technology, retention times and also weather conditions (Fent *et al.*, 2006). The partitioning of compounds between solid and liquid phases of wastewater, degradation to lower molecular weight compounds and compound transformation to conjugated forms, which may later be hydrolysed to reform the parent compound, are all factors that affect removal efficiencies. Removal rates of German WWTPs near Frankfurt/Main were investigated in 1996 and 1997 (Ternes, 1998). Thirty-two drug residues and five metabolites were analysed. Elimination rates ranged from seven percent to ninety-nine percent. For example,

carbamazepine showed the lowest removal rate at seven percent while others (fenofibrate, meclofenamic acid, tolfenamic acid, salicylic acid and acetaminophen) were undetectable in effluents despite initial concentrations of up to 54μg/L. A similar investigation of WWTPs in Rio de Janeiro in Brazil showed removal rates from thirty-four (clofibric acid) to eighty-three (indomethcin) percent (Stumpf *et al.*, 1999). In this study the performance of both activated sludge and biological filters as secondary treatments were compared. Activated sludge had a marginally higher percentage removal for the nine pharmaceutical compounds investigated. The percentage removal ranged from 6% to 71% for the biological filter while 34% to 83% was the range for the activated sludge treatment. Comparable studies in Berlin, Frankfurt and Finland were also published in 2002 and 2005 respectively (Herberer, 2002; Lindqvist *et al.*, 2005). Removal rates (Table 1.4.1) were similar to those reported for the activated sludge plant in Rio de Janeiro. For example, removal rates of ibuprofen were greater than 75% in the activated sludge treatments studied while the biological filter was less effective for the removal of ibuprofen (22%). Similarly, removal rates of clofibric acid, gemfibrozil, diclofenac, fenofibric acid, bezafibrate and naproxen were comparable in the activated sludge treatments at Frankfurt/Main and Rio de Janeiro. The removal rates determined for indomethcin in the biological filter in Rio de Janeiro were comparable with those observed for the activated sludge treatment at Rio de Janeiro and Frankfurt/Main.

Removal of pharmaceuticals due to sorption to organic material in the wastewater will be removed during primary treatment. The potential for compounds to partition to organic material is usually described by the octanol/water partitioning coefficient (K_{ow}) (Keenan, *et al*., 2008). The K_{ow} refers to absorption of compounds due to hydrophobic interactions. Therefore, it would be expected that compounds with a high partitioning coefficient would be expected to sorb to solids while compounds with low partitioning coefficients would be expected to remain in the liquid phase. Pharmaceuticals can also be removed due to adsorption which refers to the removal of a compound due to electrostatic interactions which can be

determined by the dissociation constant (pK_a) (Carballa *et al*., 2005). Remaining in the liquid phase aids the transportation of pharmaceutical compounds through WWTPs and in the release into the environment. The organic carbon coefficient (K_{oc}) refers to the concentration sorbed to organic carbon and that dissolved in water (Keenan *et al*., 2008). When available, the K_{oc} can be used to predict the partitioning of compounds between solid and liquid phases. The low pK_a and high K_{ow} of carbamazepine suggest that it could be removed by sorption to sludge but poor removal efficiencies were observed in the activated sludge plants at Frankfurt/Main (Ternes, 1998) and Berlin (Heberer, 2002). However, due to the high variability and multiplicity of input parameters the specific partitioning of a compound can only accurately be determined by analysing its concentration both the solid and liquid portion of a sample. The solid-water distribution coefficient for a particular sample can then be defined as $K_d = \frac{S}{L}$ where, S is the concentration in the solid phase and L is the concentration in the liquid phase (Carballa *et al*., 2005).

Compound	Frankfurt/Main	Rio de Janeiro	Rio de Janeiro	Berlin	Finland
	Activated sludge	Biological Filter	Activated sludge	Activated sludge	Activated sludge
Clofibric Acid	51	15	34	0	-
Ibuprofen	90	22	75	-[a]	78-100
Gemfibrozil	69	16	46	-[a]	-
Ketoprofen	-	48	69	24	51-100
Phenazone	33	-	-	44	-
Diclofenac	69	9	75	17	9-60
Fenofibric Acid	64	6	45	-	-
Bezafibrate	83	27	50	-	11-100
Indomethcin	75	71	83	0	-
Naproxen	66	15	78	82	55-98
Carbamazepine	7	-	-	8	-

Table 1.4.1 Percentage removal of pharmaceutical compounds reported in WWTPs in four different studies (Ternes, 1998; Stumpf *et al*., 1999; Heberer, 2002; Lindqvist *et al*., 2005).

[a] Compounds were detected in effluent samples but not in influent.

1.5 Ecotoxicological Effects

Most published literature to date has dealt with the occurrence of pharmaceuticals in the environment, in wastewater treatment streams and receiving waters, rather than the ecotoxicological effects resulting from these pollutants. However, pharmaceuticals are continually infused into the environment allowing them to exert a similar threat to the environment as persistent pollutants (Jones *et al.*, 2002). Moreover, the possibility of emerging strains of bacteria resistant to drugs due to constant exposure to low concentrations is a public health concern (Renew and Huang, 2004; Yang and Carlson, 2004).

WWTPs receive industrial and domestic waste streams containing low concentrations of pharmaceutical compounds and metabolites. Continual exposure to low-level residues selects for organisms resistant to the compounds present. This results in a reservoir of resistant bacteria. Studies on *Klebsiellae* strains isolated from treatment plants have shown that ninety percent show insensitivity to ampicillin and six percent show multiple resistance (Hirsh *et al.*, 1999).

Toxicity testing endeavours to predict the possible adverse effects of exposure to chemicals (Meyer, 2003). Toxicity data for compounds analysed in this study (Section 2.3) are summarised in Table 1.5.1. The effect concentration for 50% of the test population (EC_{50}) and the lethal concentration causing death in 50% of the test population (LC_{50}) are presented for several test organisms. Toxicity concentrations were in the mg/L range indicating that the low μg/L concentrations reported in aqueous samples should not impart a negative effect. However, the effects of each compound are analysed individually thus toxicity testing does not accurately reflect the fact that organisms are exposed to a cocktail of these compounds in the environment. Exposure to compounds with similar modes of action increases the potential of synergistic effects (Jones *et al.*, 2002). Two concepts, concentration addition and independent action have been used in recent years to estimate the risk associated with exposure to a complex mixtures of compounds. For example, the toxicity of clofibric

acid and carbamazepine in acute *Daphnia* studies were measured both individually and as a mixture. It was found that the combined toxicity was in agreement with the concept of concentration addition showing an increase in toxicity. Similarly, a mixture of ibuprofen and diclofenac showed a higher toxicity than expected from the individual toxicities (Cleuvers, 2003).

	Daphnid	Algae	Fish	*Hydra attenuata*	*Synechococcus leopoliensis*	*Cyclotella meneghiniana*	*Vibrio fischeri*	*Lemna gibba*	*Thamnocephalus platyurus*	*Brachionus calyciflorus*
Bezafibrate	25 [1]	18 [1]	5.3 [1]							
Caffeine	46 [1]	46 [1]	805 [1]	LC_{50} >100 [2]						
Carbamazepine	111 [1]	70 [1]	101 [1]	15.52 [2]	33.6 [3]	31.6 [3]	LC_{50} >81 [3]			
Clofibric acid	293 [1]	192 [1]	53 [1]	LC_{50} 29.4 [2]						
Diclofenac	5057 [1]	2911 [1]	532 [1]				13.5 [3]			
	22.4 [3]						0.01 [5]			
	22.4 [4]									
Furosemide	LC_{50}0.6 [10]								LC_{50} 70.6 [10]	2.5 [10]
Gemfibrozil	6 [1]	4 [1]	0.9 [1]	1.2 [2]			0.03 [5]			
Ibuprofen	38 [1]	26 [1]	5 [1]	LC_{50} 22.36 [2] 1.65 [2]			0.02 [5]			
Indomethcin	26 [1]	18 [1]	3.9 [1]	LC_{50} 22.4 [2]						
Mefenamic acid	0.428 [9*]									
Metoprolol	8 [1]	14 [1]	116 [1]							
Propranolol	2.3 [1]	5.5 [1]	29.5 [1]		0.67 [3]					
	1.6 [8]									
Salicylic acid	59 [1]	48 [1]	1.28 [1]							
Sulfamethoxazole	4.5 [1]	51 [1]	890 [1]	>100 [2]	0.03 [3]		>84 [3]	0.08 [7]		
Trimethoprim	4.8 [1]	2.6 [1]	795 [1]	LC_{50} >100 [2]				>1.0 [6]		

Table 1.5.1 Toxicity data for compounds analysed in this study.
* PNEC data. [1] Sanderson *et al*., 2003 ECOSAR data; [2] Quinn *et al*., 2008; [3] Ferrari *et al*., 2004; [4] Ferrari *et al*., 2003; [5] la Farre *et al*., 2001; [6] Crane *et al*., 2006; [7] Brian *et al*., 2004; [8] Huggett, *et al*., 2002; [9] Jones *et al*., 2002; [10] Isidori *et al*., 2006.

Environmental risk assessments (ERA) are required for pharmaceutical compounds prior to licensing in Europe to assess the potential risk of a compound to the environment. The ERA process is outlined in Figure 1.5.1. The first phase of the ERA requires that the predicted environmental concentration (PEC) of a compound be calculated. If this is less than 0.01µg/L the compound is considered to be unlikely to present a risk to the environment. The PEC (mg/L) is calculated using equation 1.

$$PEC = \frac{MDD \times F_{pen}}{V \times D} \qquad (1)$$

Where: MDD is the maximum daily dose in mg per person per day,

F_{pen} is the percentage of market penetration (percentage of population being treated with the drug),

V is the amount of wastewater in litres per person per day

D is the dilution factor

If the PEC is equal to or greater than 0.01µg/L then phase two analysis is required. Phase two involves evaluating the PEC/PNEC ratio. Predicted no effect concentration (PNEC) is estimated by assessing toxicity data on algae, *Daphnia sp.* or fish and determining the no observed effect concentration (NOEC) to which an assessment factor (AF) is applied to account for any variation in experimental parameters. If the PEC/PNEC ratio is less than one, it means that the predicted environmental concentration is less than that which will have an environmental effect and is therefore not likely to cause a risk to the environment. If this value is >1, further toxicity considerations are required and safety measures may need to be included in the product labelling (EMEC, 2006).

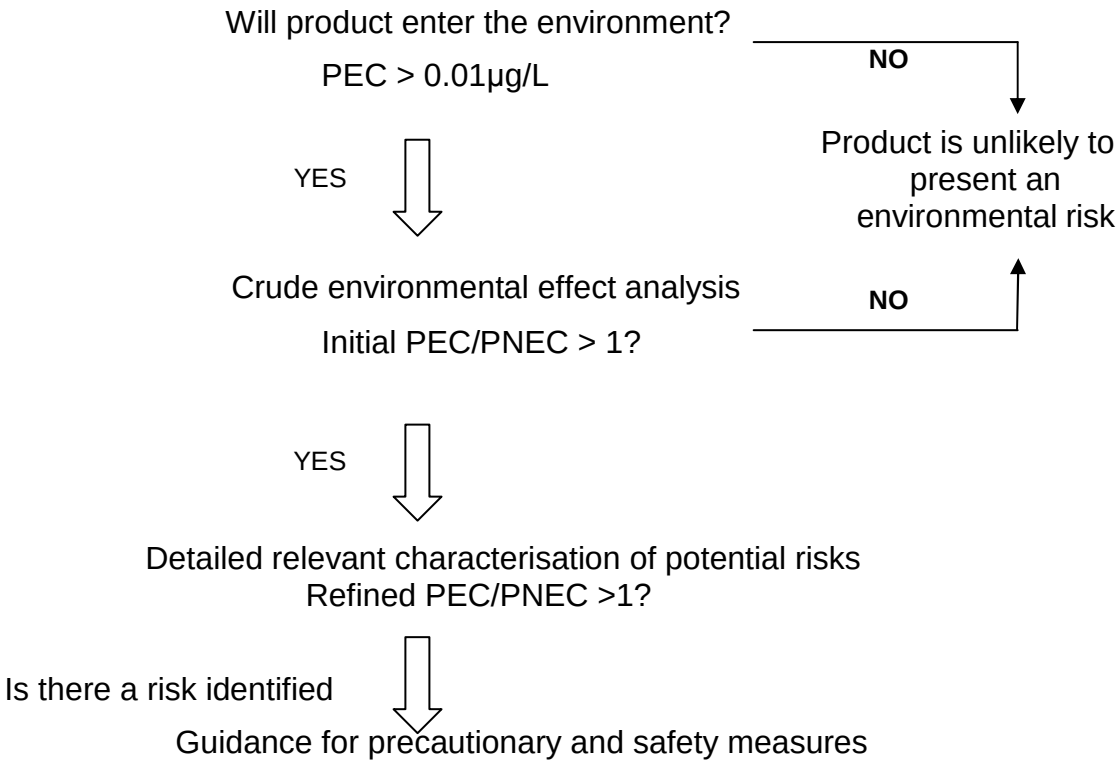


Figure 1.5.1 Environmental risk assessment scheme for human medicinal products (Knacker, 2002).

There has been a number of studies to determine the environmental risk posed by compounds frequently found in the environment. PECs for twenty-five pharmaceuticals prescribed in England predicted that twelve compounds would have an environmental concentration above 1µg/L while all would have a concentration above 0.01µg/L. Only four compounds (amoxicillin, paracetamol, mefenamic acid and oxytetracycline) had a PEC/PNEC ratio above one (Jones *et al.*, 2002). However, the calculated PECs are higher than the actual environmental concentrations reported (Table 1.5.2). Another such investigation compared PECs with measured environmental concentrations (MEC) and found that the PECs over-estimated the actual environmental concentration in all cases but both PECs and MECs were above the regulatory guide for PEC (0.01µg/L).

MEC/PNEC ratios were calculated for eleven compounds including analgesics, anti-inflamatories, antibiotics and anti-depressants, and were found to be less than one implying that they posed no potential environmental risk (Ashton *et al.*, 2004). While amoxycillin had a PEC:PNEC ratio of 588.02 indicating a potential environmental threat there was no amoxycillin measured in environmental samples.

Compound	**PEC µg/L**	**PNEC µg/L**	**PEC:PNEC Ratio**	**MEC µg/L**
Amoxycillin	2.19	0.0037 250	588.02 0.01	nd
Paracetamol	11.96	9.2 136 29	1.29 0.09 0.41	~0.5
Mefenamic Acid	0.47 0.44	0.638 0.428	0.74 1.03	0.196
Oxytetracycline	0.83	0.23 4.5	3.60 0.18	0.1

Table 1.5.2 PEC, PNEC and MEC concentrations (Ashton *et al.*, 2004).
nd = not detected

1.6 Thesis Scope

Wastewater Treatment facilities

The wastewater treatment facilities selected in this study are located at Ringsend, Swords and Leixlip. Figure 1.6.1 shows the location of the facilities and the corresponding discharge locations of each of the plants. The characteristics of each plant are shown in Table 1.6.1. Ringsend is the largest of the three facilities and it is located in Dublin Bay. The effluent from the plant is combined with the effluent from a power station prior to entry into Dublin Bay at the mouth of the River Liffey. The plant has a population equivalence of approximately 1.7 million. Leixlip wastewater treatment plant is located on the river Liffey. The plant has a total population equivalence of 90,000. While, there are two separate streams in the plant (industrial and domestic) only the domestic stream with a population equivalence of 29,000 is used for this study. The industrial stream has a population equivalence of 61,000 and was not considered for this study. The effluent from this plant is discharged directly into the river Liffey east of Leixlip village. Swords treatment plant is located in north Dublin. It has a population equivalence of 50,000. The effluent from this plant is discharged into the Broadmeadow estuary north of Dublin Bay.

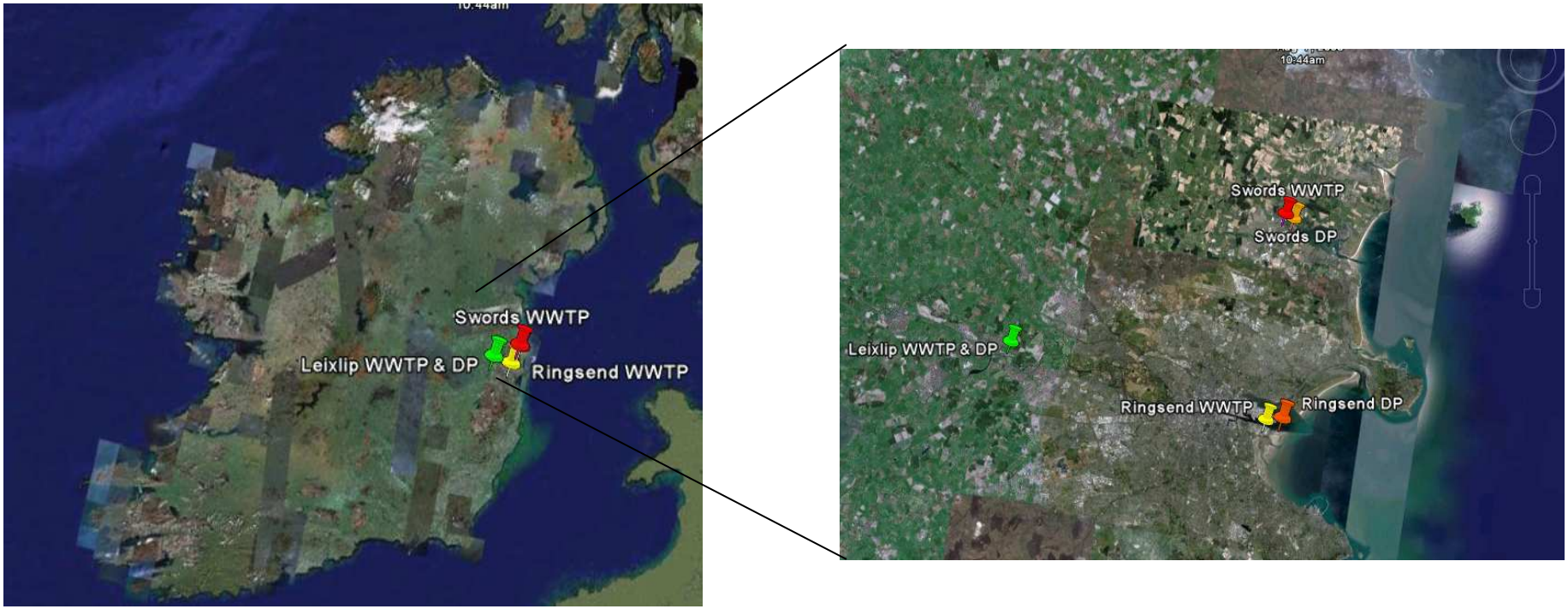


Figure 1.6.1 Location of selected wastewater treatment facilities and discharge locations (DP – Discharge point)
Source: Google Earth

	Ringsend	Leixlip	Swords
Population Equivalence	1.7 million	29,000	50,000
Mean Flow (m^3/d)	525,875	11,032	10,842
Treatment	Activated sludge	Activated sludge	Activated sludge
Additional treatment	UV (Summer months)	-	-
Discharge location	Liffey Estuary/Dublin Bay	River Liffey	Broadmeadow Estuary

Table 1.6.1 Wastewater treatment plant characteristics (Personal at Ringsend, Leixlip and Swords wastewater treatment facilities).

Selection of Analytical Techniques

Liquid chromatography tandem mass spectrometry (LC-MS) and gas chromatography tandem mass spectrometry (GC-MS) are analytical techniques involving the coupling of two individual techniques resulting in methods allowing for the separation, quantification and identification of complex mixtures of compounds. GC-MS has been used for the determination of pesticides and such pollutants in complex environmental samples. However, for GC-MS analysis analytes must be thermostable and volatile. Almost all pharmaceutically active compounds are either polar or thermally unstable molecules rendering them unsuitable for direct analysis by GC-MS. Chemical derivatisation is required prior to analysis for such compounds to enhance their volatility (Ahuja, 2001). Quantification may prove difficult if derivatisation is included in sample preparation as incomplete derivatisation and the formation of multiple products is possible. LC-MS overcomes this problem as it is suitable for analysis of a wider range of compounds including those that are thermolabile or exhibit polarity. The extensive use of LC-MS technology for the detection of pharmaceutical residues in environmental samples has proven the suitability of this technique.

LC-MS combines high performance liquid chromatography (HPLC) with mass spectrometry (MS) via a suitable interface. LC serves to separate a complex mixture of compounds while MS determines molecular ions. The resulting retention times and molecular masses allow for the identification and quantitation of compounds.

LC involves the partitioning of analytes between a liquid mobile phase and a solid stationary phase. The composition of the mobile solvent phase remains constant for isocratic chromatography while gradient elution chromatography can be achieved by using differing concentrations of solvent solutions (Hancock and Sparrow, 1984).

LC can be run in both normal and reverse phase. In normal phase chromatography, separations are generally performed between a non-polar organic mobile phase and a silica stationary phase. In reverse phase LC the separation occurs due to a non-polar stationary phase and a polar mobile phase. The separation of analytes in reversed phase chromatography depends on the hydrophobic interactions between the sample and the mobile phase (Fallon *et al.*, 1987). The molecular size of the molecule may play a role in the separation. Small polar molecules are eluted more rapidly than large non-polar molecules. In normal phase LC the size of the molecule does not determine the separation of analytes (Smith, 1988). Reverse phase LC is most frequently used for the detection of pharmaceutical compounds in aqueous matrices.

Electrospray ionisation mass spectrometry is commonly used for the identification of multi-class pharmaceutical compounds in environmental samples (Gros *et al*., 2006). Electrospray ionisation is the interface used to couple LC with the MS and involves the transfer of ions from the liquid phase to the gas phase and also as a link between the atmospheric pressure of the LC and the high vacuum MS. The sample is passed through a metal capillary to which a voltage has been applied. An electric field is obtained between the capillary and a counter-electrode. Charged droplets produced at the end of the capillary are pulled towards the oppositely charged counter electrode due to both the potential difference and the pressure gradient. The droplets are reduced in size in the

ionisation chamber due to solvent evaporation. As the droplet size reduced the Rayleigh limit is reached, where the forces of repulsion of ions in the droplet are greater than the surface tension of the liquid, and a coulombic explosion occurs. The process is repeated until gas phase ions are formed. The ions are then sampled and focused using an electrostatic lensing system into the ion trap. The ions are trapped in a stable trajectory. A varying radiofrequency (RF) voltage is applied to a ring electrode and ions become unstable and are directed towards the detector with increasing mass to charge (*m/z*) ratio. The resulting plot or mass spectrum is the relative abundance of each charged species against the *m/z* ratio. The mass analyser operates under vacuum to prevent or minimise the collision between the ions formed (Ardrey, 2003; Hoffmann and Stroobant, 2002; Pease, 1980).

The concentration of pharmaceutical compounds in environmental matrices is usually very low, in the ng/L range. For this reason a pre-concentration step is necessary prior to analysis. Solid phase extraction (SPE) is a method which is widely used for sample preconcentration and sample clean up. SPE involves the sorption of an analyte, from a sample, to a stationary solid phase followed by recovery of the analyte by elution usually in an organic phase. Therefore, SPE is used for the isolation, concentration and medium transfer of trace analytes in environmental aqueous samples (Huck and Bonn, 2000; Leon-Gonzalez *et al.*, 2000; Poole, 2002).

There are four main steps involved in SPE; conditioning, adsorption, washing and elution (Huck and Bonn, 2000).

1. Conditioning

Conditioning is the pre-treatment of the sorbent material. This is usually accomplished by passing a solvent through the sorbent material. This renders the surface more hydrophilic and therefore more compatible with the sample solution.

2. Adsorption

The liquid sample is passed through the SPE sorbent material and analytes are retained on the sorbent material.

3. Washing

This step allows for the removal of interfering compounds eg. salts without eluting the desired analyte. This is often achieved using a water wash. The solid phase is then dried usually under a stream of nitrogen to remove excess water.

4. Elution

Adsorbed analytes are removed/eluted from the solid matrix. This elution is commonly accomplished using an organic solvent however, a gas stream may be used to thermally desorb analytes.

Research Objectives

There is a lack of published research on the level of environmental contamination due to pharmaceutical compounds in Ireland. Research to date in this area has determined environmental concentrations in other countries. However, seasonal variation and the effects seasonal climate change may have on pharmaceutical concentrations have not been investigated.

The main aims of this project were to:

- Develop a multi-residue method for the detection and quantitation of selected pharmaceuticals in the WWTPs.
- Establish the level of pharmaceuticals in the influent and effluent of three WWTPs in the greater Dublin region.
- Establish the efficiency of selected wastewater treatment plants in treating contaminated water
- Ascertain any seasonal variation in pharmaceutical occurrence
- Establish if the environmental concentrations found have any potential toxicological effect.

- Investigate the potential use of artificial neural networks for the prediction the effluent concentration of pharmaceuticals.

Twenty compounds were chosen for this study. Occurrence of compounds in the environment and on the list of the top one hundred prescribed compounds were both considered (HSE, 2004). A wide range of compounds including anti-inflammatories, analgesics, antibiotics, anti-fungal agents, β-blockers, β_2 agonists and statins is included in the chosen analytes. The chosen compounds are listed in Table 2.3.1.

A twelve-month sampling regime was designed to provide a database from which to determine any seasonal relationship. As a wastewater treatment plant is a controlled system there is a limited number of influencing environmental factors, mainly rainfall and sunlight. The efficiency of each wastewater treatment plant for the removal of selected analytes was investigated. Monthly influent and corresponding effluent samples provide the relevant data to evaluate removal efficiencies. Published toxicological data are used to compare measured analyte concentrations with potential toxicological effects (Table 1.5.1 page 22).

2.0 Materials and Methods

2.1 Materials

Methanol, acetonitrile, acetonitrile with 0.1% ammonium acetate and water with 0.1% ammonium acetate were purchased from Sigma-Aldrich, Dublin, Ireland and were of HPLC grade or LC-MS grade. Dichlorodimethylsilane and toluene, HPLC grade, were also purchased from Sigma-Aldrich, Dublin, Ireland. Pharmaceuticals for investigation included trimethoprim (≥98%), caffeine (≥99%) were purchased from Fluka (Buchs, Switzerland), bezafibrate (≥98%), flurbiprofen (≥99%), indomethcin (≥99%), ibuprofen sodium salt (≥98%), mefenamic acid (≥99%), gemfibrozil (≥99%), salbutamol (≥98%), sulfamethoxazole (≥98%), furosemide (≥98%), carbamazepine (≥98%), nimesulide (≥98%) obtained from Sigma (Steinham, Germany) and salicylic acid (≥99%), propranolol hydrochloride (≥98%), clofibric acid (≥98%), diclofenac sodium salt (≥98%) and clotrimazole (≥98%) purchased from Aldrich (Steinham, Germany). Strata–X solid phase extraction cartridges were purchased from Phenomenex, United Kingdom.

1000mg/L stock solutions of each analyte were prepared in methanol and stored at 4^0C in the dark for optimum stability. Stock solutions were replaced periodically. Working standards were prepared by dilution using methanol from these stock solutions.

2.2 Glassware Preparation

All glassware used was silanised by rinsing thoroughly with a 10% (v/v) solution of dichlorodimethylsilane in toluene followed by two toluene rinses and then two methanol rinses. This was to prevent any pharmaceutical residue from adsorbing to the glassware.

2.3 Choice of Analytes

A range of analytes, representative of commonly used pharmaceuticals in human medicine, including analgesics, anti-inflammatories, β-blockers, anti-fungal agents and anti-convulsants was chosen for analysis. Selected analytes include both acidic and basic compounds with pK_a values ranging from 1.8 to 14.0. The frequency of detection in environmental aqueous samples reported in published literature and their presence on the list of the top one hundred prescribed compounds in Ireland in 2004 were also considered in the selection of analytes (HSE, 2004). The twenty selected analytes along with their respective pK_a values and chemical structures are presented in Table 2.3.1.

2.4 Sewage Samples

Amber glass bottles were used for the collection of 2.5 litres of sewage samples on site. All bottles were silanised prior to sampling. Three sewage treatment plants with six sampling sites were included in the sampling regime. Twenty-four hour composite samples were collected monthly from each site and transported to the laboratory. Auto samplers at each location were used to collect periodic aliquots. The aliquots were pooled and a two litre representative sample taken. The samples were adjusted to pH 4 using concentrated sulphuric acid and filtered through Whatman GF/C glass fibre filters to remove suspended solids on arrival. Samples were stored at 4^0C until analysed. All samples were analysed within forty-eight hours.

Compound	Therapeutic Class	Molecular Weight	pK_a	logP	$LogK_{OW}$	Reference
Bezafibrate	Anti-lipemic	361	3.6	4.2	-0.4	Bibic *et al*., 2007
						Weigel *et al*., 2004
Caffeine	CNS stimulant	194	14.0	-0.07	-0.1	Bones *et al.,* 2006
						Weigel *et al*., 2004
Carbamazepine	Anti-convulsant	236	13.9	2.45	2.7	Bones *et al.,* 2006
						Weigel *et al*., 2004
Clofibric Acid	Anti-lipemic	214	3.2	2.58	-1.3	Scheytt *et al.*, 2005
						Weigel *et al*., 2004
Clotrimazole	Anti-fungal agent	344	6.1	3.5	4.1	Bones *et al.,* 2006
						OSPAR, 2005
Diclofenac	Anti-inflammatory	318	4.2	1.13	-0.4	Bibic *et al*., 2006
						Weigel *et al*., 2004
Flurbiprofen	Anti-inflammatory	244	4.3	4.16	-	Bibic *et al.*, 2006
Furosemide	Loop diuretic	330	3.9	2.03	2.0	Bones *et al.,* 2006
Gemfibrozil	Lipid regulating agent	250	4.8	3.09	4.7	Brown *et al.*, 2007
Ibuprofen	Anti-inflammatory	206	4.3	3.97	0.3	Bibic *et al.*, 2007
						Weigel *et al*., 2004

Table 2.3.1 Therapeutic class, molecular weight, pK_a, logP and $LogK_{ow}$ values of target pharmaceuticals

Compound	Therapeutic Class	Molecular Weight	pK_a	logP	$LogK_{OW}$	Reference
Indomethcin	Anti-inflammatory	357	4.5	4.27	-	Bones *et al.,* 2006
Mefenamic Acid	Anti-inflammatory	241	4.2	5.12	5.1	Bones *et al*., 2006
Metoprolol	Beta blocker	267	9.4	1.95	0.6	Bibic *et al*., 2007 Weigel *et al*., 2004
Nimesulide	Anti-inflammatory	308	6.5	3.08		Alves *et al*., 2007
Pravastatin	Cholesterol lowering statin	446	4.7	-		Kobayashi *et al*., 2003
Propranolol Hydrochloride	Beta blocker	295	9.5	3.48	1.9	Bibic *et al*., 2007 Weigel *et al*., 2004
Salbutamol	$Beta_2$ agonist	239	9.2	-	0.01	Yamini *et al*., 2006
Salicylic Acid	Analgesic/Aspirin metabolite	138	3.5	2.36	1.2	Bones *et al.,* 2006
Sulfamethoxazole	Antibiotic	253	1.8, 5.6	6.89	0.48	Bibic *et al*., 2007 Zwiener, 2007
Trimethoprim	Antibacterial agent	290	3.2	0.91		Bibic *et al*., 2007

Table 2.3.1 Therapeutic class, molecular weight, pK_a, logP and $LogK_{ow}$ values of target pharmaceuticals

2.5 Method Development

Solid phase extraction (SPE) cartridges were used to pre-concentrate samples. A selection of six cartridges (Supelco Supelclean C8 and C18, Phenomenex Strata-X, Waters Oasis HLB, Varian Focus and Merck LiChrolut EN) was initially investigated for recovery of ten analytes. The ten selected compounds were representative of the full pK_a range. Prior to extraction the solid phase cartridges were washed with three column volumes (6 mL) of methanol followed by three column volumes of water to prepare for the sample matrix. A one litre sample that was spiked to a concentration of 5µg/L of the ten analytes was passed through each of the solid phase extraction cartridge using vacuum. Cartridges were washed with one column volume of water after the addition of sample, dried for thirty minutes using vacuum and then eluted with ten millilitres of methanol. Figure 2.5.1 outlines the main steps in the SPE protocol. After elution, samples were dried under nitrogen, re-suspended in methanol to a volume of one millilitre and analysed by high performance liquid chromatography.

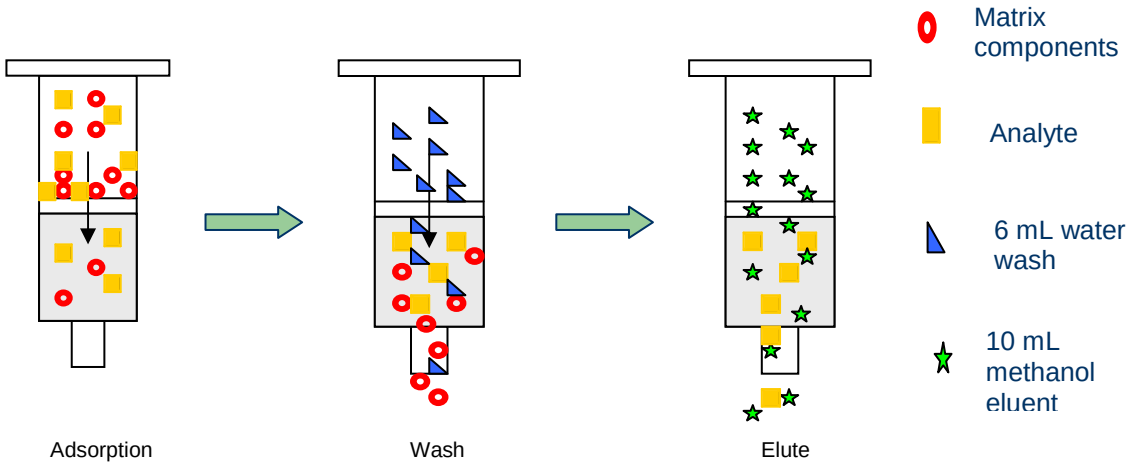


Figure 2.5.1 Schematic of SPE method

A Varian inert 9012 solvent delivery system, a Dynamax automatic sample injector model AI-200 and a Varian 9050 variable path length UV-VIS detector were used for the development of the standard HPLC separation. Resolution was achieved using a 150 x 4.6 mm end-capped Sunfire C_{18}

3.5µm reversed phase HPLC column (Waters, Ireland). A series of different mobile phases including methanol and acetonitrile as the organic phase and water with an ammonium acetate (pH 6.4) or formic acid (pH 2.8) buffer was investigated to determine the optimum. A 50µL injection volume and flow rate of 1.0 mL/min were employed and the absorbance was monitored using UV. The optimum wavelength (225nm) was determined in preliminary studies using scanning spectrometry. The optimised method was then transferred to a narrower bore Sunfire C_{18} column, 150 x 2.1 mm. The flowrate was adjusted accordingly to 0.3 mL/min and the injection volume was reduced to 10 µL. The optimised gradient is shown in Table 2.5.1.

Time	%A	%B
0	100	0
5	100	0
25	0	100
45	0	100

Table 2.5.1 HPLC Gradient. A: 80:20 (v/v water/acetonitrile) with 0.1% ammonium acetate. B: 20:80 (v/v water/acetonitrile) with 0.1% ammonium acetate.

A Bruker Daltonics Esquire~LC ion trap MS with an electrospray ionisation interface at atmospheric pressure was used for MS analysis. MS conditions were optimised separately. Standard solutions of each analyte were directly infused, using a Cole Parmer 74900 series syringe pump (Cole Parmer, Vernon Hills, IL, USA), into the mass spectrometer at a flowrate of 300µL/h. MS conditions were automatically optimised using Bruker Esquire software for each analyte. An average of the recorded parameters was used as the final focusing and ionisation parameters. The precursor peak with the greatest intensity was fragmented using tandem MS and the most abundant product ion was chosen for monitoring of the tandem MS signal.

The completed LC-ESI-MS/MS method for analysis used an Agilent 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) coupled to a Bruker Daltonics Esquire~LC ion trap MS with an electrospray ionisation interface at atmospheric pressure (Bruker Daltonics, Coventry, UK). A Waters Sunfire, narrow bore, 150 x 2.1 mm C_{18} column with 3.5µm particle size was used for separation. A flowrate of 0.3mL/min and an injection volume of 10 µL were employed. The LC-ESI-MS/MS system was controlled using Agilent Chemstation version A.06.01 and Bruker Daltonics Esquire Control version 6.08. Bruker Daltonics Data analysis software was used for data analysis. An overview of the method development is shown in Figure 2.5.2.

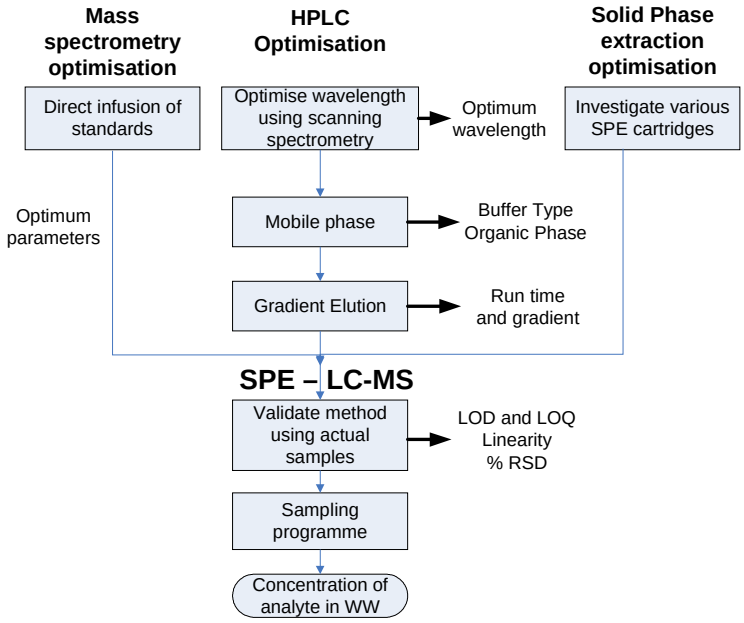


Figure 2.5.2 Overview of SPE-LC-MS/MS method development and validation

2.6 Method Validation

The SPE recovery of analytes was determined in WWTP influent and effluent sample matrices. Samples were spiked to a concentration of 5µg/L with a mixed standard of ten analytes and extracted using Strata-X cartridges in triplicate. The concentration recovered was compared to the initial spiking concentration. Blank samples (unspiked influent and effluent

samples) were also extracted to determine the concentration of analytes present in the sample before spiking and any background concentration was included in the calculation.

The precision, linearity and sensitivity of the overall SPE-LC-ESI-MS/MS method was investigated. Method precision was defined in terms of repeatability and reproducibility. Six replicate samples were spiked to a concentration of 1.40μg/L of each analyte using a mixed standard solution containing 2mg/L (of each of twenty analytes). Influent and effluent samples were similarly spiked to give six concentrations of each analyte ranging from 0.60 – 2.80 μg/L and analysed. Linearity was determined between the peak area and concentration using regression analysis. Sensitivity of the method (limits of detection (LOD) and limits of quantitation (LOQ)) was determined in both influent and effluent sample matrix. The LOD was calculated as the analyte concentration that gave a response equal to three times the signal to noise ratio. The LOQ was defined as the analyte concentration to give a response equal to ten times the signal to noise ratio (Ahuja and Scypinski, 2001). Both were calculated based on repeated injections (n=6) of a 1mg/L standard.

2.7 Matrix Effects

The effect of matrix components on the LC-MS/MS analysis was investigated. Two studies were undertaken to determine the level of signal suppression or enhancement due to matrix components. Suppression or enhancement was determined as the change in the intensity and peak area for individual analytes.

2.7.1 Addition Post Extraction

Influent and effluent samples were extracted using SPE as per the method developed for sample analysis. Each of the target pharmaceuticals (Table 2.3.1) was then added to the extract to yield a compound concentration of 2μg/L. The samples were analysed using the LC-MS/MS method outlined

in Section 2.5 and compared to a 2 μg/L standard solution. The difference in response between the two samples is used to quantify the extent of matrix effects on the compound under the given analytical conditions. Results are reported in Section 3.3.1.

2.7.2 Post Column Infusion

Both influent and effluent extracts were injected on to the LC column to achieve separation of the matrix components. A 0.5mg/L standard solution of individual compounds (nimesulide, mefenamic acid, trimethoprim and carbamazepine) was continually infused into the flow post column and pre electrospray interface (Figure 2.7.1). The analyte signal was monitored for the duration of the run. This allowed the effects of matrix components over the 45min run time to be analysed. Blank samples (sample extracts) were analysed in all cases to determine any interference from trace levels already present in the sample. The results obtained are reported in Section 3.3.2.

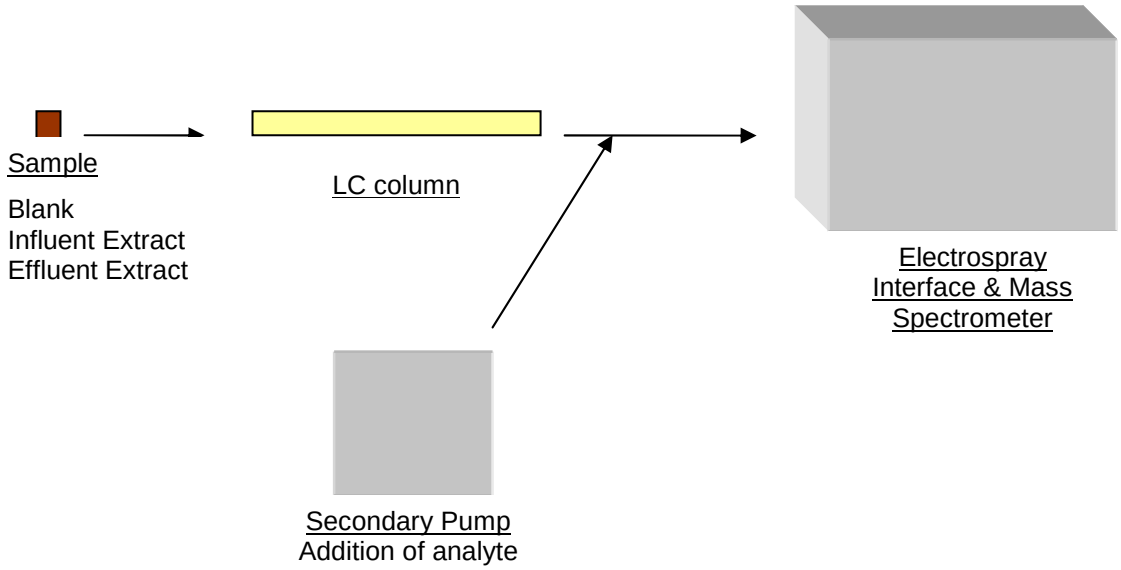


Figure 2.7.1 Post column infusion experimental setup.

2.7.3 Standard Additions

A mixed standard solution containing 5 mg/L of each of the twenty analytes was prepared and used for standard additions in monthly samples. Depending on the volume of sample collected, each sample was divided into three or four aliquots of 500 mL. One sample was extracted and analysed without any addition and the remaining samples were spiked with increasing concentrations of the analyte standard solution to yield a concentration of 1, 2 and 3 μg/L in the samples. The samples were then extracted and analysed using the LC-MS/MS method outlined in Section 2.5. The peak area counts for each analyte were determined using Bruker Daltonics EsquireLC 4.5 Data Analysis software version 3.0. Regression analysis between the peak area counts and concentration was used to calculate the concentration of each analyte in the raw sample. The variation of analyte signal suppression between influent and effluent matrices could be determined by comparison of the same addition in both matrices. Also, the standard additions method identified the complete suppression of analyte signal due to matrix effects in certain cases.

2.8 Metal Analysis

The presence of metals in wastewater streams was investigated to assess the potential for metal related interference in analysis of samples. ICP-AES analysis was completed on influent and effluent samples from the three wastewater treatment plants. As this facility was not available the analysis was performed by an INAB (Irish National Accreditation Board) accredited laboratory, TMS Environment Ltd. Results are presented in Section 3.4.

2.9 Surfactant Analysis

The effect of the surfactant linear alkyl benzene sulphonate (LAS) (Figure 2.9.1) on LC-MS/MS analysis was investigated. LAS is an anionic surfactant commonly used in detergents and cleaners (Clara *et al.*, 2007). An almost complete removal (>99%) of LAS during wastewater treatment has been reported previously (Temmink and Klapwijk, 2004). LAS is a highly polar compound which can be concentrated on SPE cartridges (Schröder, 1999) and is known to impair electrospray ionisation signals of various compounds (Ishihama *et al.*, 2000). For these reasons the effect of LAS on the signal suppression of four compounds (nimesulide, mefenamic acid, carbamazepine and trimethoprim) was investigated.

Analyte standard solutions were prepared and analysed as above. The same concentration of analytes was also prepared in a 2% LAS solution and analysed. The results were compared with respect to signal intensity, peak shape and retention time. Results are presented in Section 3.5.

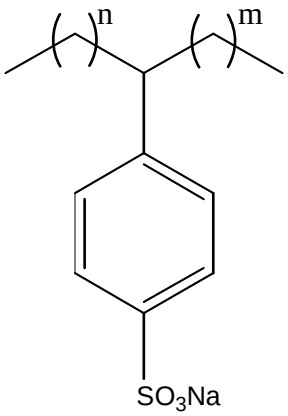


Figure 2.9.1. LAS structure

3.0 Results

3.1 Method Development

Sample pre-treatment

Analyte recoveries from six commercially available solid phase extraction cartridges were investigated initially. The mechanism of retention for both C_8 and C_{18} cartridges is the same as that of reverse phase chromatography - non-specific hydrophobic interactions between the analytes and the hydrocarbon chain of the stationary phase. Strata-X has both a hydrophobic and hydrophilic entity and is suitable for the preconcentration of both polar and non-polar analytes (Figure 3.1.1). Oasis HLB also has hydrophilic and hydrophobic entities and is suitable for both polar and non-polar analyte recovery. The Focus cartridge produced by Varian is also suitable for the concentration of polar and non-polar compounds. It incorporates four retention mechanisms: proton donor, proton acceptor, polar and hydrophobic. Merck LiChrolut EN is a mixed polarity copolymer sorbent and is suitable for the preconcentration of both polar and non-polar compounds. Recoveries in excess of 100% were found for some analytes. This may be a result of variances in the matrix as a real sample matrix was used for this investigation (Section 3.3). Strata-X yielded the highest average recovery for the analytes investigated and was used for further investigations (Table 3.1.1). An extraction pH of 4 was used as it had been shown previously to give optimum recovery for similar compounds (Bones *et al.*, 2006).

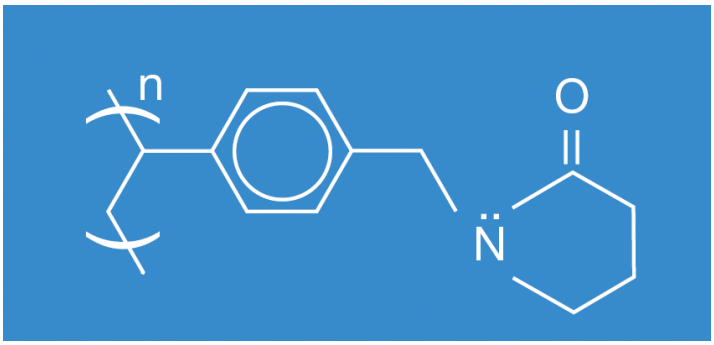


Figure 3.1.1 Structure of Strata-X (Phenomenex)

	C_8	C_{18}	Strata-X	HLB	Focus	LiChrolut
Bezafibrate	99.3	65.0	110.3	110.2	97.3	71.7
Clofibric acid	93.7	77.3	94.5	90.5	61.7	61.5
Diclofenac	75.3	43.4	76.6	71.1	59.4	42.1
Flurbiprofen	137.8	89.5	130.5	119.8	104.7	87.5
Gemfibrozil	90.3	58.5	87.5	84.1	60.7	37.3
Ibuprofen	75.9	59.8	73.6	76.2	56.9	49.9
Indomethcin	117.7	64.1	123.4	112.7	102.9	49.0
Mefenamic acid	101.7	62.7	101.2	92.0	84.9	45.9
Salicylic acid	5.7	16.3	107.8	90.5	21.3	38.9

Table 3.1.1 Percentage recovery of analytes from six SPE cartridges**.**

LC-ESI-MS/MS

LC-MS/MS was the method of choice for this work due to its applicability to a wide range of compounds including polar, thermo-labile compounds. Also, MS allows for the positive identification and quantification of compounds.

A Waters Sunfire C_{18}, 150 x 2.1 mm column with a 3.5 μm particle size was used for chromatographic separation of selected analytes. Separation was monitored by UV at 270nm which was determined as the optimum using scanning spectrometry. A series of different mobile phases including methanol and acetonitrile as the organic phase and water with an ammonium acetate or formic acid buffer was investigated. A simple gradient of 20-80% acetonitrile with 0.1% ammonium acetate in both the aqueous (pH 6.4) and organic phase gave sufficient separation of the 20 analytes for detection.

Over the course of the separation two analytes (nimesulide and flurbiprofen) were found to co-elute at 22.3 and 22.5 mins. Caffeine and trimethoprim also have close retention times of 2.0 and 2.1 mins. Due to the complexity of the sample matrix and presence of matrix components UV detection was not sensitive enough for the quantitation of analytes and therefore MS detection was used. The separation and detection of analytes using this method are illustrated in Figure 3.1.2 a and b.

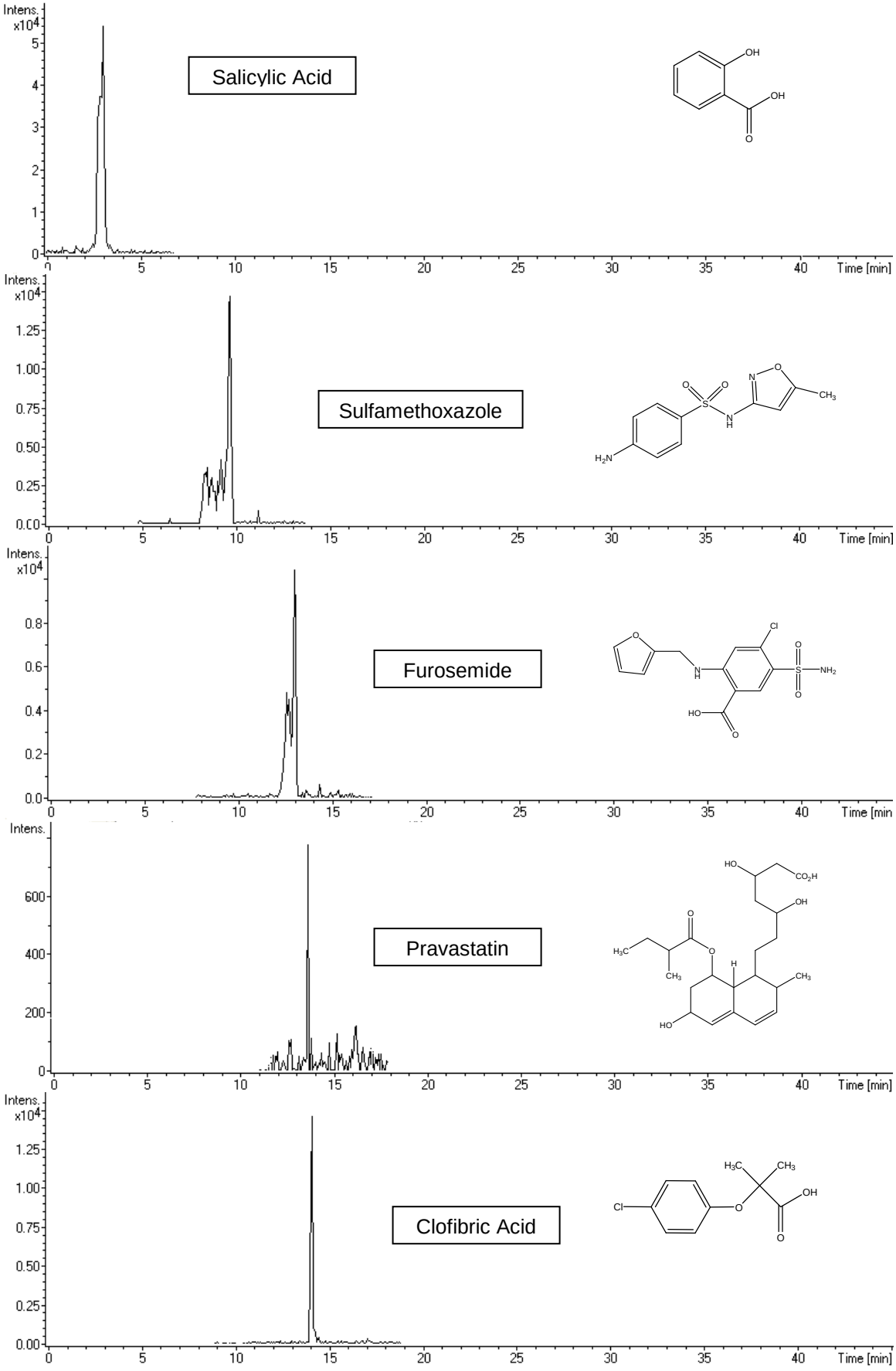


Figure 3.1.2a Chromatograms of a 5µg/mL standard in influent matrix analysed using negative ionisation

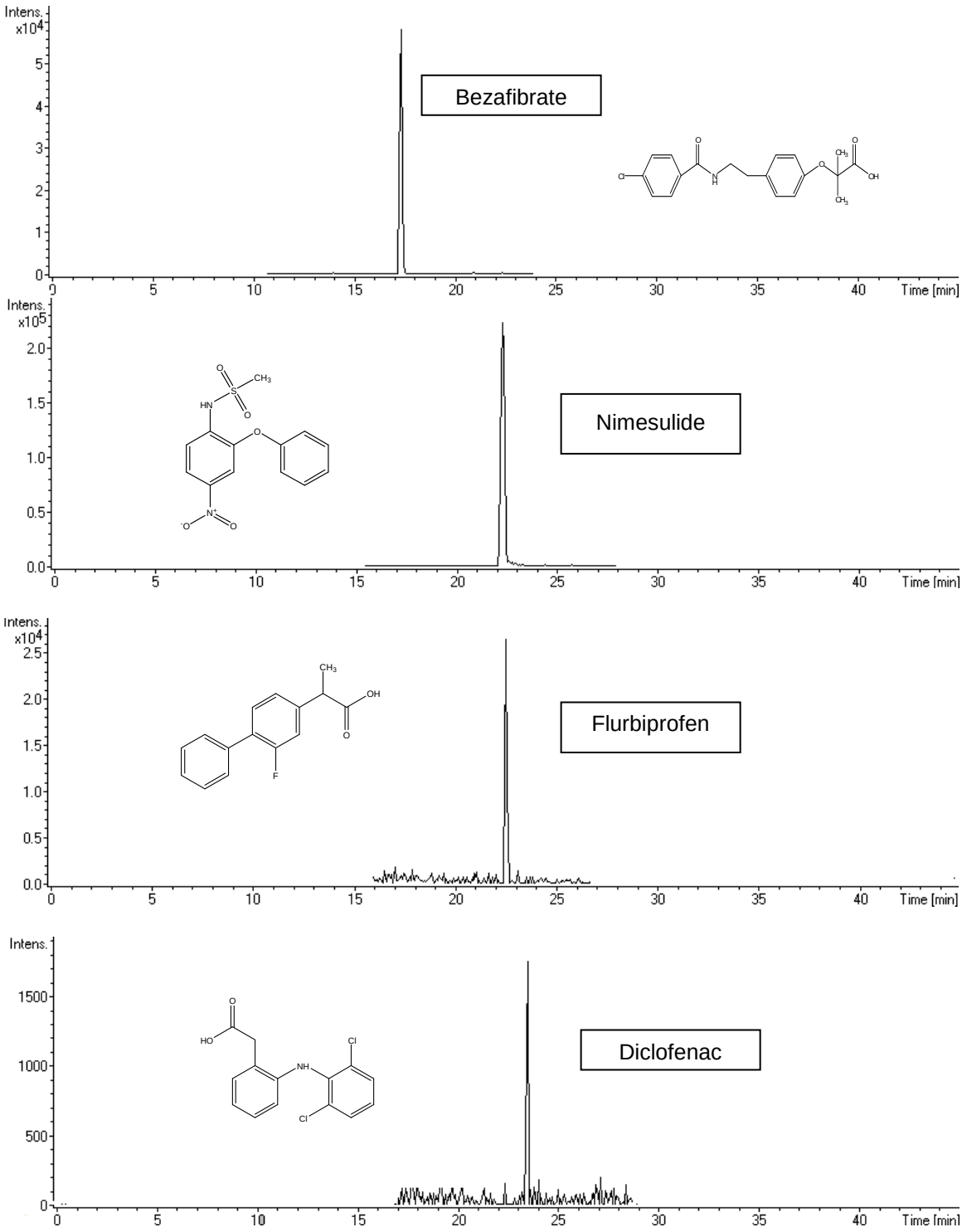


Figure 3.1.2a (continued) Chromatograms of a 5µg/mL standard in influent matrix analysed using negative ionisation

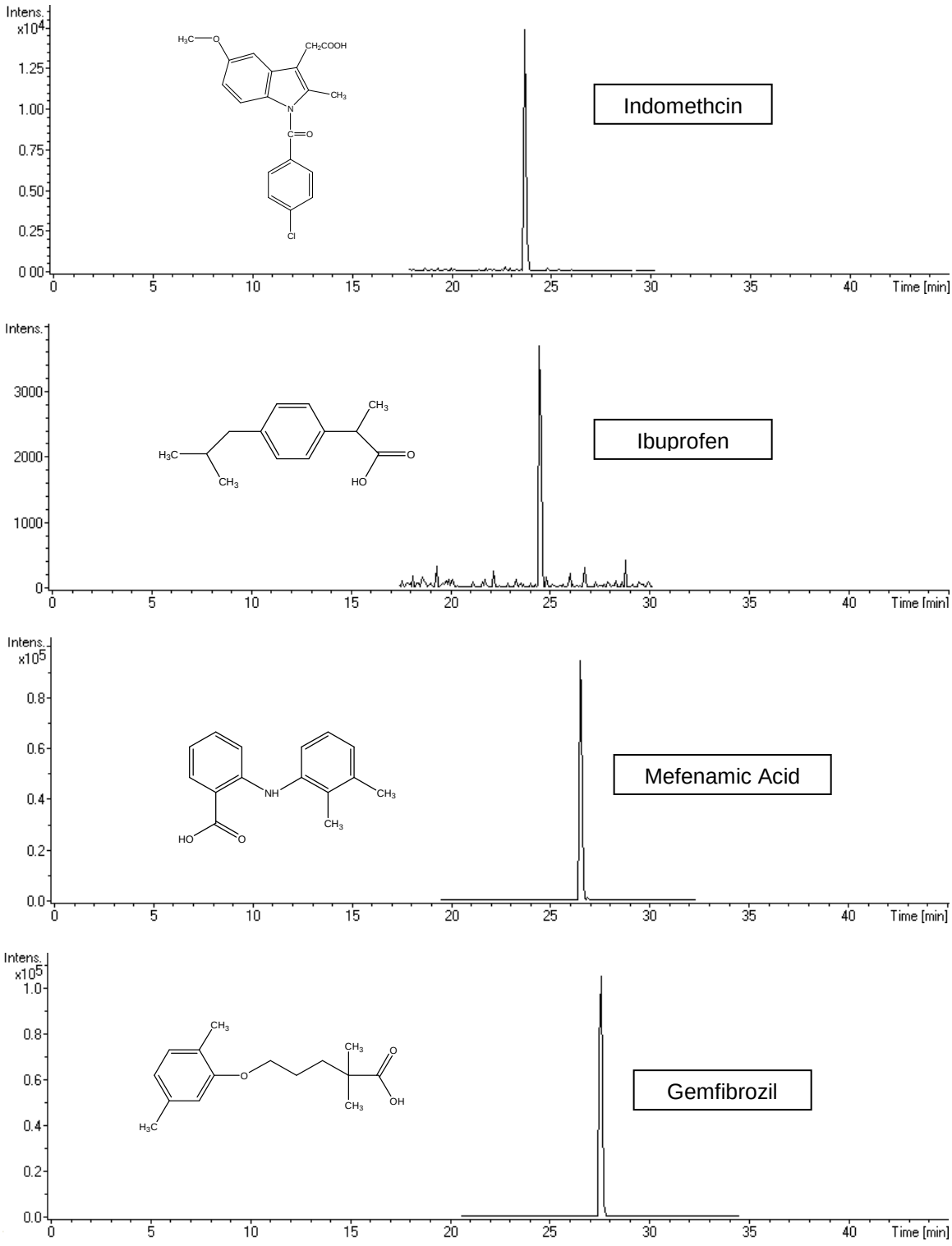


Figure 3.1.2a (continued) Chromatograms of a 5µg/mL standard in influent matrix analysed using negative ionisation

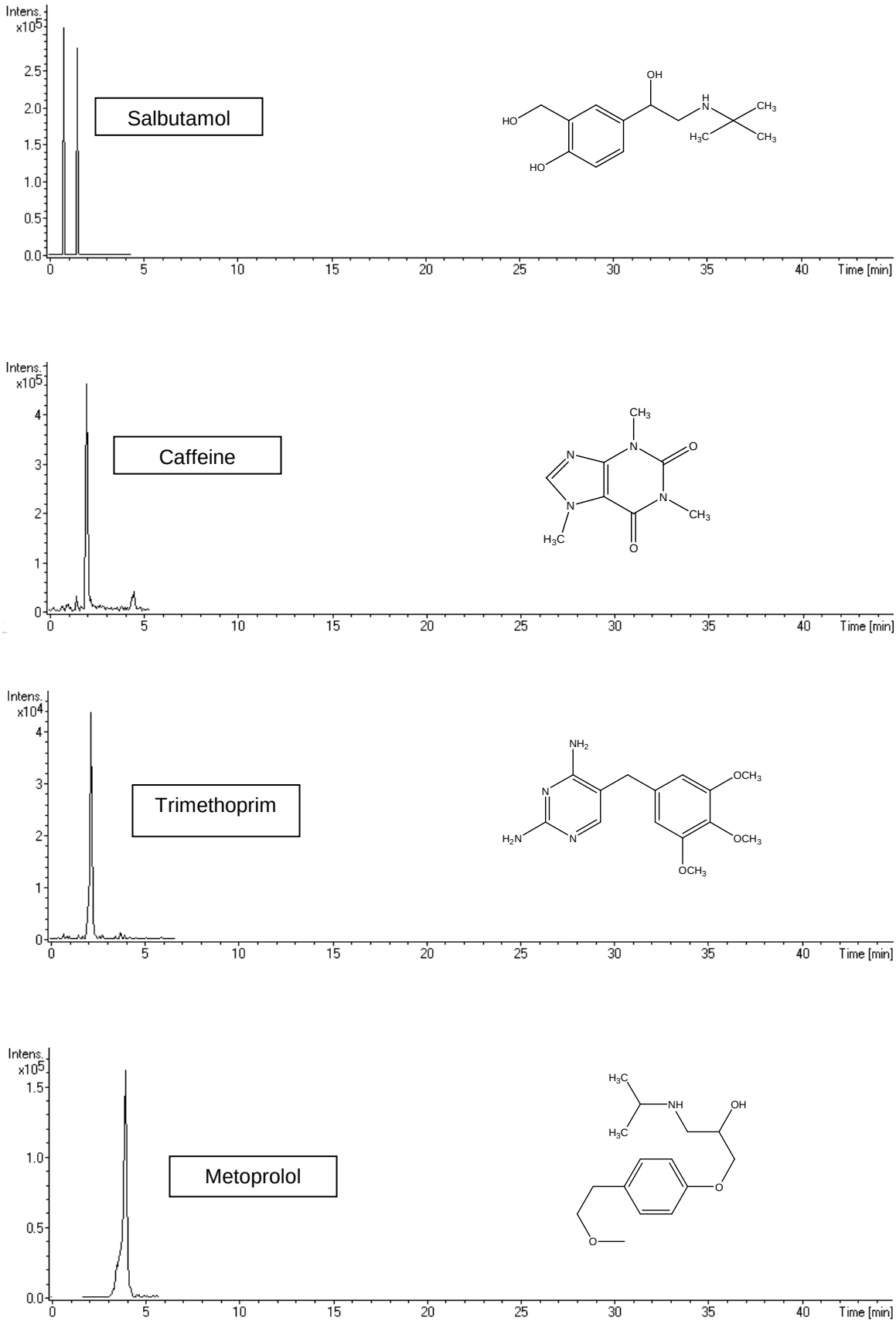


Figure 3.1.2b Chromatograms of a 5µg/mL standard in influent matrix analysed using positive ionisation.

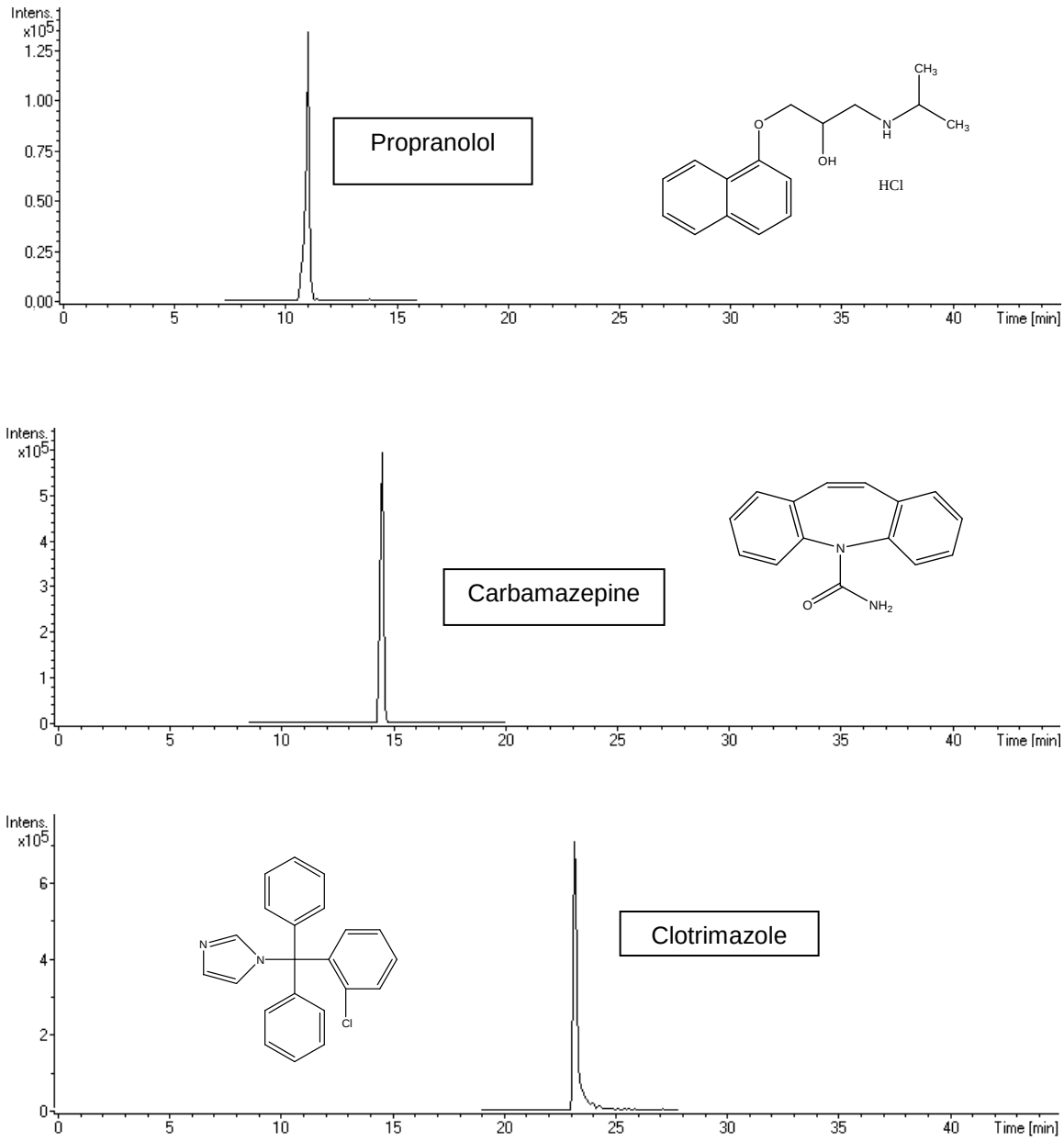


Figure 3.1.2b (continued). Chromatograms of a 5µg/mL standard in influent matrix analysed using positive ionisation.

Mass spectrometry parameters were optimised by direct infusion of standards for each analyte individually. The parent ion, with the strongest intensity for each analyte, $[M\text{-}H]^-$, $[M\text{-}COOH]^-$ and $[M+H]^+$ ions were selected in negative and positive mode respectively. Initial samples, from September 2006 to February 2007, were analysed using only parent ions and the results are reported in Appendix A. To improve the identification of analytes, monitoring of product ions was included in the method. Product ions were determined by fragmentation of the parent ion under MS/MS

conditions. Parent and product ions are listed in Table 3.1.2. Optimum MS parameters for the analytes are tabulated in Tables 3.1.3 and 3.1.4. No product ions were observed for salicylic acid, flurbiprofen or sulfamethoxazole in negative mode ionisation. Caffeine also yielded no fragmentation ion in positive mode ionisation. Monitoring of these compounds was consequently restricted to the precursor ions.

	R_t	Parent ion	Product ion
Negative ionisation			
Salicylic acid	3.0	137	-
Sulfamethoxazole	9.7	252	-
Furosemide	13.0	329	285
Pravastatin	13.6	423	321
Clofibric acid	14.1	213	127
Bezafibrate	17.3	360	274
Nimesulide	22.3	307	229
Flurbiprofen	22.5	199	-
Diclofenac	23.5	250	214
Indomethcin	23.7	213	297
Ibuprofen	24.5	205	159
Mefenamic acid	26.5	240	196
Gemfibrozil	27.5	249	121
Positive ionisation			
Salbutamol	0.8/1.5	240	195
Caffeine	2.0	194	-
Trimethoprim	2.1	291	230
Metoprolol	4.0	268	159
Propranolol	11.0	260	183
Carbamazepine	14.5	237	194
Clotrimazole	23.1	277	165

Table 3.1.2 Retention times (R_t) and ions for LC-MS/MS monitoring.

	Salicylic acid	Clofibric acid	Bezafibrate	Flurbiprofen	Diclofenac	Indomethcin
Capillary	4500	4500	4500	4500	4500	4500
End plate offset	626	592	1085	500	1200	649
Skim 1	15	46	15	28	32	42
Skim 2	5.4	13.5	4.7	8.1	6.4	5.9
Lens 1	2.0	4.3	3.0	1.3	1.5	6.1
Lens 2	41.5	50.7	39.2	30.0	30.0	81.6
Cap Exit Offset	53.3	50.0	50.0	50.0	50.0	68.0
Octopole	1.1	1.6	1.0	1.8	1.9	1.0
Octopole Δ	1.9	2.2	1.4	2.1	2.4	1.5
Octopole RF	127.9	50.0	300.0	181.1	230.3	189.3
Trap drive	29.4	37.9	33.3	35.9	34.7	28.9

Table 3.1.3 Optimum parameters for analytes analysed in negative ESI mode**.**

	Ibuprofen	Mefenamic acid	Nimesulide	Furosemide	Sulfamethoxazole	Gemfibrozil
Capillary	4500	4500	4500	4500	4500	4500
End plate offset	649	695	672.1	867.2	500	500
Skim 1	15.0	15.0	19.2	15.0	15.0	26.1
Skim 2	5.9	4.2	5.7	4.7	4.9	6.9
Lens 1	2.7	2.3	1.4	2.4	1.3	1.5
Lens 2	36.9	46.1	30.0	42.6	30.0	30.0
Cap Exit Offset	69.7	51.6	54.9	51.6	50.0	50.0
Octopole	1.9	1.0	1.9	2.0	1.5	1.9
Octopole Δ	2.3	1.4	1.7	1.8	1.5	2.5
Octopole RF	160.7	222.1	181.2	254.9	164.8	201.6
Trap drive	30.3	29.7	39.4	39.4	34.9	33.8

Table 3.1.3 (Continued) Optimum parameters for analytes analysed in negative ESI mode.

	Clotrimazole	Propranolol	Metoprolol	Carbamazepine	Trimethoprim	Caffeine	Salbutamol
Capillary	4500	4500	4500	4500	4500	4500	4500
End plate offset	707	672	752	534	718	500	741
Skim 1	15	15	94	15	26	15	15
Skim 2	6.4	6.9	4.7	6.6	5.9	6.2	6.4
Lens 1	0.8	3.8	1.7	1.0	1.5	0.9	3.2
Lens 2	30.0	59.8	30.0	30.0	30.0	38.1	51.8
Cap Exit Offset	73.0	63.1	66.4	71.3	76.2	51.6	50.0
Octopole	3.0	3.2	2.8	2.9	2.6	2.6	2.7
Octopole Δ	2.0	2.3	2.0	2.1	1.9	1.5	1.5
Octopole RF	205.7	209.8	173.0	160.7	160.7	173.0	185.3
Trap drive	38.4	38.0	36.1	35.9	34.7	33.5	36.3

Table 3.1.4 Optimum parameters for analytes analysed in positive ESI mode.

3.2 Method Validation

The SPE-LC-MS/MS method was validated in both influent and effluent sample matrices and validation data are presented in Tables 3.2.1. and 3.2.2. Linearity was determined using regression analysis between the area ratios and concentration. Correlations of R^2>0.9, with the exception of ibuprofen, were obtained over a concentration range of 0.60-2.90μg/L. Limits of detection and quantitation (LOD and LOQ) were defined as the concentration yielding a signal to noise ratio of 3:1 and 10:1 respectively. As seen in Table 3.2.1 the LOD ranged from 0.002 – 0.855 μg/L in influent and 0.001 – 0.743μg/L in effluent samples. LOQ ranged from 0.005 – 2.850 μg/L in influent samples and 0.003 – 2.478 μg/L in effluent samples. The precision of the overall method was determined from six replicates of low-level spiked samples (1.40μg/L). Precision varied by less than 10% in most cases. High variability (34.7%) was observed for reproducibility of ibuprofen while repeatability varied by only 9% as shown in Table 3.2.2. Bezafibrate, clofibric acid, flurbiprofen, furosemide, gemfibrozil, indomethcin, metoprolol, pravastatin and salicylic acid had percentage reproducibility values above 10%. With the exception of caffeine (10.2%) repeatability was below 10% for all analytes. Variability in precision has been shown to increase with increased complexity in a matrix (Bones *et al*., 2006). Six samples were used to determine the precision of the overall method. As there is sample to sample variation in matrix components a precision of ~10% in results was seen as acceptable in this study.

	Linearity (R^2)	LOD (µg/L)		LOQ (µg/L)	
		Influent	Effluent	Influent	Effluent
Bezafibrate	0.9854	0.033	0.050	0.112	0.150
Caffeine	0.9894	0.280	0.138	0.934	0.460
Carbamazepine	0.9951	0.010	0.004	0.034	0.013
Clofibric acid	0.9813	0.222	0.335	0.740	1.118
Clotrimazole	0.9932	0.010	0.004	0.034	0.013
Diclofenac	0.9972	0.855	0.743	2.850	2.478
Flurbiprofen	0.9907	0.743	0.489	2.478	1.629
Furosemide	0.9205	0.094	0.109	0.313	0.365
Gemfibrozil	0.9749	0.026	0.010	0.086	0.032
Ibuprofen	0.8558	0.228	-	0.760	-
Indomethcin	0.9712	0.263	0.283	0.877	0.792
Mefenamic acid	0.9222	0.020	0.004	0.060	0.013
Metoprolol	0.9831	0.633	0.097	2.111	0.324
Nimesulide	0.9655	0.002	0.001	0.005	0.003
Pravastatin	0.9371	0.072	0.047	0.239	0.156
Propranolol	0.9618	0.007	0.017	0.022	0.057
Salbutamol	0.9558	0.008	0.155	0.027	0.518
Salicylic acid	0.9864	0.028	0.115	0.093	0.383
Sulfamethoxazole	0.9799	0.072	0.166	0.241	0.553
Trimethoprim	0.9126	0.171	0.020	0.570	0.067

Table 3.2.1 Linearity and detection and quantitation limits of the method.

	% RSD	
	Reproducibility	Repeatability
Bezafibrate	13.7	3.5
Caffeine	4.7	10.2
Carbamazepine	3.6	2.6
Clofibric acid	14.4	4.9
Clotrimazole	6.3	4.4
Diclofenac	5.8	3.4
Flurbiprofen	13.5	1.1
Furosemide	18.2	0.1
Gemfibrozil	17.7	3.7
Ibuprofen	34.7	9.0
Indomethcin	11.4	2.5
Mefenamic acid	5.3	0.2
Metoprolol	14.5	2.4
Nimesulide	8.1	5.0
Pravastatin	13.9	4.7
Propranolol	6.6	5.3
Salbutamol	8.4	2.0
Salicylic acid	15.6	1.4
Sulfamethoxazole	8.0	1.0
Trimethoprim	8.7	4.0

Table 3.2.2 Validation data for precision of overall method

3.3 Matrix Effects

The negative effect of matrix components on electrospray mass spectrometry has been identified previously in environmental and clinical samples (Petrović *et al.*, 2005; Taylor, 2005). The effect of influent and effluent matrix components on the LC-MS/MS method used in this study was investigated using two methods - 1) addition post extraction and 2) post column infusion.

3.3.1 Addition Post Extraction

The analyte response from a standard solution of selected compounds was compared to that obtained from the same concentration of the compounds in influent and effluent SPE extract. The results of these experiments are presented in Table 3.3.1.

Analyte signal suppression was observed in both influent and effluent matrices. Suppression in influent samples was in general greater than that observed in effluent samples. For example, the signal for clotrimazole was suppressed by 25.8% in the effluent matrix with a corresponding suppression of 43.9% in the influent matrix. Similarly, the suppression observed for nimesulide reduced significantly from influent to effluent where the suppression in effluent matrices was <5% while in the influent it was 18.5%. Ibuprofen was most affected by the presence of matrix components with >70% suppression observed in both influent and effluent matrices. Trimethoprim and gemfibrozil both had significant levels of suppression in influent samples (32.8% and 45.6%) while that in effluent samples was <5%. Carbamazepine, mefenamic acid, nimesulide and trimethoprim (in bold in Table 3.3.1) were selected for further investigations on matrix components as they represent a range of retention times and ionisation modes.

	% Ion Suppression	
	Influent	Effluent
Bezafibrate	<5	<5
Caffeine	28.3	5.4
Carbamazepine	**37.7**	**23.0**
Clofibric acid	15.6	29.7
Clotrimazole	43.9	25.8
Diclofenac	23.7	27.7
Flurbiprofen	60.5	37.2
Furosemide	-48.7	10.7
Gemfibrozil	45.6	<5
Ibuprofen	77.6	72.0
Indomethcin	11.8	44.2
Mefenamic acid	**<5**	**25.8**
Metoprolol	52.8	39.1
Nimesulide	**18.5**	**<5**
Pravastatin	15.6	27.5
Propranolol	88.7	32.7
Salbutamol	<5	77.0
Salicylic acid	<5	56.3
Sulfamethoxazole	-60.3	33.8
Trimethoprim	**38.2**	**<5**

Table 3.3.1 % Ion suppression due to matrix components.

3.3.2 Post Column Infusion

These four compounds (carbamazepine, mefenamic acid, nimesulide and trimethoprim), two detected using positive mode ionisation and two using negative mode ionisation and with varying retention times were chosen as representatives for this investigation. The results are presented in Figures 3.3.1, 3.3.2, 3.3.4, 3.3.5. In general signal suppression was greater in influent samples. Signals for nimesulide and mefenamic acid were almost completely suppressed for the duration of the run with the addition of influent matrix components and at the retention times of 22.3 and 26.5 minutes respectively. The signal suppression observed with effluent samples was less than that in influent samples. There was minimal suppression for the majority of the run time with short time frames showing complete suppression. This indicates a significant removal of suppressing compounds during treatment. Similarly the signal intensity for carbamazepine (Rt = 14.5 min) and trimethoprim (Rt = 2.1 min) in the influent matrix was suppressed to a greater degree than that observed in the effluent matrix.

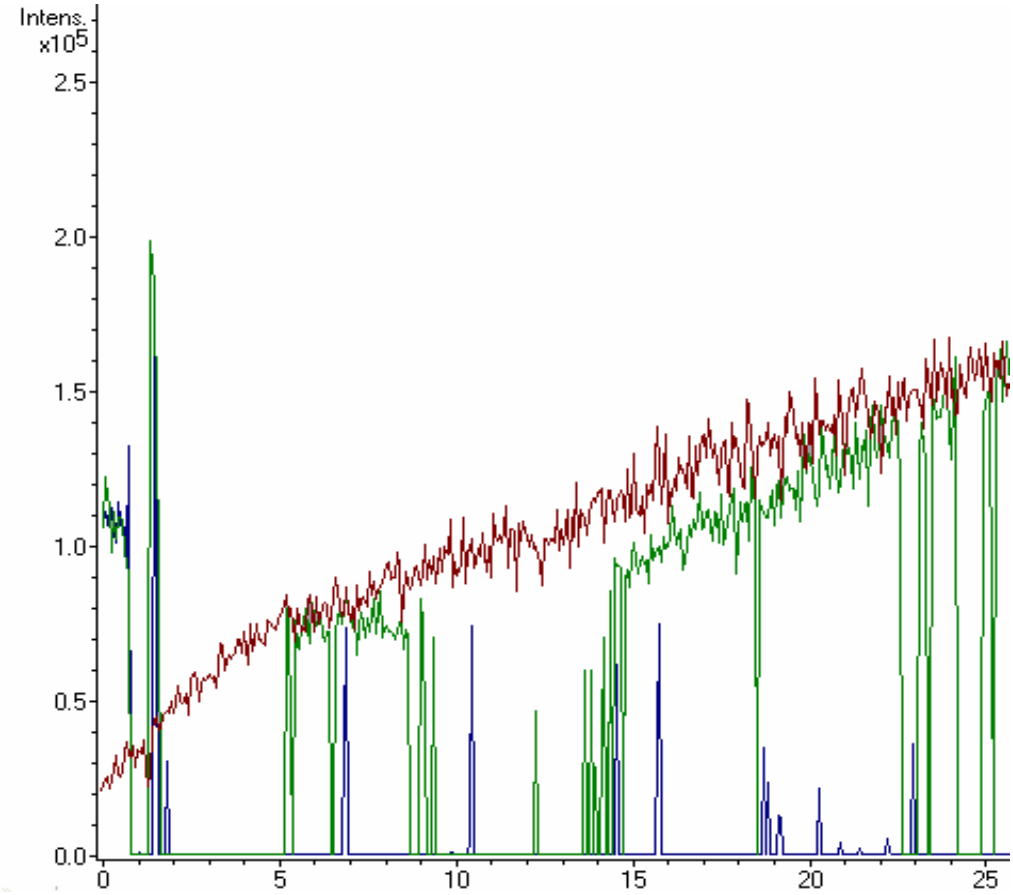


Figure 3.3.1 Effect of signal suppression on nimesulide (negative mode).

Brown line: Infused nimesulide standard (0.5mg/L); Blue line: Infused nimesulide standard (0.5mg/L) + extracted influent sample; Green line: Infused nimesulide standard (0.5mg/L) + extracted effluent sample.

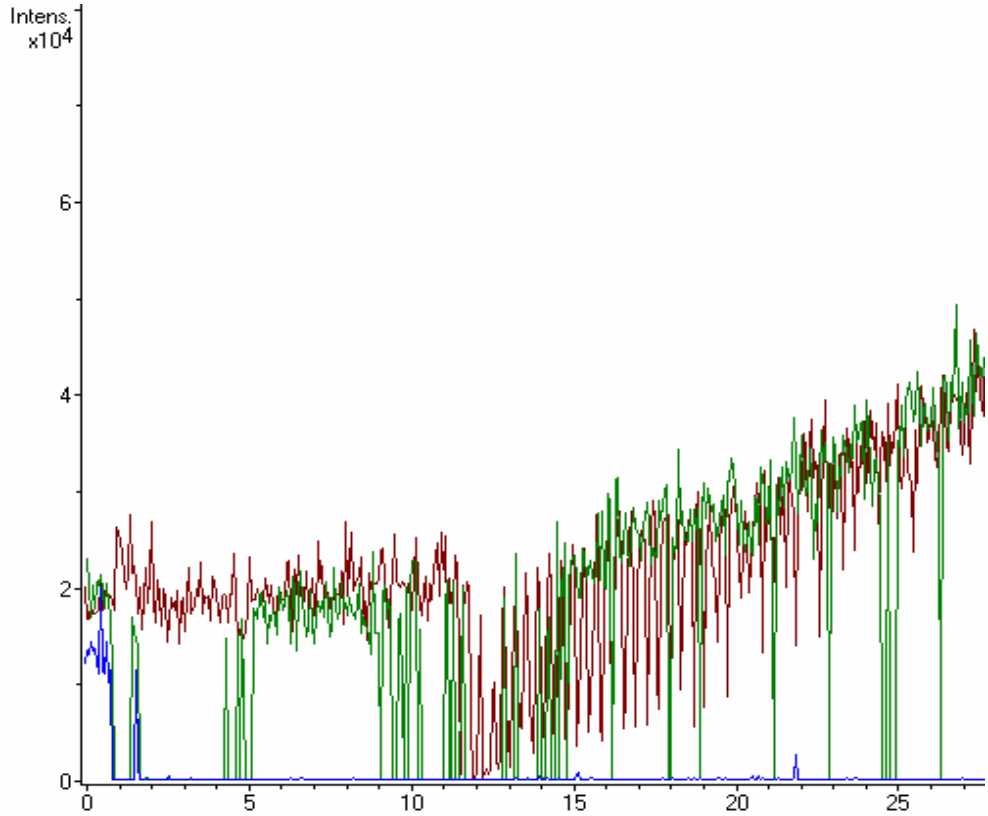


Figure 3.3.2 Effect of signal suppression on mefenamic acid (negative mode).

Brown line: Infused mefenamic acid standard (0.5mg/L); Blue line: Infused mefenamic acid standard (0.5mg/L) + extracted influent sample; Green line: Infused mefenamic acid standard (0.5mg/L) + extracted effluent sample.

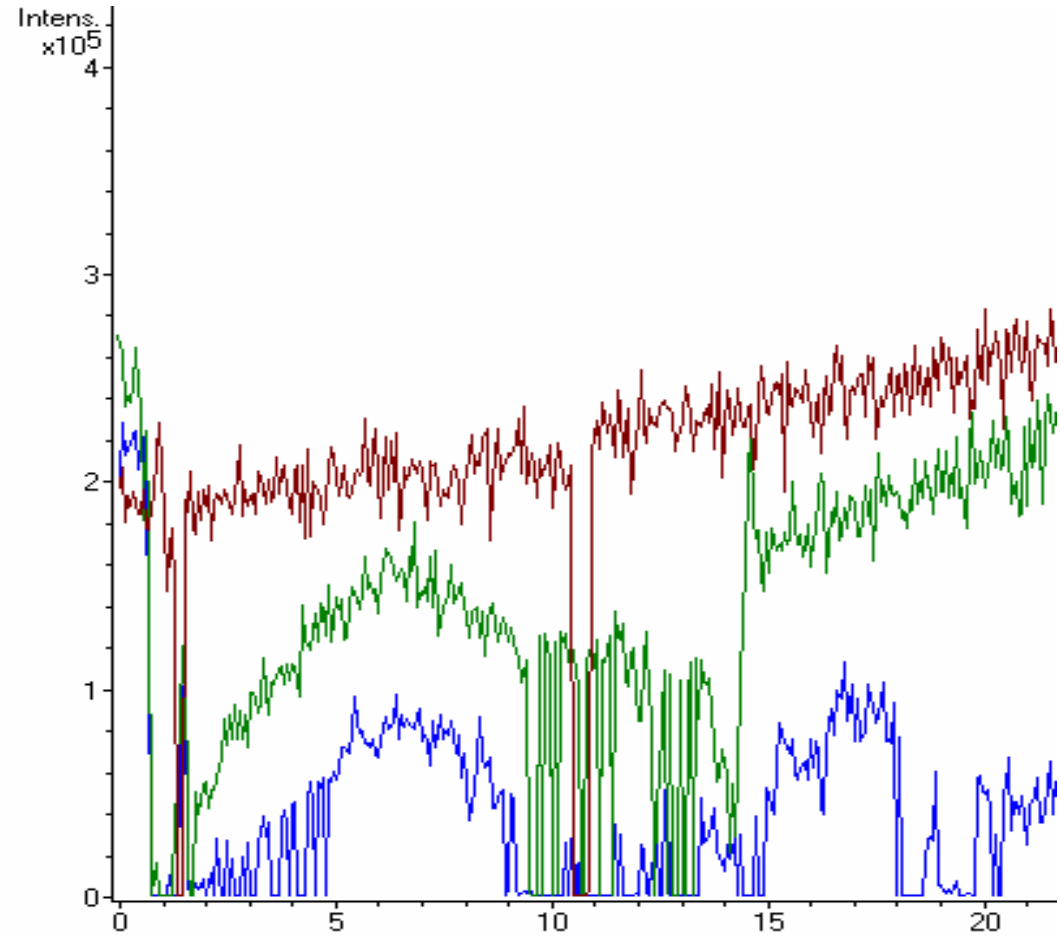


Figure 3.3.3 Effect of signal suppression on Carbamazepine (Positive mode).

Brown line: Infused carbamazepine standard (0.5mg/L); Blue line: Infused carbamazepine standard (0.5mg/L) + extracted influent sample; Green line: Infused carbamazepine standard (0.5mg/L) + extracted effluent sample.

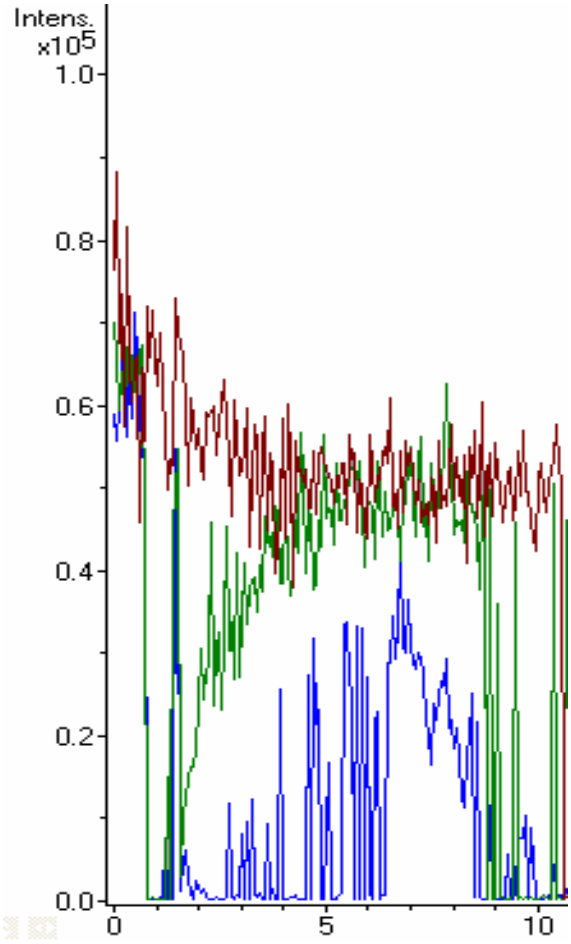


Figure 3.3.4 Effect of signal suppression on trimethoprim (Positive mode).
Brown line: Infused trimethoprim standard (0.5mg/L); Blue line: Infused trimethoprim standard (0.5mg/L) + extracted influent sample; Green line: Infused trimethoprim standard (0.5mg/L) + extracted effluent sample.

3.3.3 Standard Additions

To allow for accurate quantification of compounds in influent and effluent samples, an internal standard or standard additions can be used to correct any analyte signal suppression or enhancement. In this study standard additions were used because in addition to allowing analyte quantification they further illustrate the effect of signal suppression. Graphical representation of the data from the three plants in November 2007 was chosen to illustrate the method of standard additions.

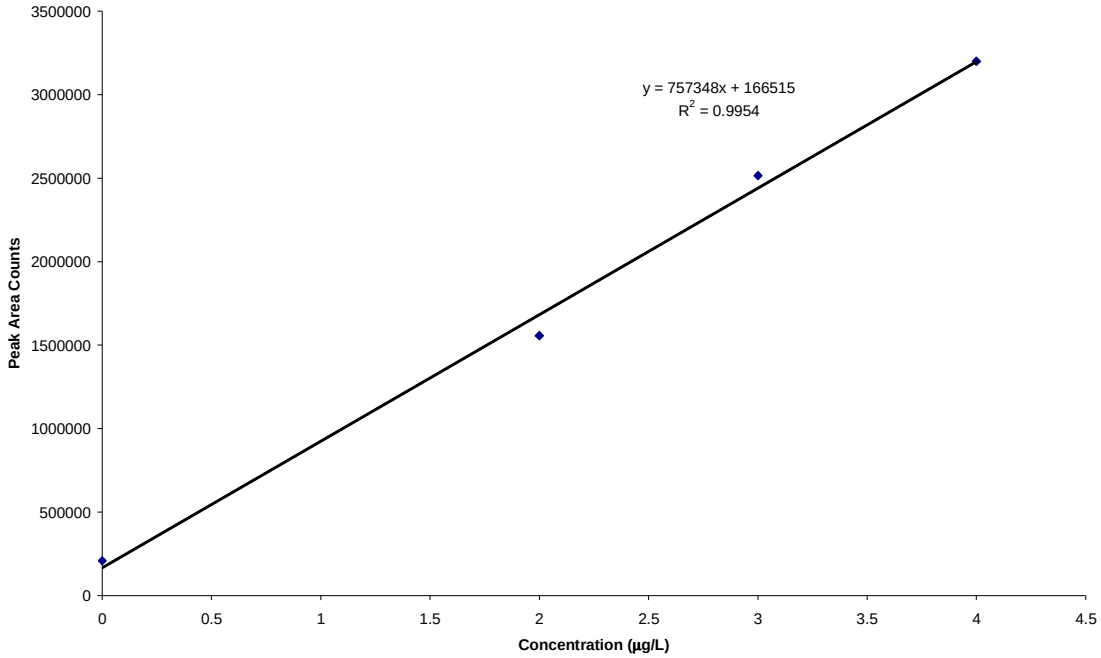
Figures 3.3.5 – 3.3.9 show data from the Leixlip plant. The linear graphs (Figures 3.3.5 – 3.3.8) highlight the >0.9 regression achieved from standard additions in both influent and effluent samples. Quantification of individual compounds was possible using these graphs. Also, the effect of matrix suppression is evident. For example, the peak area counts for the standard additions of carbamazepine were ~15% greater in effluent samples than influent samples (Figure 3.3.5). Figure 3.3.6 also highlights a difference in analyte response between influent and effluent samples. The peak area count for the 2 μg/L addition in the influent sample is ~1750000 while the equivalent in the effluent sample is 100% greater at ~3500000. Trimethoprim was detected and quantified in the effluent sample collected from Leixlip in November 2007 (Figure 3.3.8a), however, it was not detected in the corresponding influent. Figure 3.3.9 shows the extracted ion chromatograms for trimethoprim from the influent sample and three subsequent standard additions. It is clear that trimethoprim with a retention time of 2.1 minutes is absent from all chromatograms which clearly demonstrates that complete signal inhibition occurred.

The November data from the Swords plant are presented in Figures 3.3.10 - 3.3.18. Again, linear plots show the high degree of linearity obtained for individual compounds detected and quantified. Peak areas obtained for the standard additions in effluent samples are greater than those observed for the same addition in influent samples for clotrimazole, carbamazepine and nimesulide (Figures 3.3.10, 3.3.11 and 3.3.12). Linear plots showing the linearity of the additions in effluent samples where the compound is absent in the raw sample are also included (Figures 3.3.11

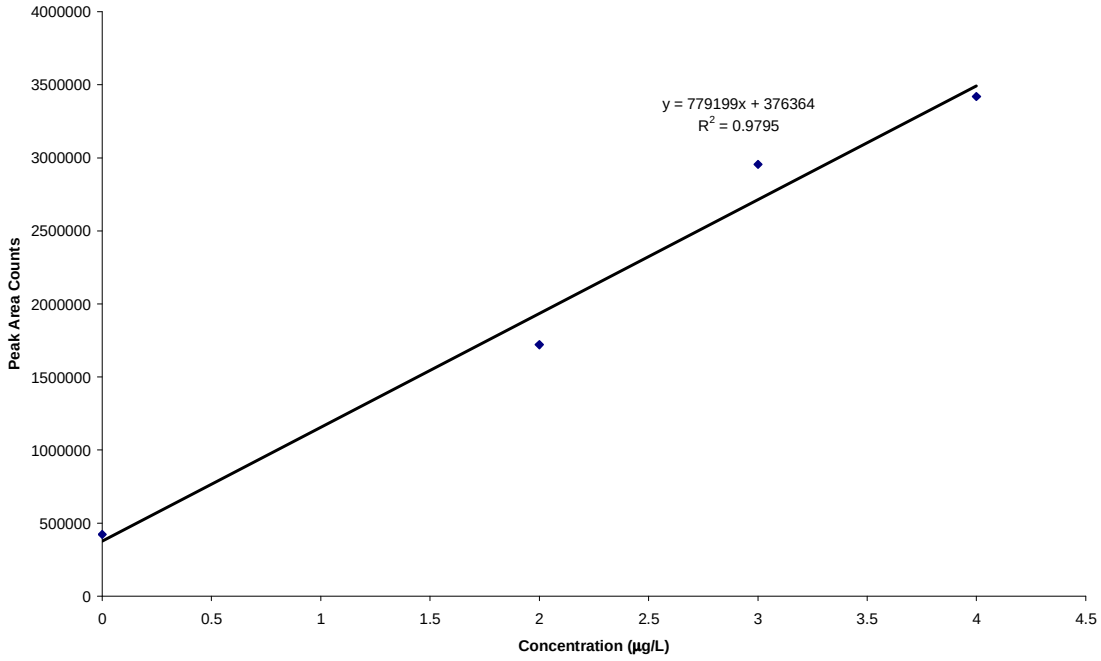
and 3.3.12). For example, carbamazepine and nimesulide were not detected in the influent sample from Swords in November but the linearity of the additions was > 0.9. Figures 3.3.15 – 3.3.18 are extracted ion chromatograms illustrating the suppression of the analyte signal in influent samples.

Data from the Ringsend November sample are presented in Figures 3.3.19 – 3.3.25. Good linearity was obtained for compounds detected in influent and effluent matrices and the peak area counts for the same standard additions were generally greater in effluent samples than influent samples. For example, peak area counts for additions of carbamazepine were ~25% greater in effluent samples than in influent samples. The extracted ion chromatograms in Figures 3.3.24 and 3.3.25 show the absence of signal in influent samples for furosemide and mefenamic acid while both compounds were quantifiable in corresponding effluent samples.

The standard additions method highlighted complete suppression of flurbiprofen, mefenamic acid, diclofenac, clofibric acid, sulfamethoxazole and ibuprofen in influent samples from the WWTPs. Indomethcin and salbutamol were also completely suppressed in influent matrix from the Swords plant. Suppression of the indomethcin signal was ~50% and ~70% in the Leixlip and Ringsend treatment plants respectively and salbutamol was suppressed by ~ 90% in both Leixlip and Ringsend influent samples. No analyte was completely suppressed in effluent matrices. Other analyte signals were significantly suppressed in influent samples compared to effluent signals. The signal for metoprolol was suppressed by ~50% in Leixlip influent samples, ~40% in Ringsend samples and ~80% in Swords samples. Signal suppression of bezafibrate varied from ~30% to ~70% in the three plants. The amount of signal suppression varied both between WWTPs and also between samples. Therefore it is important that standard additions are used in every sample for quantification of analytes.

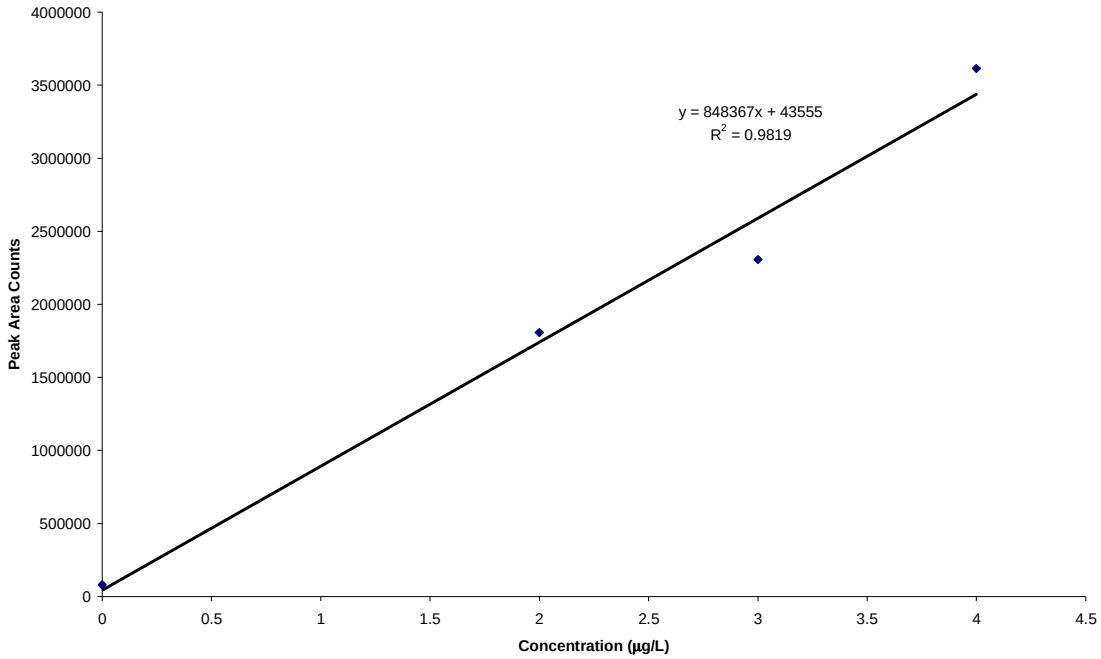


a) Influent

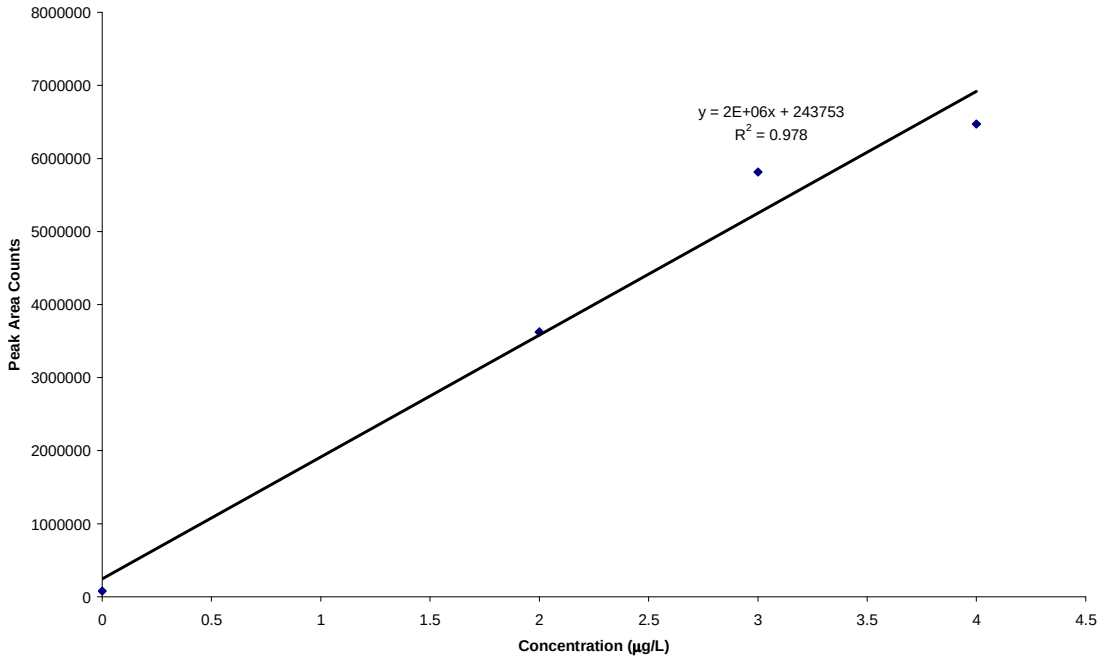


b) Effluent

Figure 3.3.5 Carbamazepine: standard additions in influent (a) and effluent (b) samples from Leixlip, November 2007.

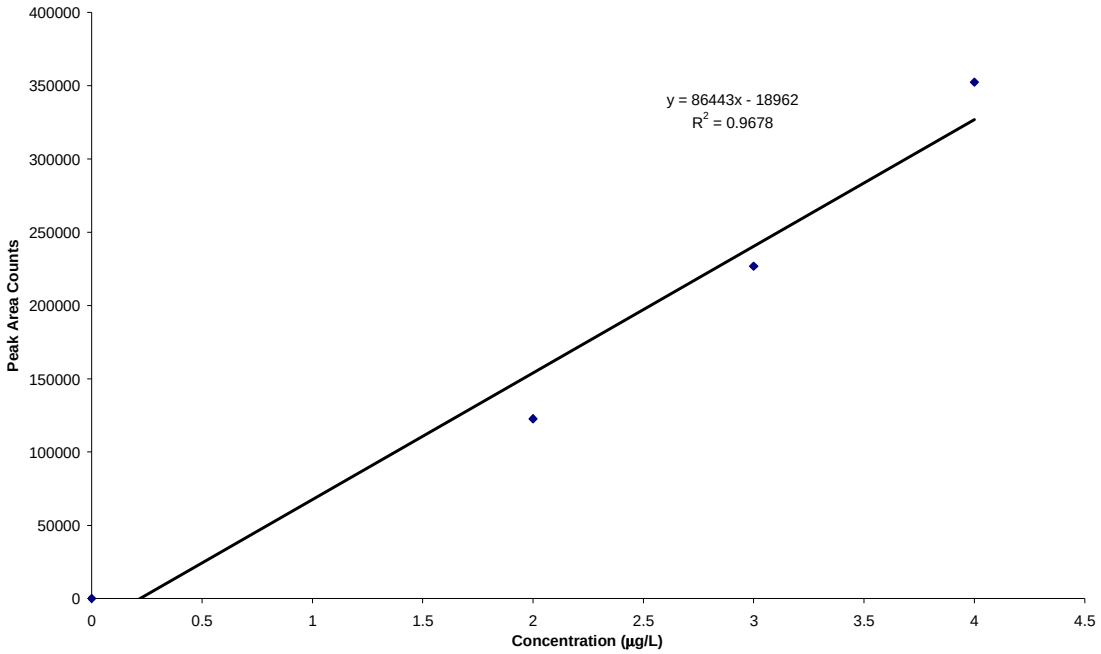


a) Influent

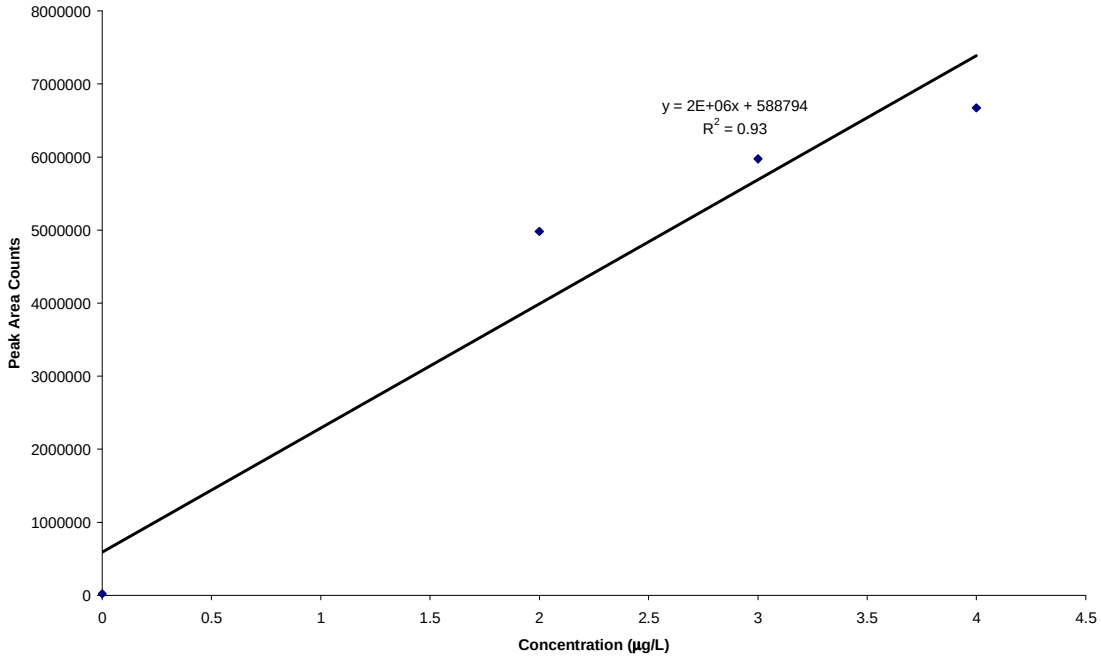


b) Effluent

Figure 3.3.6 Clotrimazole: standard additions in influent (a) and effluent (b) samples from Leixlip, November 2007.

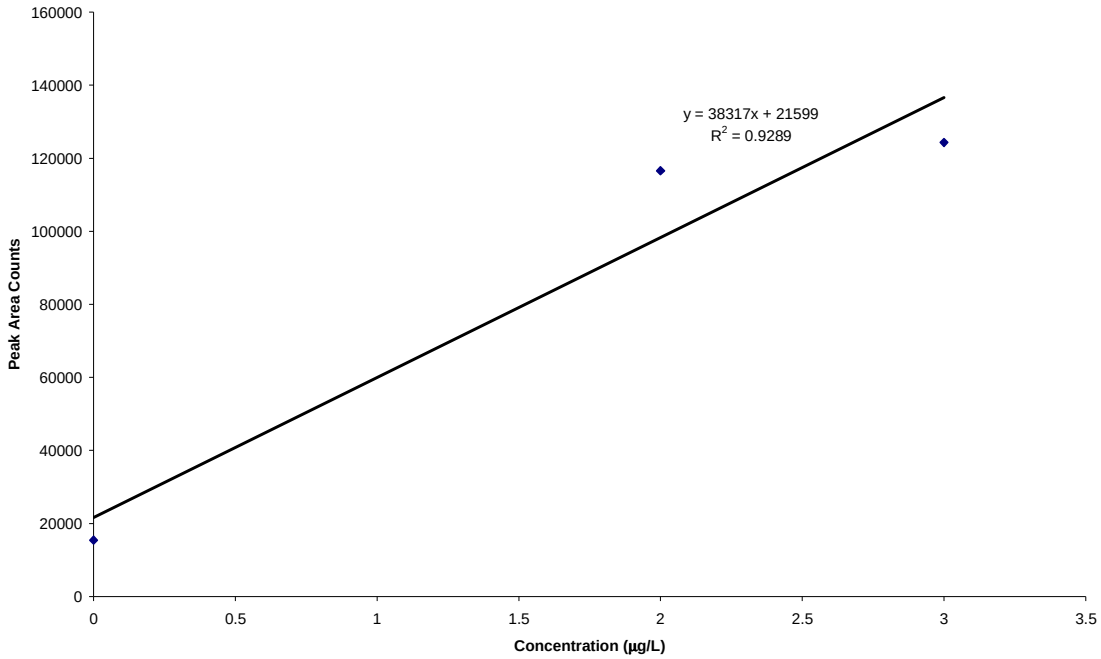


a) Influent

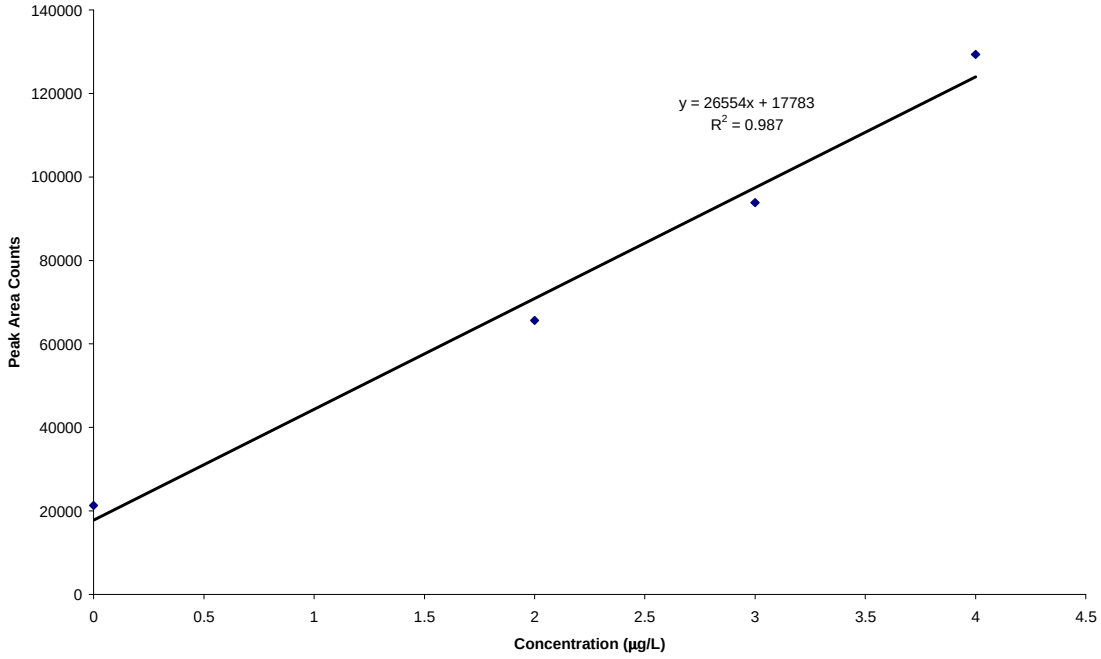


b) Effluent

Figure 3.3.7 Nimesulide: standard additions in influent (a) and effluent (b) samples from Leixlip, November 2007.

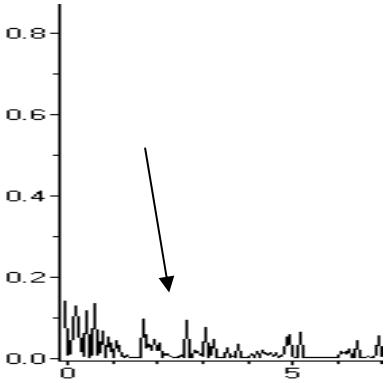


a) Trimethoprim

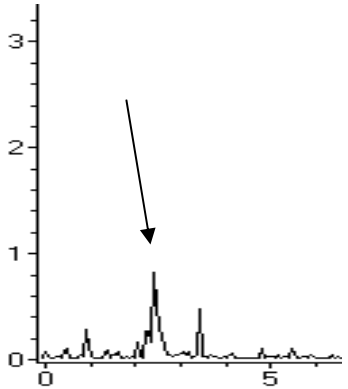


b) Furosemide

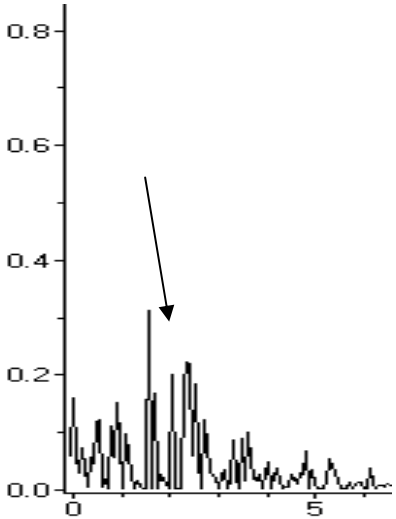
Figure 3.3.8 Trimethoprim (a) and Furosemide (b): standard addition in effluent sample from Leixlip, November 2007.



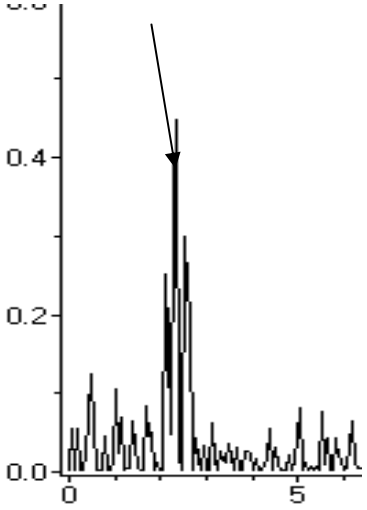
a) Trimethoprim: Influent



c) Trimethoprim: Influent + 3 μg/L standard

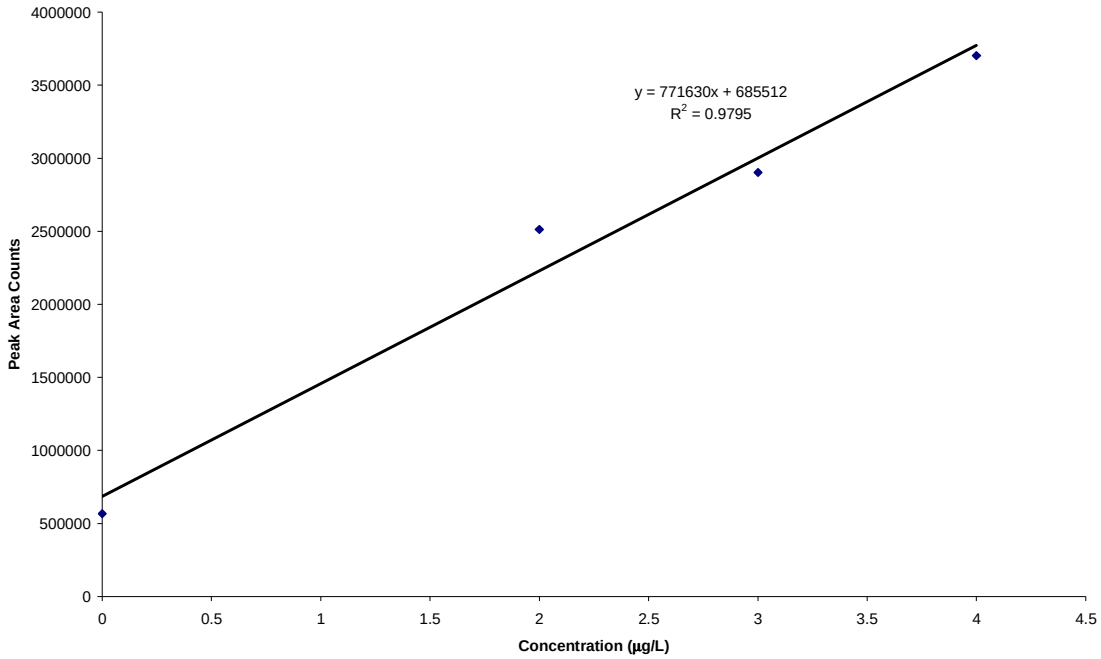


b) Trimethoprim: Influent + 2μg/L standard

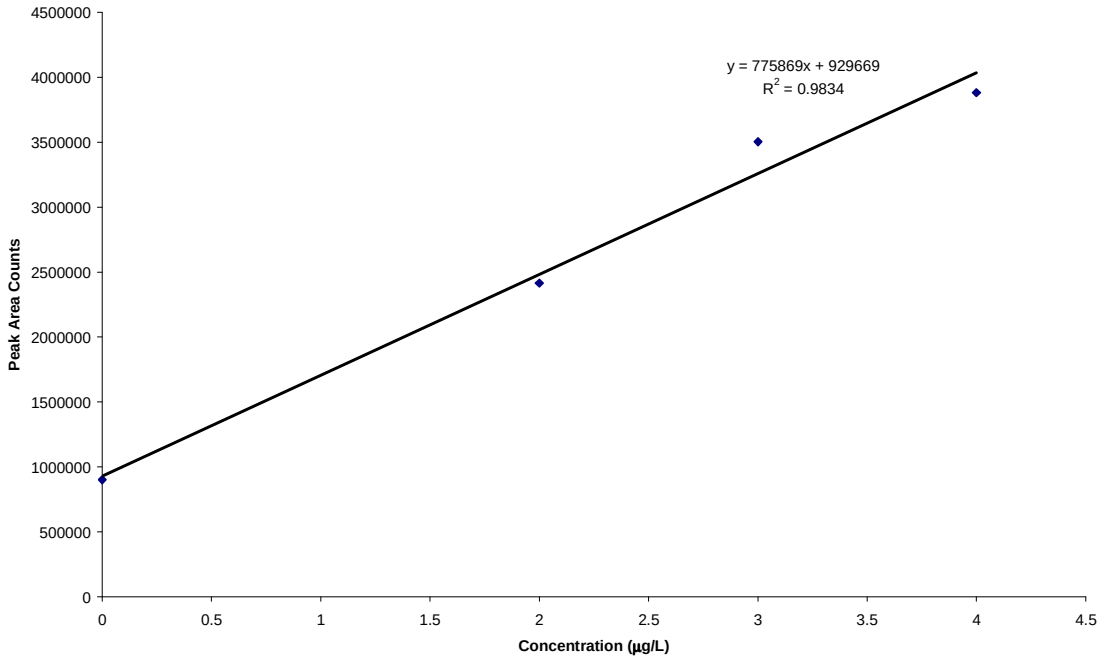


d) Trimethoprim: Influent + 4 μg/L standard

Figure 3.3.9 Chromatograms to illustrate the absence of signal for standard addition of trimethoprim in influent sample (Leixlip November 2007). Arrow indicates R_t for trimethoprim.

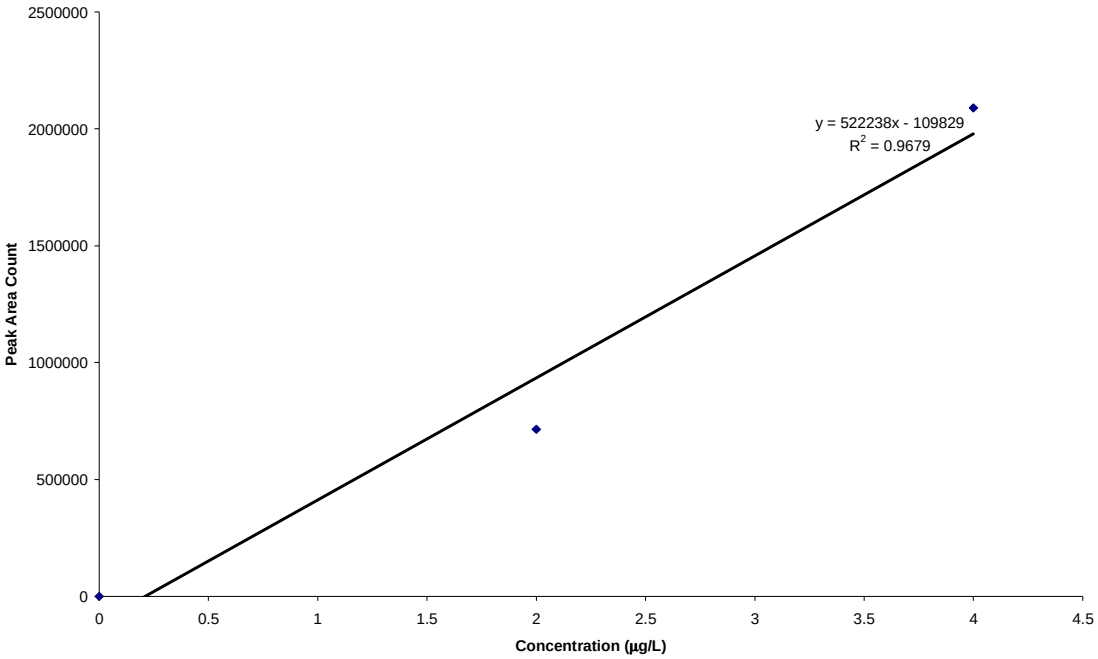


a) Influent

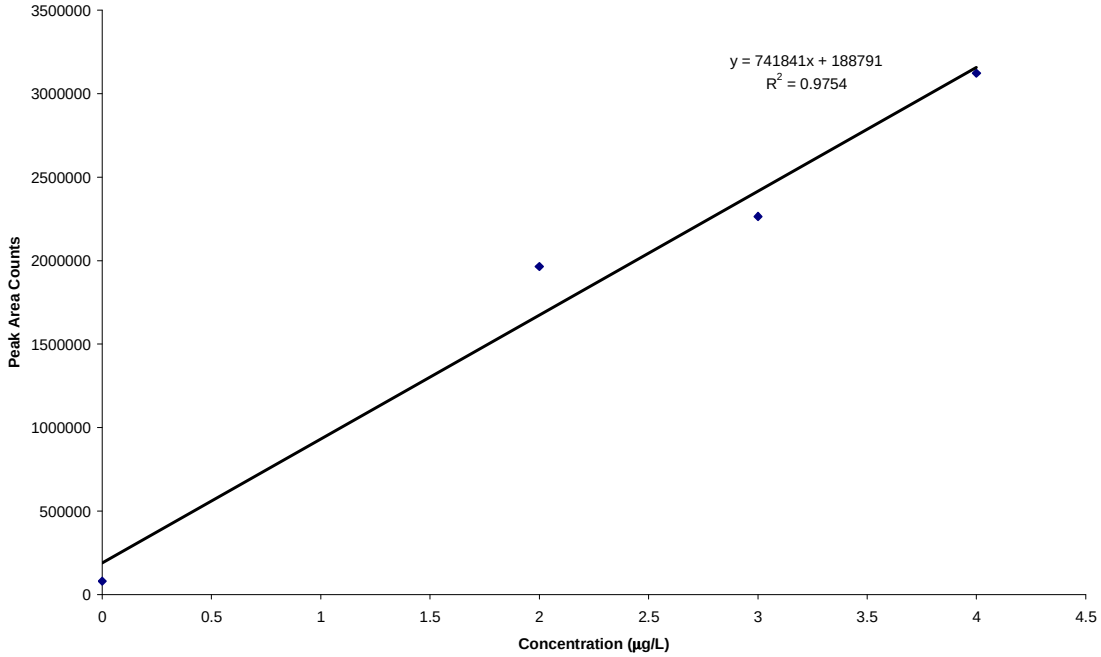


b) Effluent

Figure 3.3.10 Clotrimazole: standard addition in influent (a) and effluent (b) samples from Swords, November 2007.

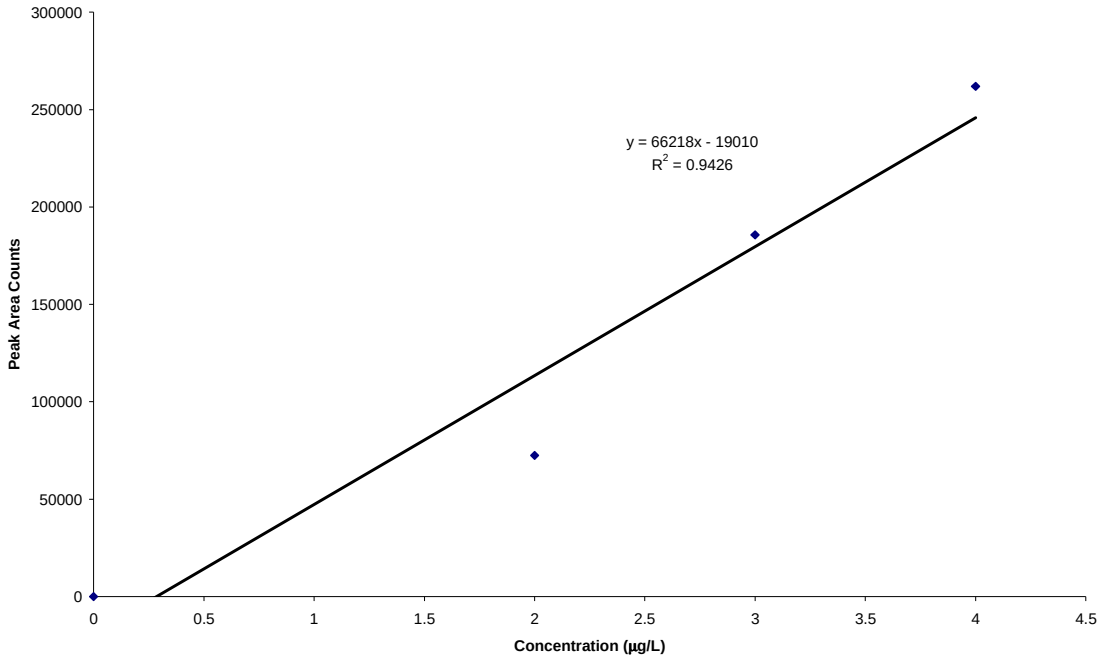


a) Influent

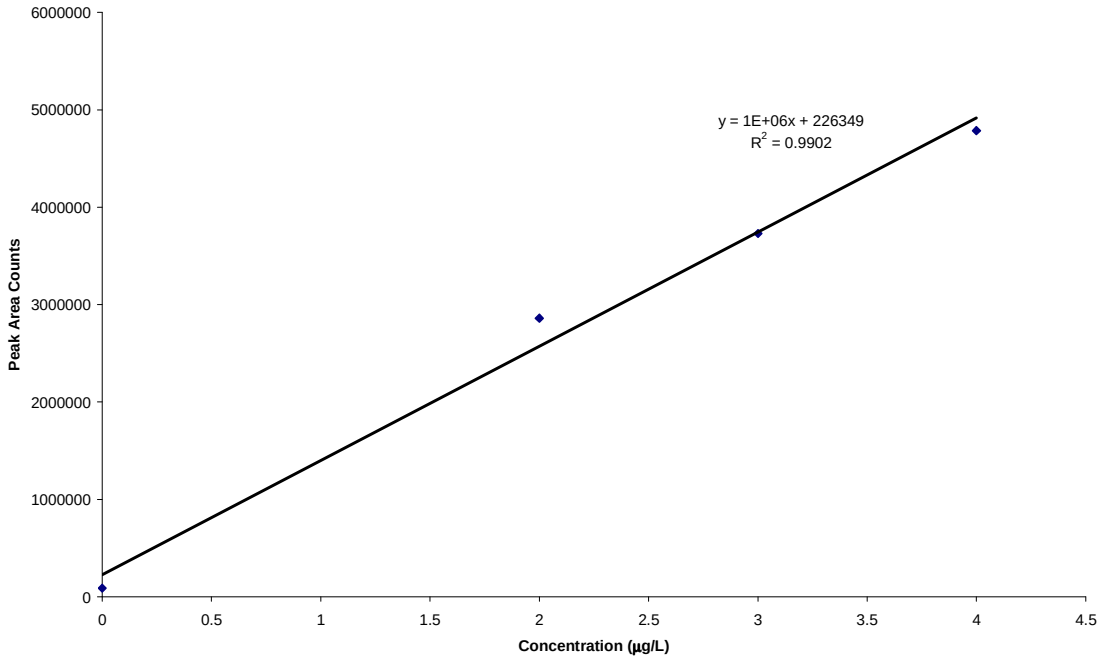


b) Effluent

Figure 3.3.11 Carbamazepine: standard addition in influent (a) and effluent (b) samples from Swords, November 2007.

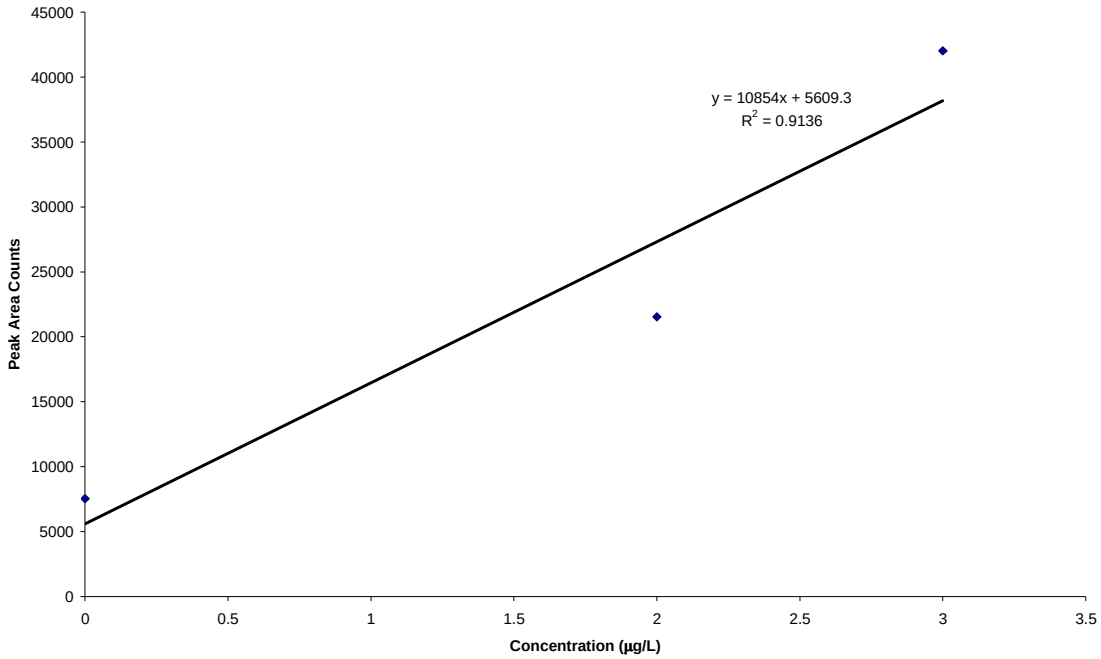


a) Influent

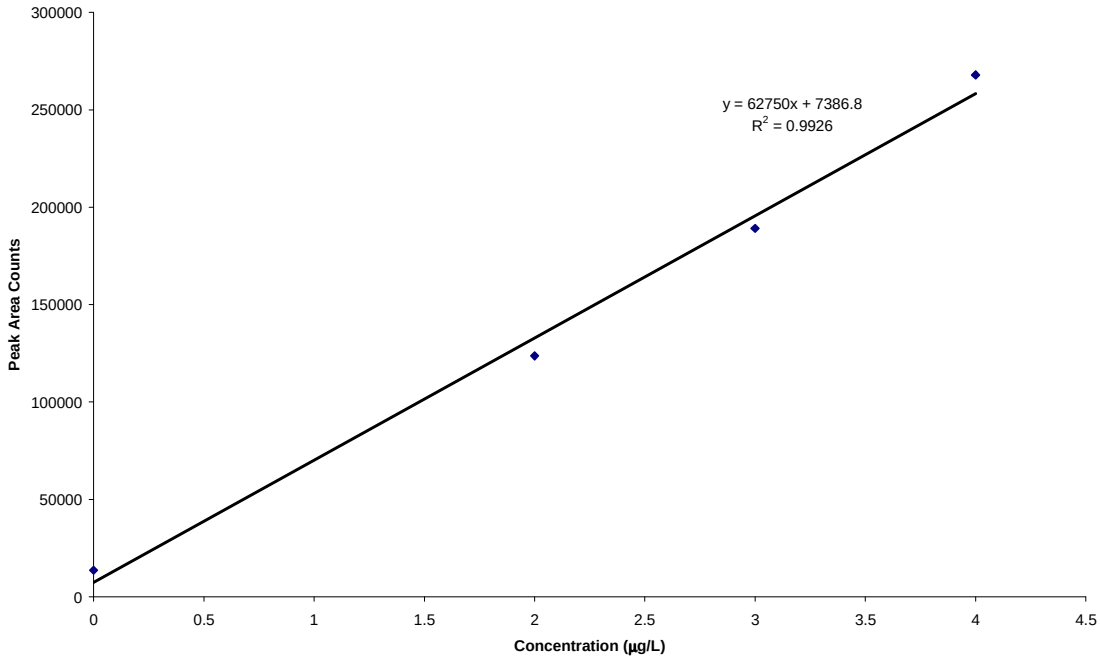


b) Effluent

Figure 3.3.12 Nimesulide: standard addition in influent (a) and effluent (b) samples from Swords, November 2007.

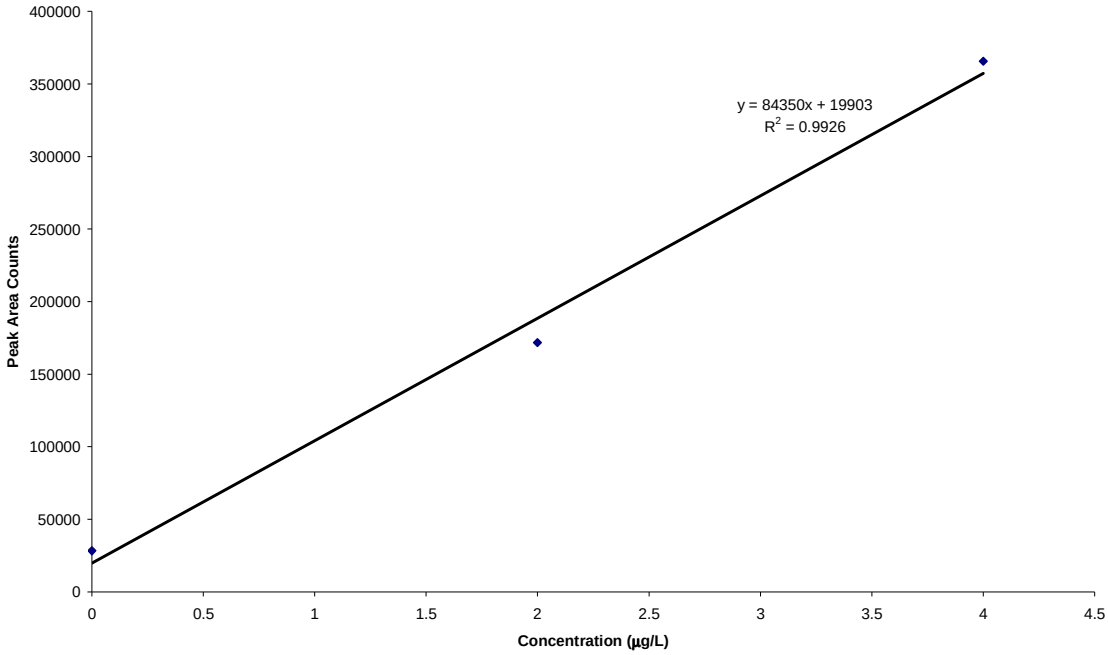


a) Furosemide

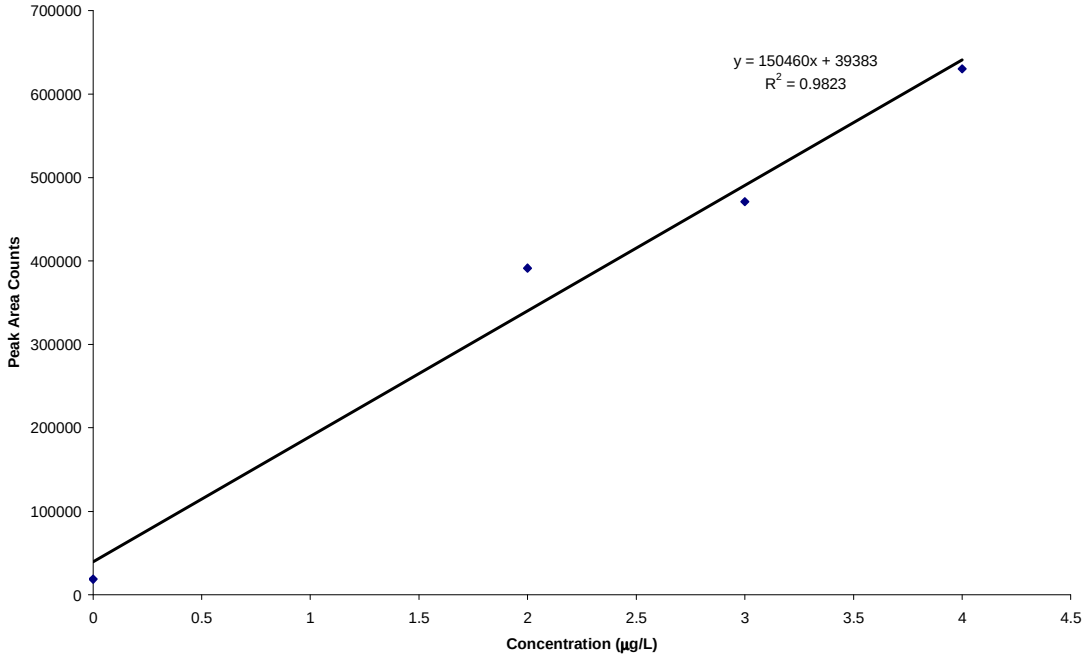


b) Trimethoprim

Figure 3.3.13 Furosemide (a) and trimethoprim (b): standard additions in effluent samples from Swords, November 2007.

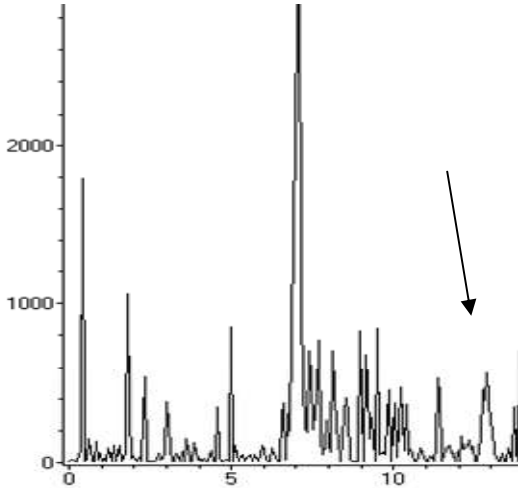


a) Mefenamic acid

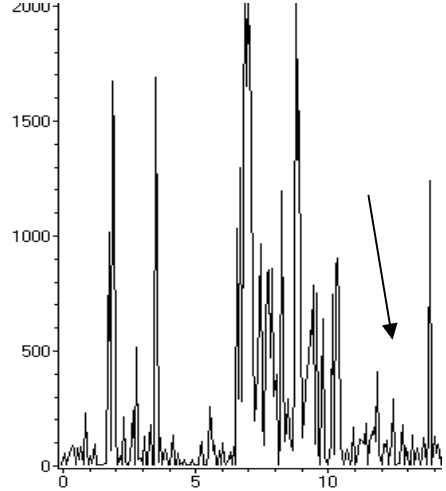


b) Propranolol

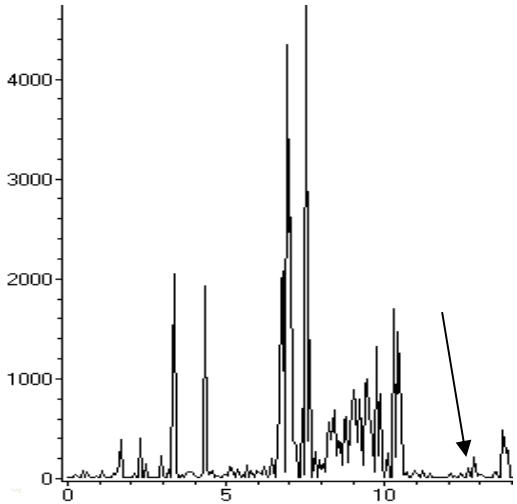
Figure 3.3.14 Mefenamic acid (a) and propranolol (b): standard additions in effluent samples from Swords, November 2007.



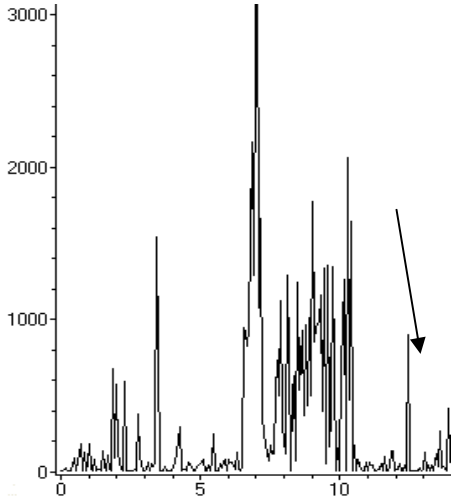
a) Furosemide: Influent



c) Furosemide: Influent + 3µg/L standard

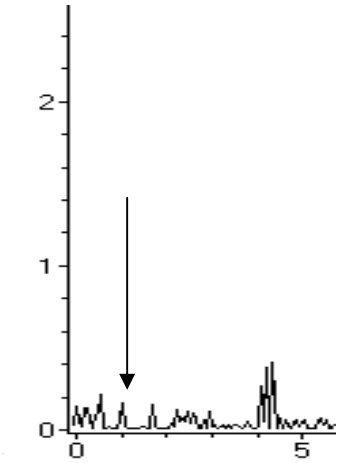


b) Furosemide: Influent + 2µg/L standard

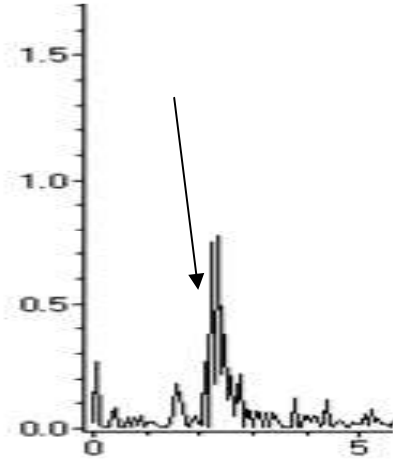


d) Furosemide: Influent + 4µg/L standard

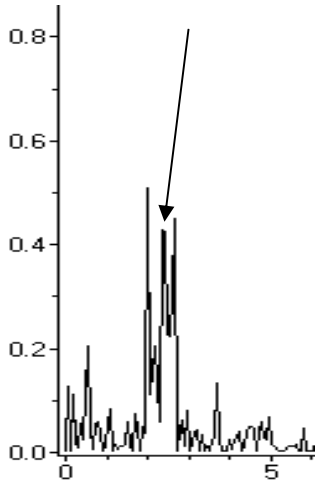
Figure 3.3.15 Chromatograms illustrating the absence of signal for standard additions of furosemide in influent samples. (Swords November 2007). Arrow indicates R_t for furosemide.



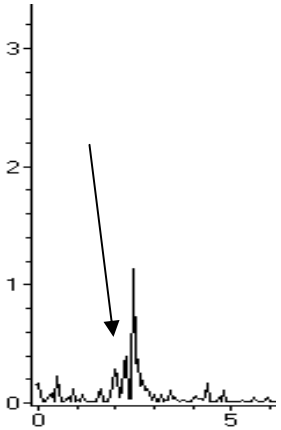
a) Trimethoprim: Influent



b) Trimethoprim: Influent+ 2 μg/L standard

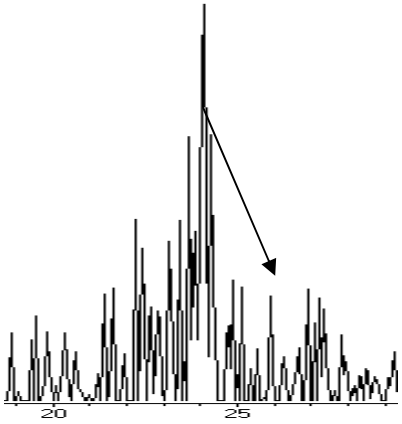


c) Trimethoprim: Influent + 3 μg/L standard

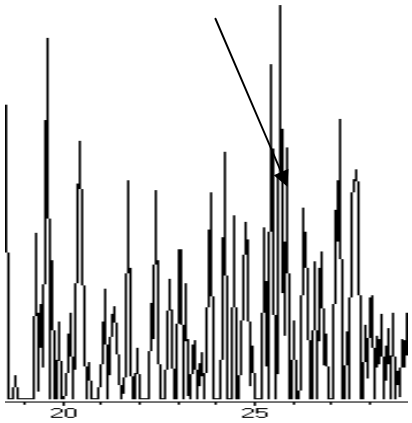


d) Trimethoprim: Influent + 4 μg/L standard

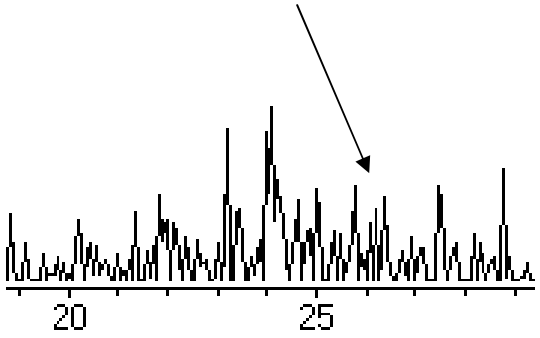
Figure 3.3.16 Chromatograms illustrating the absence of signal for standard additions of trimethoprim in influent samples. (Swords November 2007). Arrow indicates R_t for trimethoprim.



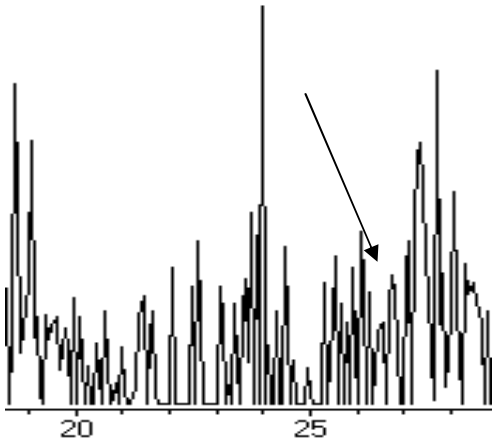
a) Mefenamic acid: Influent



c) Mefenamic acid: Influent + 3 μg/L standard

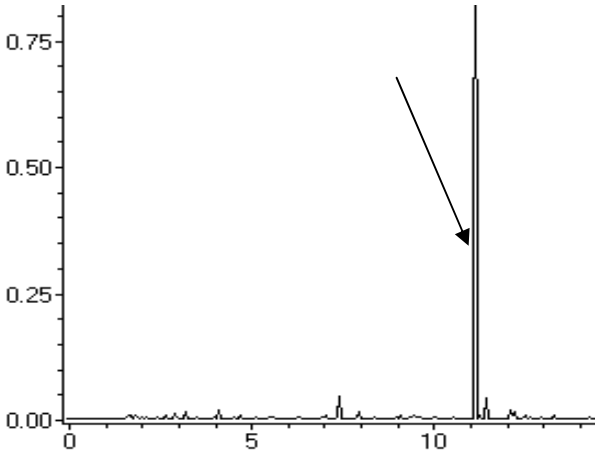


b) Mefenamic acid: Influent + 2 μg/L standard

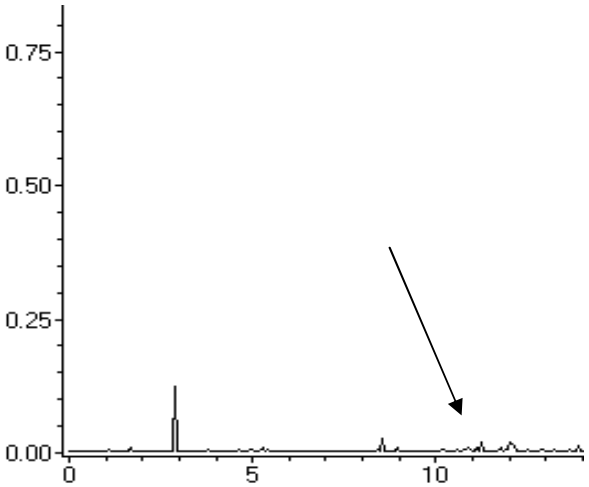


d) Mefenamic acid: Influent + 4 μg/L standard

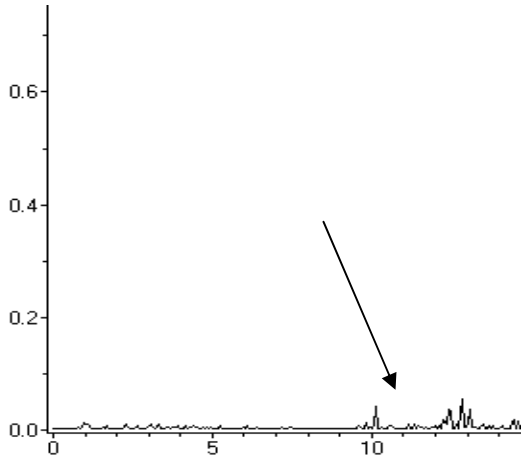
Figure 3.3.17 Chromatograms illustrating the absence of signal for standard additions of mefenamic acid in influent samples. (Swords November 2007). Arrow indicates R_t for mefenamic acid.



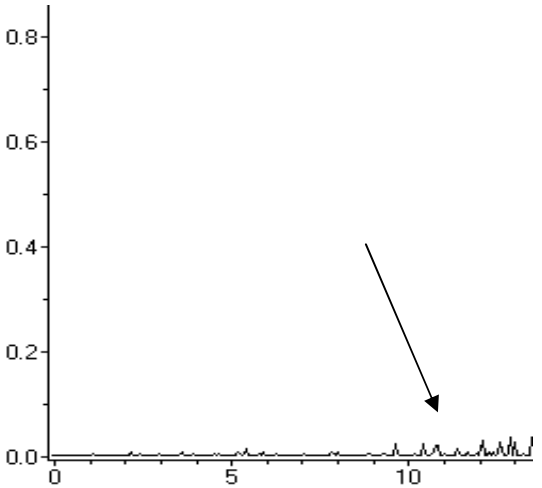
a) Propranolol: Influent



b) Propranolol: Influent + 2 μg/L standard

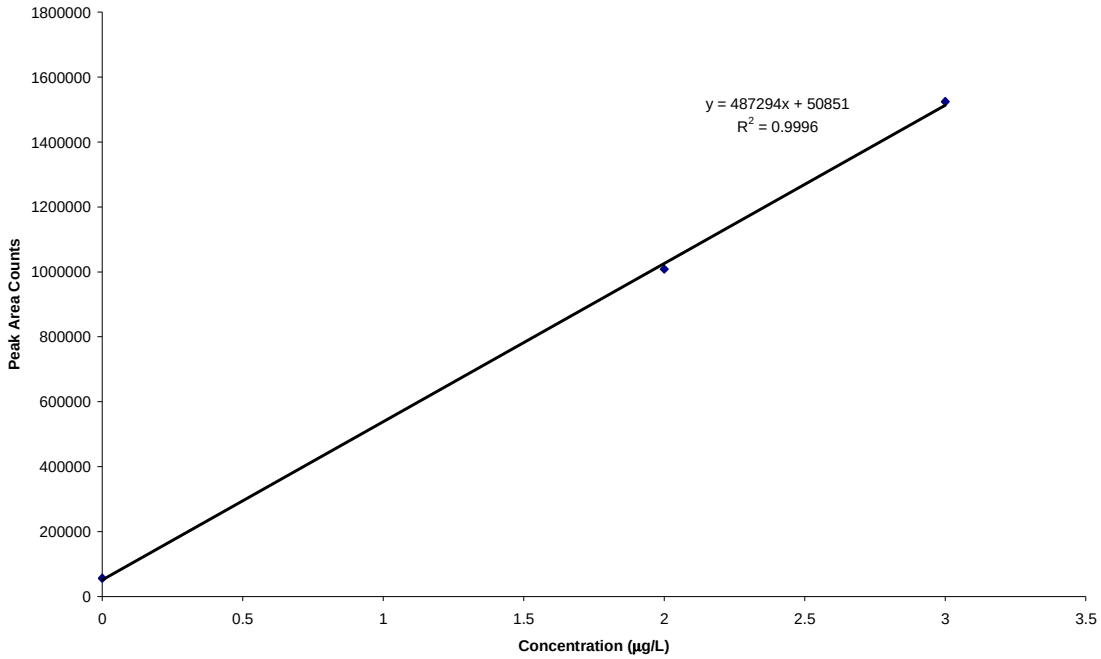


c) Propranolol: Influent + 3 μg/L standard

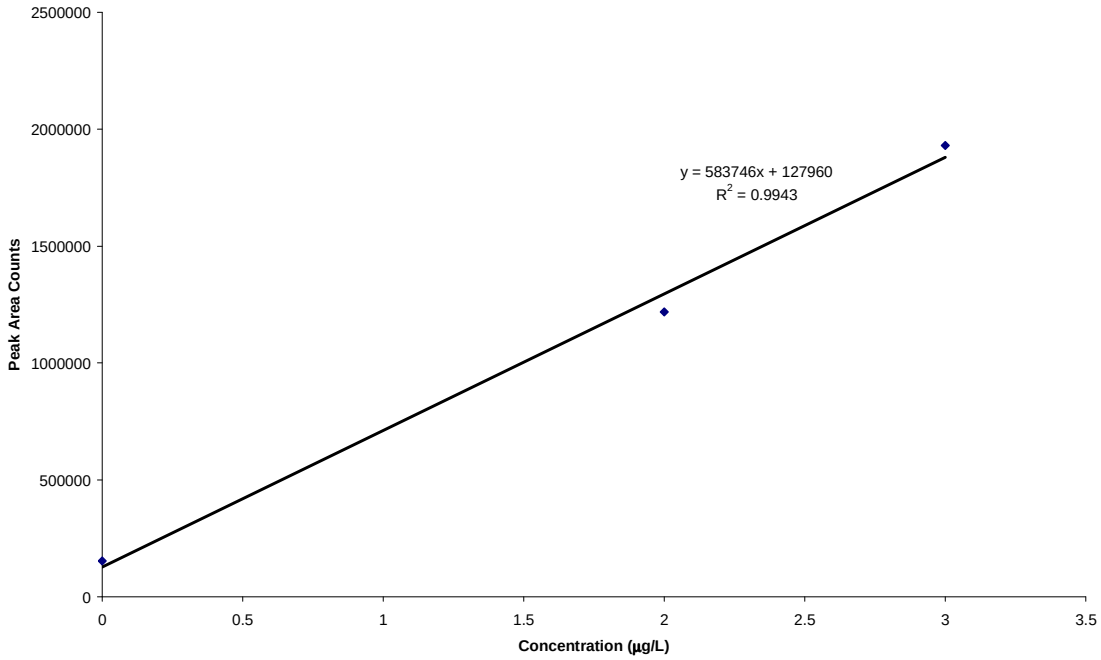


d) Propranolol: Influent + 4 μg/L standard

Figure 3.3.18 Chromatograms illustrating the absence of signal for standard additions of propranolol in influent samples. (Swords November 2007). Arrow indicates R_t for propranolol.

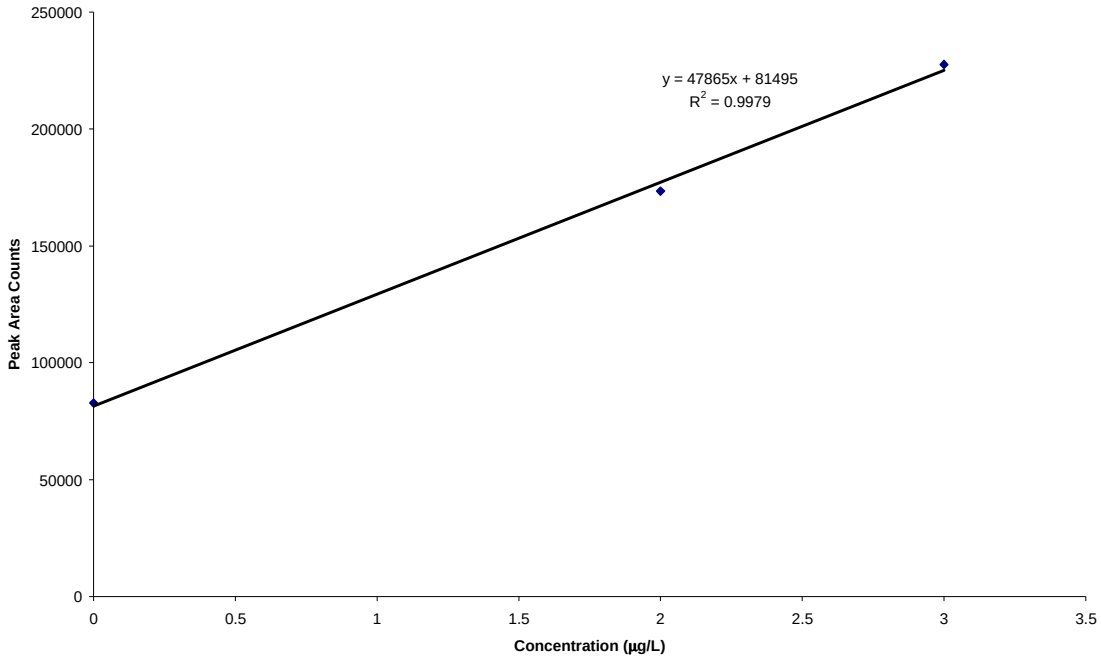


a) Influent

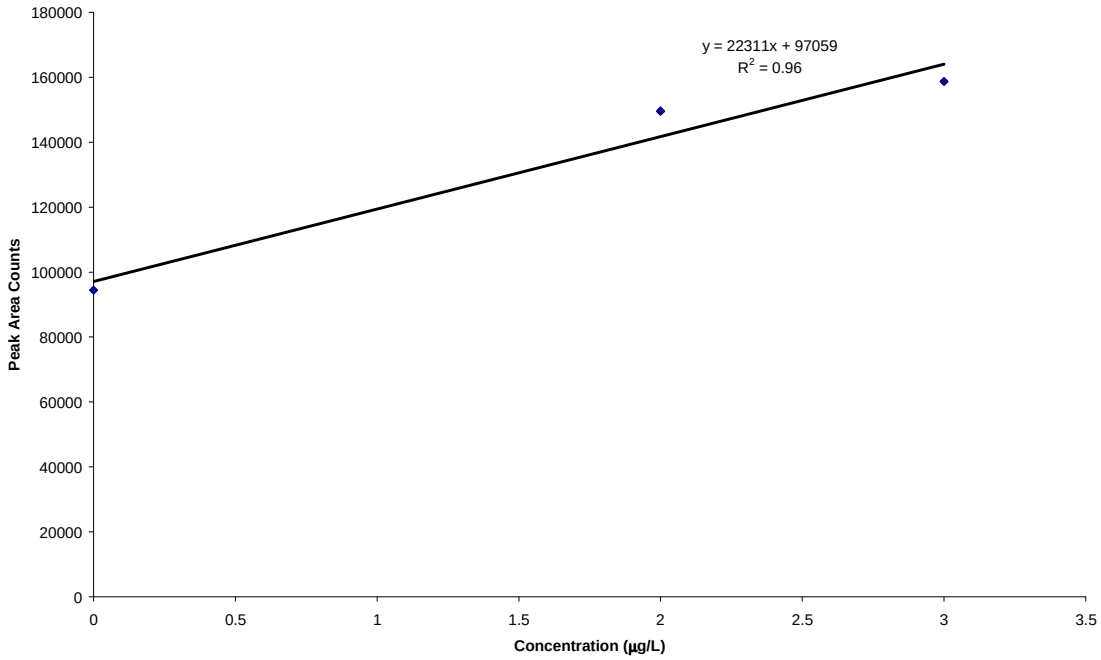


b) Effluent

Figure 3.3.19 Carbamazepine: standard additions in influent (a) and effluent (b) samples from Ringsend, November 2007.

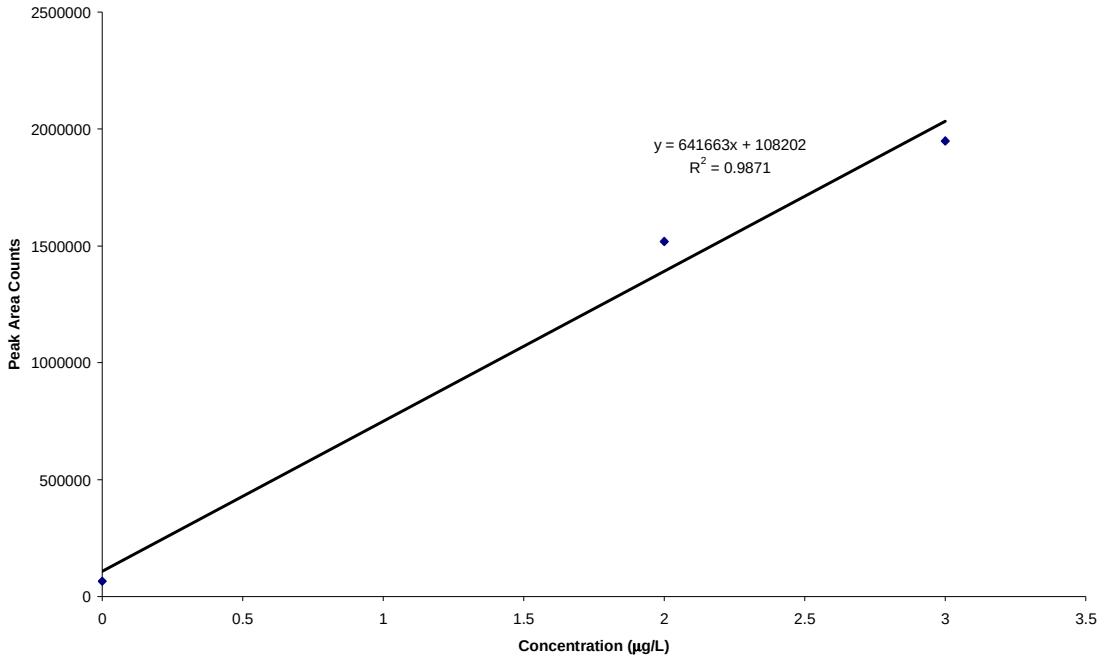


a) Influent

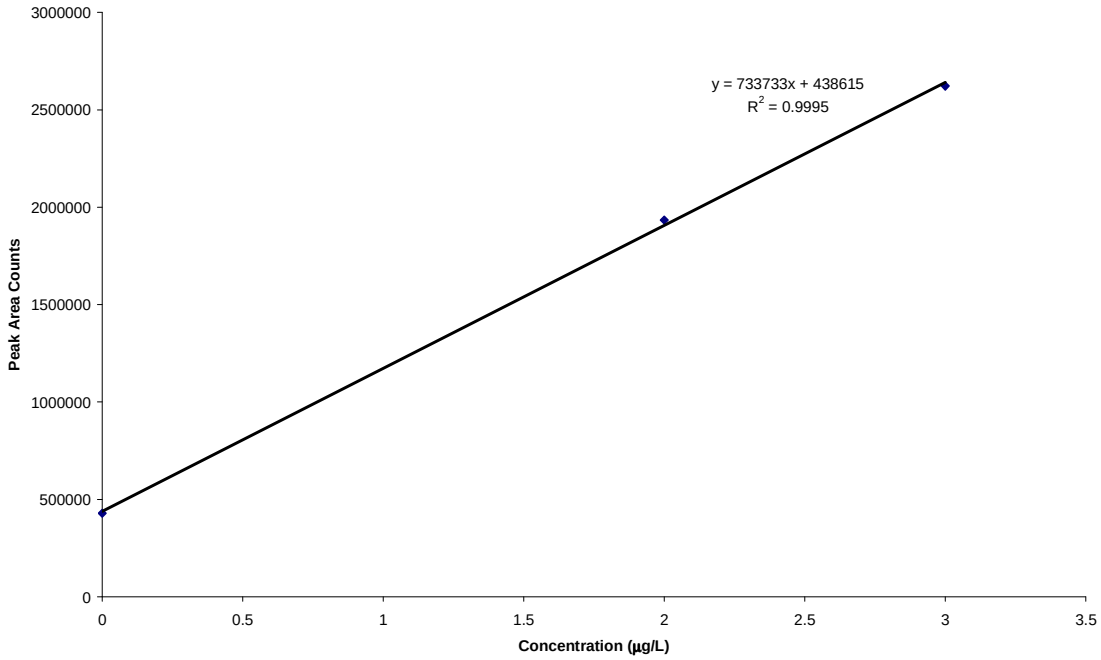


b) Effluent

Figure 3.3.20 Metoprolol: standard additions in influent (a) and effluent (b) samples from Ringsend, November 2007.

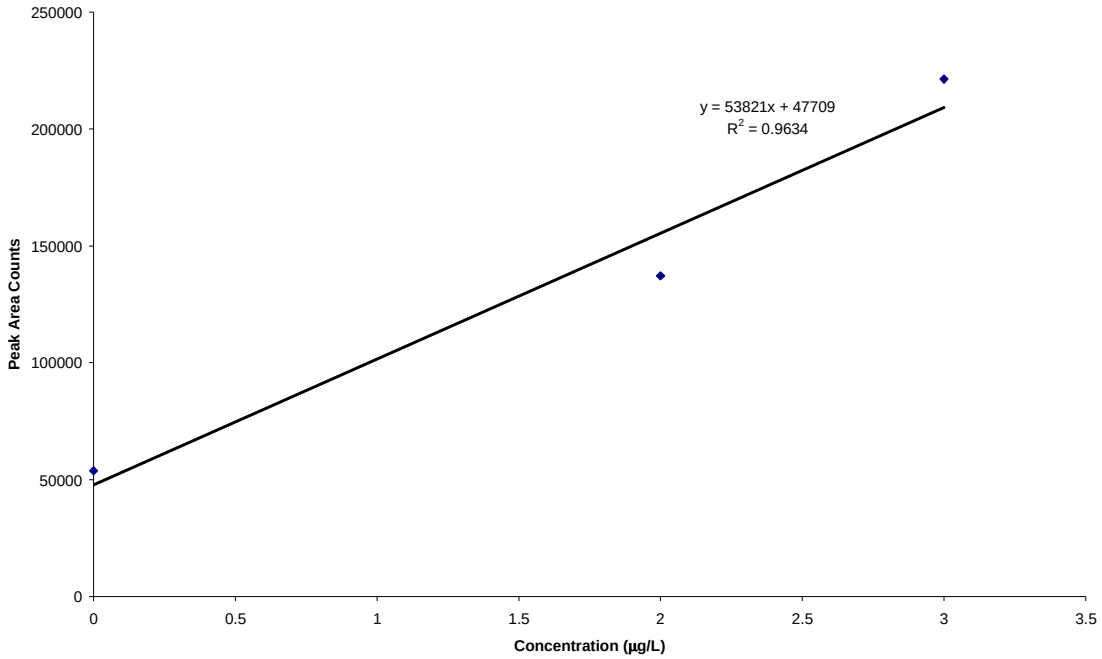


a) Influent

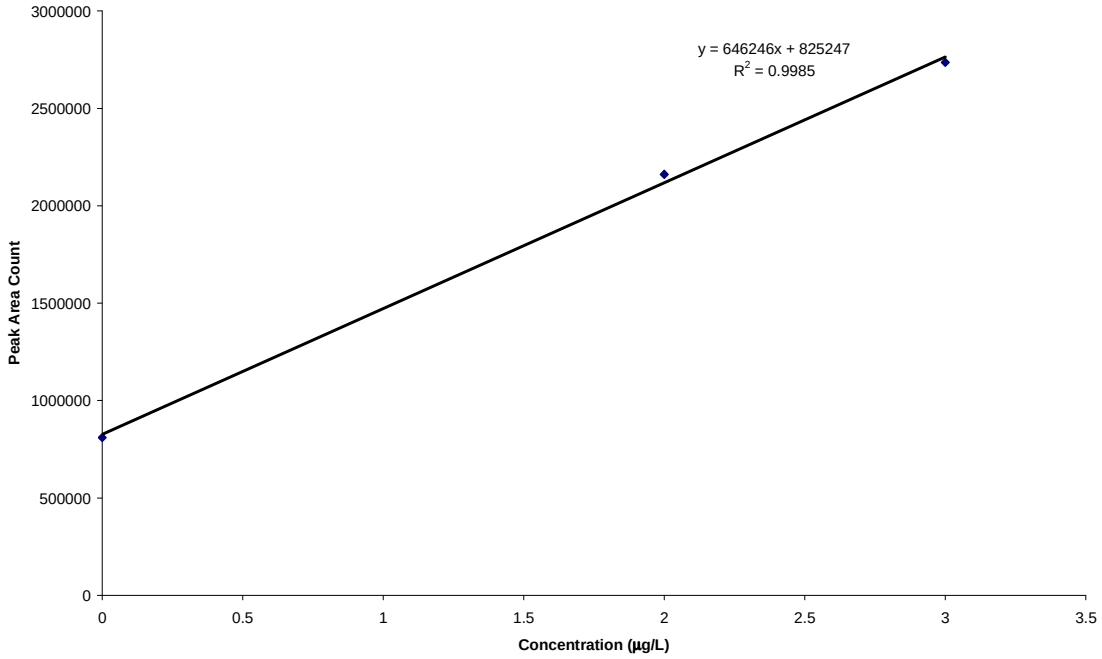


b) Effluent

Figure 3.3.21 Clotrimazole: standard additions in influent (a) and effluent (b) samples from Ringsend, November 2007.

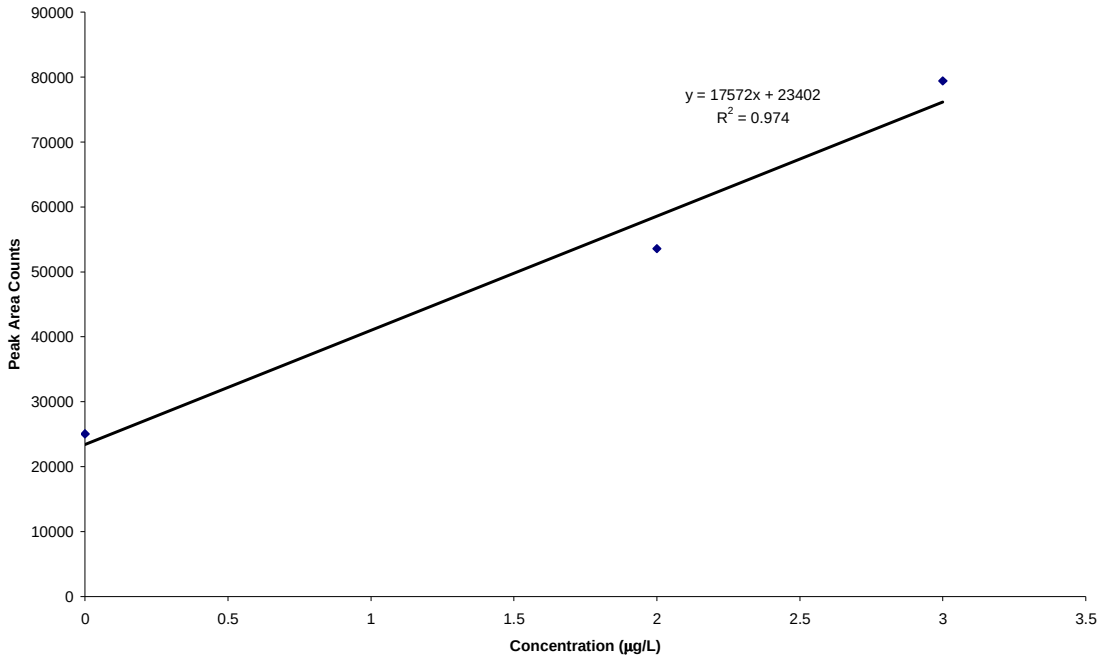


a) Influent

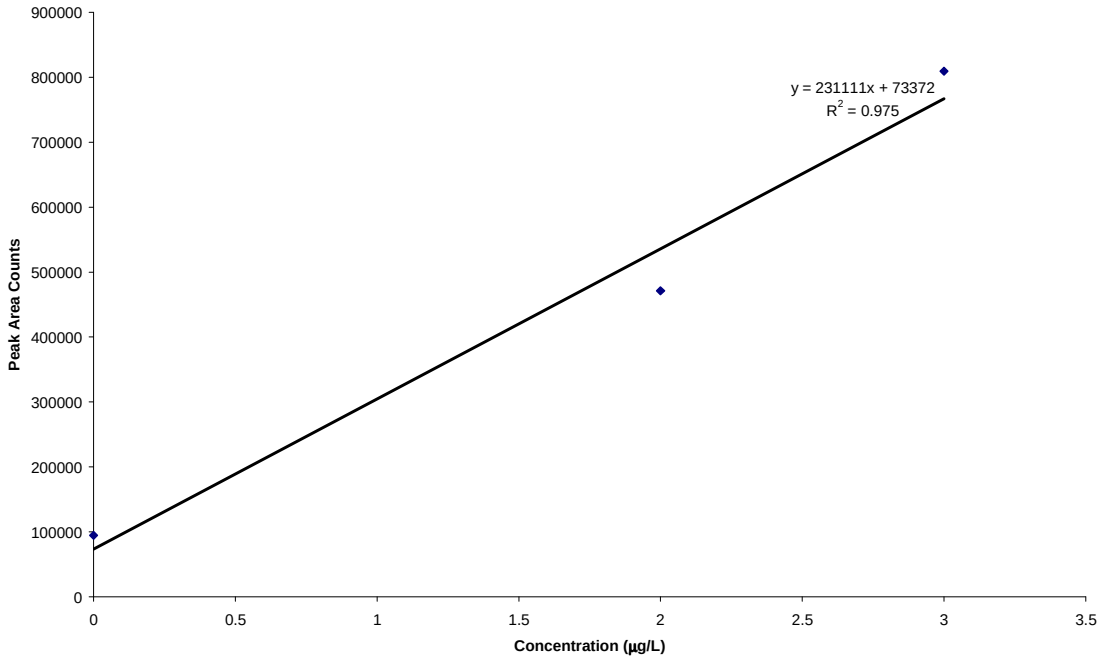


b) Effluent

Figure 3.3.22 Nimesulide: standard additions in influent (a) and effluent (b) samples from Ringsend, November 2007.

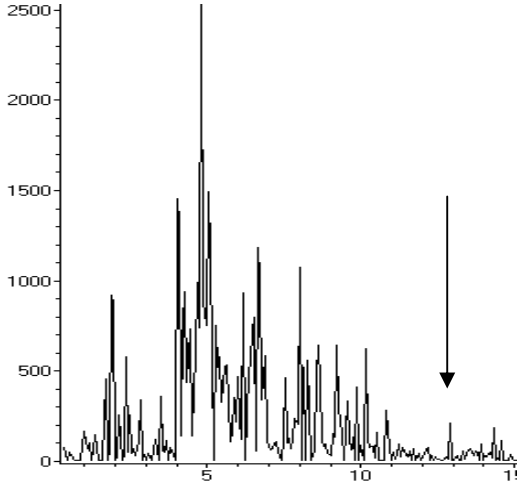


a) Furosemide

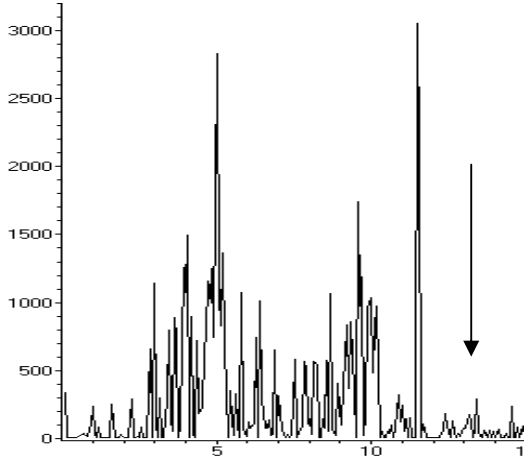


b) Mefenamic acid

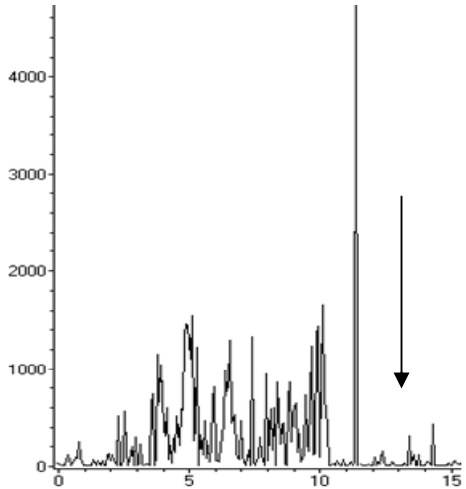
Figure 3.3.23 Furosemide (a) and mefenamic acid (b): standard additions in effluent samples from Ringsend, November 2007.



a) Furosemide: Influent

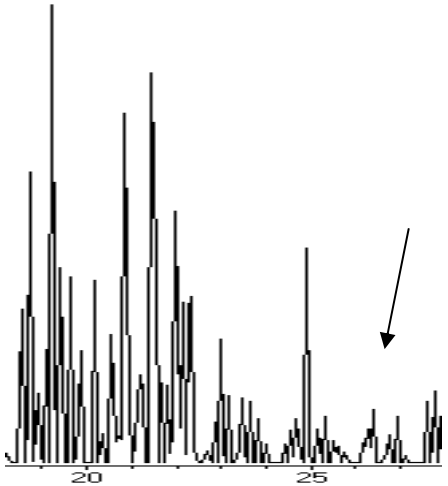


b) Furosemide: Influent + 2μg/L standard

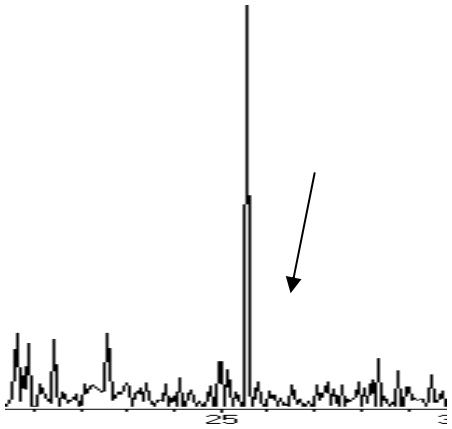


c) Furosemide: Influent + 3 μg/L standard

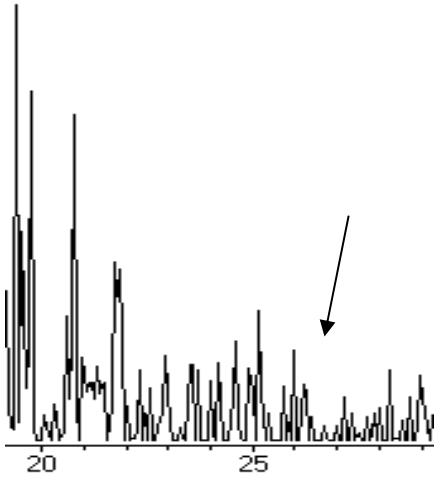
Figure 3.3.24 Chromatograms illustrating the absence of signal for standard additions of furosemide in influent samples (Ringsend, November 2007). Arrow indicates R_t for furosemide.



a) Mefenamic acid: Influent



b) Mefenamic acid: Influent + 2 μg/L



c) Mefenamic acid: Influent + 3 μg/L

Figure 3.3.25 Chromatograms illustrating the absence of signal for standard additions of mefenamic acid in influent samples (Ringsend, November 2007). Arrow indicates R_t for mefenamic acid.

3.4 Metal Analysis

ICP-AES metal analysis results from TMS Environment Ltd. are presented in Table 3.4.1. Influent and corresponding effluent samples from the three plants were analysed for twelve metals. The concentrations were very low in both influent and effluent samples. The only metals present above detection limits were chromium, copper, iron, manganese and lead.

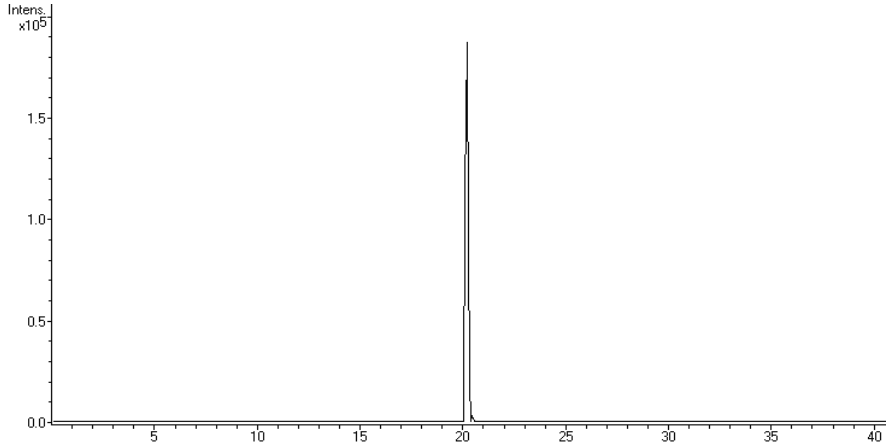
	Influent (mg/L)			Effluent (mg/L)		
	Swords	Leixlip	Ringsend	Swords	Leixlip	Ringsend
Silver	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Arsenic	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Cadmium	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Chromium	0.0059	0.0059	0.0072	0.0066	0.0052	<0.005
Copper	0.061	0.074	<0.05	<0.05	<0.05	<0.05
Iron	0.054	0.083	0.43	0.014	0.025	0.086
Mercury	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Manganese	0.005	0.084	0.081	0.057	0.041	0.036
Nickel	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Lead	0.026	<0.01	0.025	0.024	<0.01	0.022
Tin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Zinc	<0.01	<0.01	<0.01	0.029	0.029	<0.01

Table 3.4.1 Metal concentrations detected in influent and effluent samples at three wastewater treatment plants

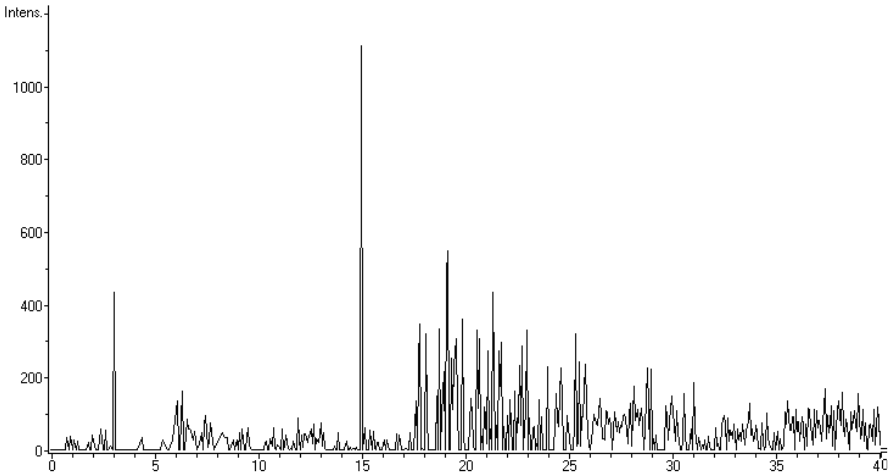
3.5 Surfactant Analysis

The effect of linear alkyl benzene sulphonate on the LC-MS/MS method was investigated in this study. The experimental design was similar to the that used for addition post extraction in that a standard solution was compared to the same concentration in a LAS solution. The results are presented in Figures 3.5.1 and 3.5.2. The analysis of nimesulide was significantly affected by the presence of LAS. The signal was completely suppressed and no analyte peak was observed (Figure 3.5.1b). The

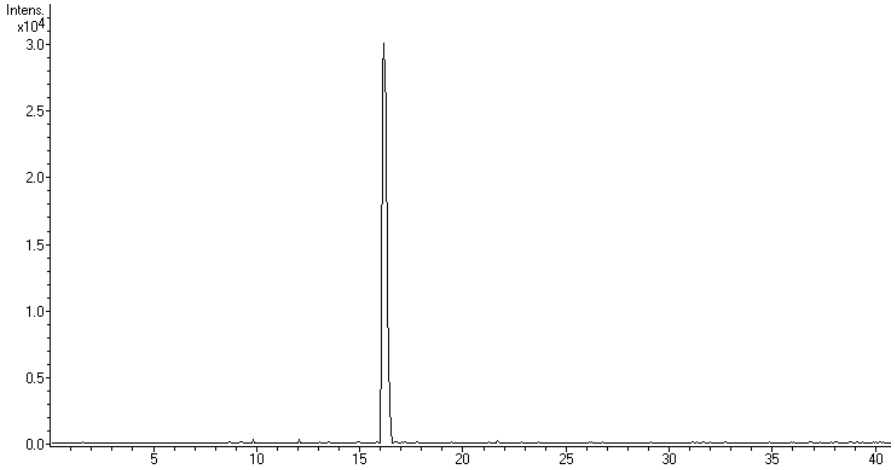
retention time of mefenamic acid was altered in the presence of LAS by approximately one minute but the peak area count was unaffected (Figure 3.5.1d). The retention of trimethoprim was altered with the addition of the surfactant. The peak broadened and was eluted over three minutes and separated to give multiple peaks (Figure 3.5.2b). The analyte response for carbamazepine was not affected by the addition of the surfactant (Figure 3.5.2c and d). The effect of LAS on signal suppression does not appear to be influenced by the mode of ionisation but rather is compound specific.



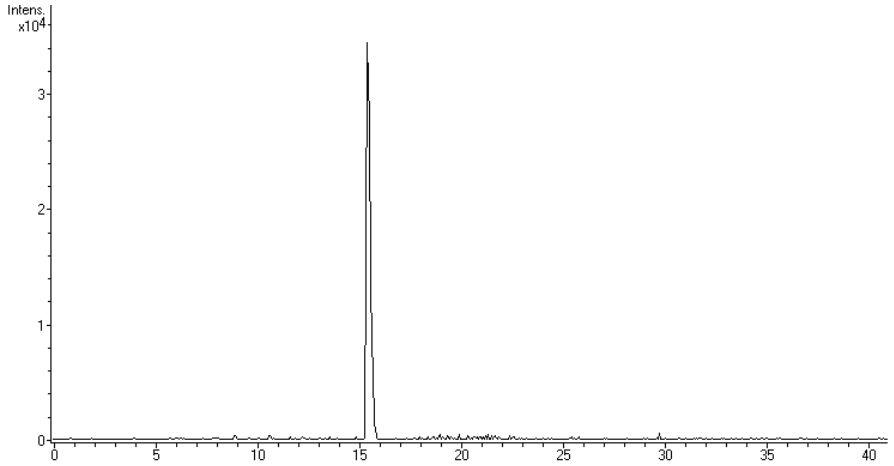
a) Nimesulide Standard



b) Nimesulide Standard + LAS

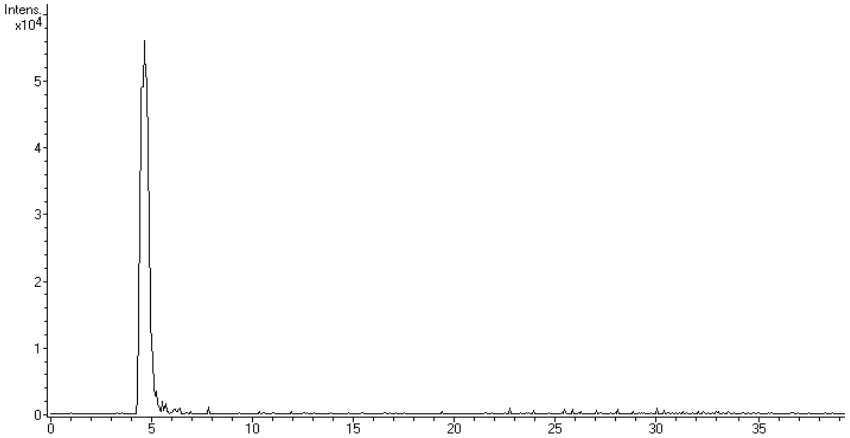


c) Mefenamic acid standard

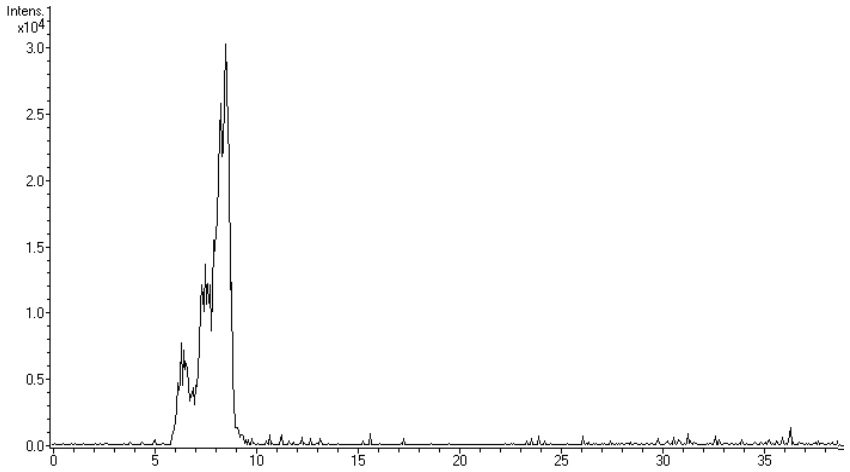


d) Mefenamic acid + LAS

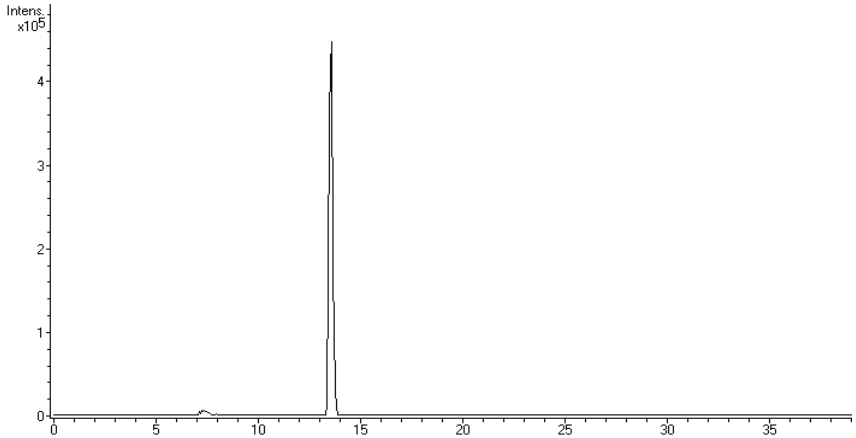
Figure 3.5.1 Effect of LAS on analysis of nimesulide and mefenamic acid in negative mode ionisation.



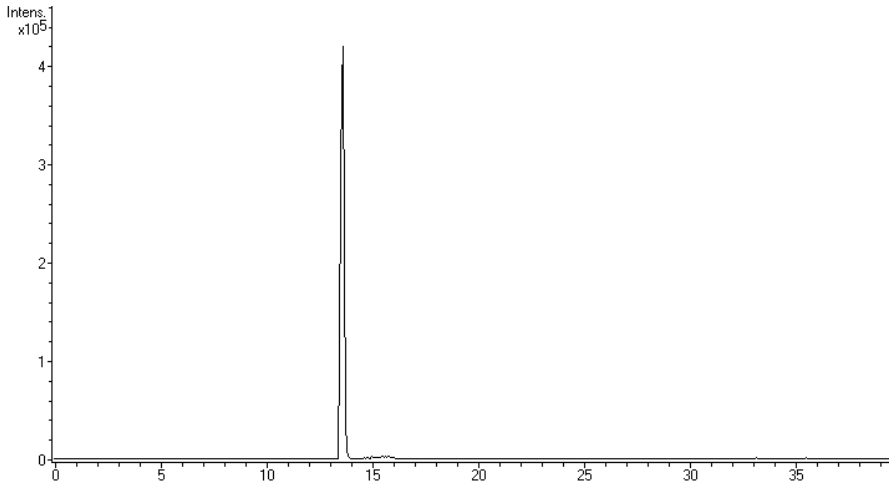
a) Trimethoprim standard



b) Trimethoprim standard + LAS



c) Carbamazepine standard



d) Carbamazepine standard + LAS

Figure 3.5.2 Effect of LAS on analysis of trimethoprim and carbamazepine in positive mode ionisation.

3.6 Monthly Sampling

Twenty four hour composite influent and effluent samples were collected from each of the three wastewater treatment facilities each month from July 2007 to June 2008. Samples were analysed using the SPE-LC-MS/MS method outlined in Section 2.6. Results are presented in Tables 3.6.1, 3.6.2 and 3.6.3. Operational data from the three treatment plants, on the day of sampling and corresponding weather data are also included in the tables. Thirteen of the selected compounds were present at concentrations above the LOD in samples collected from the Swords treatment plant. Carbamazepine and clotrimazole had the highest frequency of occurrence. Apparent concentrations of both these compounds exhibited higher values in effluent streams. Bezafibrate (2 samples), caffeine (4 samples), diclofenac (1 sample), metoprolol (2 samples), pravastatin (4 samples), propranolol (1 sample) and salicylic acid (2 samples) had limited occurrence in samples collected from Swords. Trimethoprim was identified and quantified in five samples during the sampling period. Influent and corresponding effluent concentrations were found only in March 2008. The rate of removal for trimethoprim was 94.6%. Similarly, influent and corresponding effluent concentrations of furosemide and nimesulide were observed in only one month (January 2008 and June 2008 respectively). No removal of furosemide was observed while removal of nimesulide was greater than 99%. Mefenamic acid was found only in effluent samples collected from the Swords plant.

In the samples collected from the Leixlip treatment plant, thirteen of the selected compounds were found. Clotrimazole and carbamazepine were present most often in the samples. In general the concentration of these two compounds in effluent samples was greater than that observed in corresponding influent samples. A reduction in carbamazepine concentration during treatment was observed once in May 2008, while a reduction in clotrimazole concentration occurred in both August 2007 and May 2008. Caffeine was present in influent samples with no corresponding effluent concentration with the exception of July 2007. Diclofenac (4

samples), gemfibrozil (5 samples), mefenamic acid (4 samples), metoprolol (3 samples), pravastatin (3 samples), propranolol (2 samples), and salicylic acid (2 samples) occurred infrequently during the sampling period. Furosemide and trimethoprim occurred more frequently. However, no pattern of removal or increase in concentration was apparent.

Thirteen compounds were also found to be present in samples collected from the Ringsend treatment facility and again carbamazepine and clotrimazole were detected more often than the other eleven compounds. The concentrations of carbamazepine and clotrimazole were frequently higher in effluent than influent samples. Bezafibrate, diclofenac, salicylic acid, propranolol and pravastatin were detected only on one or two occasions. Mefenamic acid was detected in four effluent samples and never in corresponding influent samples. The occurrence of furosemide was inconsistent in influent or effluent samples. In July 2007 and March 2008 furosemide was detected in both influent and effluent samples and removal varied from 34% to 95%. Metoprolol was detected in four months samples (July, August and November 2007 and June 2008). The concentration apparently increased during treatment. There was no trend in the pattern of occurrence for either nimesulide or trimethoprim. Both compounds were detected in effluent samples and not in corresponding influent samples. However, nimesulide was detected in both influent and corresponding effluent samples in March 2008.

In total fourteen of the twenty analytes were found in wastewater samples. Caffeine, carbamazepine, clotrimazole, diclofenac, furosemide, mefenamic acid, metoprolol, nimesulide, pravastatin, propranolol, salicylic acid and trimethoprim were detected in the three treatment plants. Bezafibrate was found in the Swords and Ringsend plants but absent in samples collected from the Leixlip plant while gemfibrozil was found only in samples from the Leixlip plant.

	Aug 2007		Sept 2007		Oct 2007		Nov 2007		Dec 2007	
	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff
Bezafibrate	<0.03	<0.05	7.25	<0.15	<0.03	<0.05	<0.03	<0.05	<0.03	<0.05
Caffeine	<0.93	<0.14	<0.28	<0.14	34.9	<0.46	<0.93	<0.14	<0.28	<0.14
Carbamazepine	<0.03	0.26	<0.01	0.25	0.1	1.09	<0.01	0.25	<0.03	0.35
Clotrimazole	<0.01	<0.01	<0.01	<0.01	0.54	0.45	0.90	1.20	<0.03	0.33
Diclofenac	Sup	<0.74	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74
Furosemide	<0.09	<0.37	Sup	<0.37	<0.09	<0.37	<0.09	0.50	Sup	<0.37
Mefenamic acid	Sup	0.53	Sup	0.20	Sup	<0.13	Sup	0.25	Sup	0.57
Metoprolol	2.57	<0.10	Sup	<0.10	<0.63	<0.10	<0.63	<0.10	Sup	<0.10
Nimesulide	Sup	1.07	Sup	0.07	<.005	<0.003	<.005	0.02	Sup	<0.003
Pravastatin	<0.07	<0.05	<0.24	<0.05	<0.24	<0.05	<0.07	<0.16	<0.07	<0.16
Propranolol	Sup	<0.02	Sup	<0.02	Sup	<0.02	Sup	0.26	Sup	<0.02
Salicylic acid	<0.09	<0.11	<0.09	<0.11	<0.09	<0.11	<0.09	<0.11	<0.09	<0.11
Trimethoprim	<0.17	<0.07	<0.17	0.25	<0.17	0.32	<0.17	0.1	<0.17	<0.07
BOD (mg/L)	300	4	330	2	350	5			270	2
COD (mg/L)	545	34.1	712	28.6	927	30.6			562	38.8
pH	7.75	7.52	7.8	7.43	7.71	7.23			7.6	7.52
SS (mg/L)	401	17	247	11	407	13			253	9
Flowrate (m^3/d)	11991		9570		10216				12259	
Rainfall (mm)	1.3		0		0		0.4		7.8	
Temperature (^{0}C)	17.8		18.3		16.2		13.6		9.7	
Sunshine (h)	4.8		8.8		5.0		0		3.6	

Table 3.6.1 Concentration of analytes (µg/L) detected in Swords WWTP samples (Inf = Influent; Eff = Effluent; Sup = Suppressed signal). BOD, COD, SS and flowrate data were obtained from the WWTP. Rainfall, temperature and sunshine data were obtained from the Met Éireann

	Jan 2008		Feb 2008		Mar 2008		April 2008		June 2008	
	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff
Bezafibrate	<0.03	0.12	<0.03	<0.05	<0.03	<0.05	<0.03	<0.05	<0.03	<0.05
Caffeine	<0.28	<0.46	<0.28	<0.46	<0.28	<0.46	<0.28	<0.46	<0.28	<0.46
Carbamazepine	<0.03	0.59	0.15	0.30	<0.03	0.25	<0.03	0.60	1.52	<0.01
Clotrimazole	<0.03	0.12	<0.03	<0.01	<0.03	<0.01	<0.03	<0.01	<0.03	<0.01
Diclofenac	Sup	<0.74	Sup	<0.74	Sup	2.95	<0.86	<0.74	<0.86	<0.74
Furosemide	0.45	0.44	<0.09	<0.11	<0.09	0.30	<0.09	0.85	<0.09	<0.11
Mefenamic acid	Sup	<0.004	Sup	<0.004	Sup	<.004	Sup	<.004	Sup	<.004
Metoprolol	Sup	<0.10	Sup	<0.10	Sup	0.12	Sup	<0.10	Sup	<0.10
Nimesulide	<.002	<.001	<.002	<.001	<.002	<.001	<.002	<.001	0.25	<0.003
Pravastatin	<0.07	<0.05	<0.07	<0.05	1.85	<0.16	<0.07	<0.05	<0.07	<0.05
Propranolol	Sup	<0.02	Sup	<0.02	Sup	<0.02	<.007	<0.02	<.007	<0.02
Salicylic acid	<0.03	Sup	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12
Trimethoprim	<0.17	<0.02	Sup	<0.02	15.7	0.85	<0.17	<0.02	Sup	<0.02
BOD (mg/L)	420	7	350	2	450	4			410	
COD (mg/L)	1220	51.4	1011	67.9	482	31	678	44.2	1087	99.6
pH	7.91	7.49	7.64	7.24	7.64	7.58	7.76	7.59	7.48	7.93
SS (mg/L)	475	10	331	22	446	17	308	13	489	27
Flowrate (m^3/d)	10945		9826		9666		11573		8714	
Rainfall (mm)	4.2		0		3.2		0.1		0	
Temperature (^{0}C)	7.3		12.1		11.9		8.7		18.4	
Sunshine (h)	1.7		8.2		3.7		1.7		14.4	

Table 3.6.1 (continued) Concentration of analytes (μg/L) detected in Swords WWTP samples (Inf = Influent; Eff = Effluent; Sup = Suppressed signal). BOD, COD, SS and flowrate data were obtained from the WWTP. Rainfall, temperature and sunshine data were obtained from the Met Éireann.

	July 2007		August 2007		Sept 2007		Oct 2007		Nov 2007		Dec 2007	
	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff
Caffeine	<0.28	3.05	3.79	<0.14	<0.93	<0.46	3.30	<0.14	<0.93	<0.46	<0.93	<0.14
Carbamazepine	<0.03	0.12	0.15	0.35	0.20	0.52	<0.03	<0.01	<0.03	0.50	0.28	0.32
Clotrimazole	<0.03	0.13	0.19	<0.01	<0.03	0.30	<0.03	<0.01	0.22	0.13	<0.03	0.11
Diclofenac	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74
Furosemide	<0.09	<0.37	<0.09	<0.37	<0.09	<0.11	<0.09	<0.37	<0.09	0.68	<0.09	<0.11
Gemfibrozil	0.14	<0.01	<0.03	<0.03	<0.03	<0.01	<0.03	<0.01	<0.03	<0.01	<0.03	<0.03
Mefenamic acid	Sup	<0.01	Sup	0.67	Sup	<.004	Sup	<.004	Sup	<.004	Sup	1.73
Metoprolol	Sup	<0.10	Sup	<0.10	Sup	<0.10	Sup	4.09	Sup	<0.10	Sup	<0.10
Nimesulide	0.02	<.003	<.005	0.14	Sup	<.003	<.005	<.003	Sup	0.35	<.005	0.13
Pravastatin	<0.07	<0.05	<0.07	<0.05	<0.07	<0.05	<0.07	<0.05	<0.07	<0.05	<0.24	<0.05
Propranolol	Sup	<0.02	Sup	<0.02	Sup	<0.02	Sup	<0.02	Sup	<0.02	Sup	<0.02
Salicylic acid	0.30	<0.12	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12	<0.09	<0.12
Trimethoprim	<0.17	<0.02	<0.17	0.20	<0.17	<0.02	<0.17	<0.02	<0.57	0.57	0.87	0.41
BOD (mg/L)			61	3.5			198	2.8	101	2.5	97	6.4
COD (mg/L)			125	9	309	28	408	23	295	31	253	23
pH			7.52	7.49	7.38	7.18	7.63	7.12	7.41	7.72	7.34	7.67
SS (mg/L)			42	4	254	8	374	3	116	7	163	8
Flowrate (m^3/d)	11168		14711		9446		8179		9516		16714	
Rainfall (mm)			16.6		0.2		1.3		0.4		0	
Temperature (^{0}C)			17		13.2		15.8		7.5		6.6	
Sunshine (h)			4.5		9.7		0.7		4.7		6.2	

Table 3.6.2 Concentration of analytes (μg/L) detected in Leixlip WWTP samples (Inf = Influent; Eff = Effluent; Sup = Suppressed signal). BOD, COD, SS and flowrate data were obtained from the WWTP. Rainfall, temperature and sunshine data were obtained from the Met Éireann

	Jan 2008		Feb 2008		Mar 2008		April 2008		May 2008		June 2008	
	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff
Caffeine	<0.93	<0.14	<0.28	<0.14	<0.93	<0.14	<0.28	<0.14	<0.93	<0.14	<0.28	<0.14
Carbamazepine	<0.03	<0.01	<0.03	0.58	0.72	0.70	0.13	1.20	0.70	<0.01	0.77	1.20
Clotrimazole	<0.03	<0.01	<0.03	<0.01	<0.03	<0.01	<0.03	0.21	0.5	<0.01	<0.03	<0.01
Diclofenac	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74	<0.86	0.73	<0.86	<0.74
Furosemide	<0.09	<0.11	<0.09	<0.11	<0.09	<0.11	1.00	<0.11	0.90	<0.37	1.70	<0.11
Gemfibrozil	<0.03	<0.01	<0.03	<0.01	<0.03	0.15	<0.03	0.12	<0.03	<0.01	<0.03	<0.01
Mefenamic acid	Sup	<.004	Sup	<.004	Sup	<.004	Sup	<.004	Sup	0.90	Sup	<.004
Metoprolol	Sup	<0.32	Sup	<0.32	Sup	<0.10	Sup	<0.10	Sup	<0.10	Sup	<0.10
Nimesulide	<.002	<.001	<.002	<.001	<.002	<.003	<.002	<.001	<.002	<.001	<.002	<.001
Pravastatin	<0.07	<0.02	<0.07	<0.02	3.25	0.40	<0.07	<0.02	<0.07	<0.02	<0.07	<0.02
Propranolol	Sup	<0.06	Sup	<0.06	Sup	<0.02	Sup	<0.02	Sup	<0.02	Sup	<0.02
Salicylic acid	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12
Trimethoprim	<0.17	0.46	<0.17	<0.02	<0.17	<0.02	<0.17	<0.02	0.25	<0.02	<0.17	<0.02
BOD (mg/L)	114	2	200	2	102	5			206	3.8	299	7.7
COD (mg/L)	398	23	414	23	343	26			650	67	447	57
pH	7.40	7.09	7.38	7.10	7.47	7.26	7.38	7.15	7.28	7.24	7.43	7.21
SS (mg/L)	182	4	104	4	102	2	278	9	452	14	190	13.5
Flowrate (m^3/d)	16920		13518		10518		10632		8812		8140	
Rainfall (mm)	1.8		1		0.7		1.5		0		0	
Temperature (^{0}C)	8.6		9.4		4.7		11.1		19.9		23.3	
Sunshine (h)	0.1		5.1		3.4		6.6		9.4		9.7	

Table 3.6.2 (continued) Concentration of analytes (μg/L) detected in Leixlip WWTP samples (Inf = Influent; Eff = Effluent; Sup = Suppressed signal). BOD, COD, SS and flowrate data were obtained from the WWTP. Rainfall, temperature and sunshine data were obtained from the Met Éireann

	July 2007		Aug 2007		Sept 2007		Oct 2007		Nov 2007		Dec 2007	
	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff
Bezafibrate	<0.03	<0.05	<0.03	<0.05	<0.03	<0.05	<0.03	<0.05	<0.03	<0.05	<0.03	<0.15
Caffeine	<0.28	<0.14	<0.93	22.7	<0.93	<0.46	2.10	2.15	<0.28	<0.14	<0.28	<0.46
Carbamazepine	0.45	0.60	0.23	0.49	0.45	0.70	<0.01	6.50	0.10	0.22	0.24	0.78
Clotrimazole	<0.01	0.50	0.65	8.65	0.35	0.28	<0.01	<.004	0.17	0.61	<0.01	0.10
Diclofenac	<0.86	0.50	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74
Furosemide	6.45	0.30	<0.09	<0.11	1.45	<0.11	<0.09	<0.37	<0.09	1.33	<0.09	<0.37
Mefenamic acid	Sup	9.1	Sup	0.29	Sup	<.004	Sup	<.004	Sup	0.30	Sup	<.004
Metoprolol	Sup	<0.32	<2.11	4.19	Sup	<0.10	Sup	<0.10	<2.11	4.34	Sup	<0.10
Nimesulide	Sup	3.05	Sup	0.50	<.002	<0.003	0.88	<.001	<.002	<.001	<0.005	<.001
Pravastatin	1.55	<0.05	<0.07	<0.05	<0.07	<0.05	<0.07	<0.05	<0.07	<0.05	<0.07	<0.05
Propranolol	Sup	<0.02	Sup	<0.02	Sup	<0.02	Sup	<0.02	Sup	<0.02	Sup	<0.02
Salicylic acid	12.8	<0.12	5.1	<0.12	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12
Trimethoprim	<0.17	0.62	<0.17	0.22	<0.17	<0.07	<0.17	<0.02	<0.17	<0.02	<0.17	<0.02
BOD (mg/L)	191	5	186	15	323	16	259	7	227	10	232	13
COD (mg/L)	409	59	426	46	843	71	566	50	615	49	530	56
pH	7.5	7.6	7.6	7..8	7.6	7.7	7.6	7.6	7.6	7.5	7.5	7.6
SS (mg/L)	240	18	176	18	396	26	316	19	276	19	241	19
Flowrate (m^3/d)	509245		391576		799043		349516		733549		368793	
Rainfall (mm)	5.3		0		0		1.3		8.9		0	
Temperature (^{0}C)	15.4		17.9		19.0		15.8		8.8		7.3	
Sunshine (h)	4.9		2.4		8.4		0.7		0.1		6.9	

Table 3.6.3 Concentration of analytes (µg/L) detected in Ringsend WWTP samples (Inf = Influent; Eff = Effluent; Sup = Suppressed). BOD, COD, SS and flowrate data were obtained from the WWTP. Rainfall, temperature and sunshine data were obtained from the Met Éireann

	Jan 2008		Feb 2008		Mar 2008		April 2008		May 2008		June 2008	
	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff
Bezafibrate	<0.03	<0.05	<0.03	<0.05	<0.03	<0.05	<0.03	<0.05	<0.03	<0.05	<0.03	<0.05
Caffeine	<0.28	<0.14	<0.28	<0.14	<0.28	10.9	<0.28	<0.14	13.9	<0.14	0.50	<0.14
Carbamazepine	0.30	0.45	<0.03	0.47	<0.03	0.25	0.51	<.004	0.20	<0.01	0.55	<0.01
Clotrimazole	<0.01	<0.01	<0.01	<0.01	<0.01	0.15	<0.03	<.004	<0.03	<0.01	<0.01	<.004
Diclofenac	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74	<0.86	<0.74
Furosemide	<0.09	<0.11	<0.09	<0.11	3.45	2.28	<0.09	<0.11	<0.31	0.35	<0.09	0.14
Mefenamic acid	Sup	<.004	Sup	<.004	Sup	<.004	Sup	<.004	Sup	<.004	Sup	<0.01
Metoprolol	Sup	<0.10	Sup	<0.10	Sup	<0.10	Sup	<0.10	Sup	<0.10	Sup	2.0
Nimesulide	<.002	<.003	0.15	<.003	0.64	<.003	<.002	<.003	<.002	<.003	<.002	<.003
Pravastatin	<0.07	<0.05	<0.07	<0.05	<0.07	0.82	<0.07	<0.05	<0.07	<0.05	<0.07	<0.05
Propranolol	Sup	<0.02	Sup	0.31	Sup	<0.02	Sup	<0.02	Sup	<0.02	Sup	<0.02
Salicylic acid	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12	<0.03	<0.12
Trimethoprim	1.6	<0.02	<0.17	<0.02	<0.17	<0.07	<0.17	<0.02	0.10	<0.02	<0.17	<0.02
BOD (mg/L)	70	15	278	6	183	39	220	13	565	15	248	8
COD (mg/L)	159	53	543	66	352	130	438	62	1030	63	570	53
pH	7.7	7.6	7.4	7.4	220	98	7.6	7.6	7.3	7.5	7.4	7.7
SS (mg/L)	70	33	278	29	7.4	7.4	274	30	346	35	272	22
Flowrate (m^3/d)	837565		399936		492370		434566		352482		352043	
Rainfall (mm)	4.5		0		3.2		0.1		0		0	
Temperature (^{0}C)	12.2		12.1		11.9		8.7		17.4		18.4	
Sunshine (h)	1.9		8.2		3.7		1.7		9.7		14.4	

Table 3.6.3 (continued) Concentration of analytes (μg/L) detected in Ringsend WWTP samples (Inf = Influent; Eff = Effluent; Sup = Suppressed signal). BOD, COD, SS and flowrate data were obtained from the WWTP. Rainfall, temperature and sunshine data were obtained from the Met Éireann

4.0 Discussion

4.1 Matrix Effects

One of the main characteristics of the method used in this study and of electrospray mass spectrometry in general is that it is subject to interference from organic and inorganic components in the matrix (Kasprzyk-Hrodern *et al.*, 2008). This interference usually results in the suppression of analyte signals affecting the overall sensitivity of the method (Petrović *et al.*, 2005).

4.1.1 Addition Post Extraction

The effects of matrix suppression/enhancement in influent and effluent matrices are presented in Table 3.3.1 (page 61). Four compounds (flurbiprofen, ibuprofen, metoprolol and propranolol) were suppressed by more than fifty percent in the presence of influent matrix components. Three compounds (salicylic acid, ibuprofen and salbutamol) were suppressed, also, by more than fifty percent in effluent matrix components. Ibuprofen exhibited the most intense suppression in both matrices at 77.7% and 72.0% respectively. This may account for the poor linearity (0.8558) and reproducibility (34.7%) (Table 3.2.1 and 3.2.2, pages 58 and 59) obtained during method validation. Ibuprofen was not detected in any sample during the twelve months which may also be due to the reduced analyte signal. The apparent high removal of salicylic acid observed in the three WWTPs may also be a result of increased suppression in effluent samples. To correct for any suppression or enhancement of analyte signal, internal standards or alternatively a method of standard additions can be used. In this study a wide range of compounds are being analysed and therefore a large number of internal standards would be required. The use of an inadequate number of internal standards for quantification may lead to an inaccurate quantification of signal suppression of each compound

and may result in under- or over-estimation of compound concentration. Therefore, standard addition was the chosen method for quantification.

4.1.2 Post column infusion

An additional study to illustrate the effects of signal suppression was undertaken for four compounds and the results are presented in Figures 3.3.1. – 3.3.4 (pages 63 and 64). Signal suppression of nimesulide was quantified at 18.5% (influent) and <5% (effluent) in the addition post extraction experiment (Table 3.3.1). However, post column infusion showed almost complete suppression of the analyte signal at its retention time of 22 minutes. Suppression of the signal in the presence of the effluent extract confirmed the <5% suppression observed in the addition post extraction experiment. Mefenamic acid, also monitored in negative mode ionisation, showed extensive signal suppression in the presence of influent matrix components. The suppressive effect was less in the effluent sample. This is contradictory to what was observed in the addition post extraction study where no suppressive effect was determined in influent samples and 25.8% suppression in the presence of effluent matrix components. The complete suppression of signal in influent samples may account for the detection of mefenamic acid in effluent samples despite it being undetected in corresponding influent samples.

Carbamazepine and trimethoprim were the two analytes monitored in positive mode ionisation which were selected for post column infusion studies. The results for carbamazepine show signal suppression in both influent and effluent samples (Figure 3.3.3). While the level of suppression was more pronounced than that observed in the post extraction addition study complete suppression of the analyte signal did not occur. The analyte signal suppression for trimethoprim was also greater in the post column infusion experiment than in the post extraction addition study. At the retention time of approximately two minutes there is significant suppression of both influent and effluent signals. The level of suppression caused by the influent matrix was greater than that caused by the effluent

matrix which is consistent with what was seen with the compounds analysed in negative mode. Variation in signal suppression observed for the four analytes investigated is due to the variation in matrix components between samples.

Overall, the effect of matrix suppression is more pronounced in negative mode ionisation than positive mode ionisation. This has been observed in a previous study on the effect of environmental sample matrix components on electrospray ionisation (Benijts *et al.*, 2004). Also, the suppressive effect is reduced from influent to effluent samples. This decrease is due to the reduction in organic and inorganic loading. Competition between the analytes and matrix components for access to the droplets surface and gas phase emission is one possible cause for matrix suppression in ESI (Benijts *et al.*, 2004). Matrix effects were seen to be compound-dependent and previous studies on the suppressive effects of plasma have shown a decrease in matrix suppression with increased polarity of the compound (Bonfiglio *et al.*, 1999). In this study, matrix suppression was seen to be compound-dependent but correlation between compound polarity and matrix suppression was not as clear. Carbamazepine is more polar than nimesulide and mefenamic acid. However the matrix effects observed for carbamazepine are lower. Of the two compounds analysed in positive mode ionisation trimethoprim, the more polar compound is suppressed to a greater degree. This is in line with what was reported by Bonfiglio *et al.*, 1999. In the absence of a database of mass spectra for substances, two further investigations were completed in an attempt to elucidate the compounds which may be responsible for the increased matrix suppression. Firstly, the effects of surfactants in influent and effluent samples was investigated and secondly, the presence of metals was established (Section 4.2).

4.1.3 Standard additions

A method of standard additions was used in this analysis for quantification to ensure the accurate reporting of concentrations present in wastewater

streams. The linear plots used for quantification of analytes in November 2007 are included in Figures 3.3.5 – 3.3.8, 3.3.10 – 3.3.14, 3.3.19 – 3.3.23 (pages 67-70, 72-76, 81-85) for illustration. Good linearity of standard addition was required for quantification in this analysis. Only plots with a correlation coefficient of ~0.9 or above were used for quantification during the twelve months sampling. Using this method the emergence of compounds during treatment could be investigated. For example, nimesulide was detected in the effluent sample from Leixlip in November 2007 while the concentration present in the influent stream was below detection limits. This was considered a true result as an analyte response was observed for the standard additions in the influent sample (Figure 3.3.7, page 69). In the same month, trimethoprim was also detected in the effluent sample but was absent from the influent. When the standard additions for trimethoprim in the influent matrix were analysed, no signal for trimethoprim was detected (Figure 3.3.9, page 71). This result indicates that there may in fact have been trimethoprim in the influent stream but that it was masked by the suppressive effects of matrix components. This result is in line with what was observed in the addition post extraction and post column infusion investigations. The same observation was made for furosemide in the November sample at Leixlip. Similarly, some compounds detected in the effluent and not in influent samples from Swords treatment facility had complete signal suppression of standard additions in the influent (furosemide, trimethoprim, mefenamic acid and propranolol). This suppressive effect was also observed for furosemide and mefenamic acid in influent streams at Ringsend.

The significant matrix suppression identified in the analysis of influent samples in this study indicates that the extent of pharmaceutical contamination in influent samples cannot be determined for specific analytes. Flurbiprofen, mefenamic acid, diclofenac, clofibric acid, sulfamethoxazole and ibuprofen analyte signals were completely suppressed in influent samples from the three treatment plants. Complete signal suppression was also observed for indomethcin and salbutamol in influent samples from the Swords treatment plant. It also calls into question

the hypothesis put forward by numerous authors that metabolites are deconjugated, during wastewater treatment, to yield the parent compound as an explanation of increasing effluent concentrations (Ternes, 1998; Heberer *et al.*, 2002; Miao *et al.*, 2002; Lishman *et al.*, 2006).

4.2 Metal and Surfactant Analysis

The metal analysis completed by TMS Environmental Ltd. showed that only trace quantities of metal were present in influent and effluent streams. Also, where metals were detected there was no significant variation between influent and effluent samples. This indicates that suppression of analyte signal during analysis is not associated with metal related interferences.

The effect of LAS on the signal suppression of four compounds (nimesulide, mefenamic acid, carbamazepine and trimethoprim) was investigated. LAS products contain a mixture of homologues with alkyl chain lengths from C_{10} to C_{13}. However, for the purpose of this investigation, monitoring of the individual species was not included because an overall picture of the presence and suppressive effect of the surfactant on analysed standards was required. Signal suppression of >90% was observed for three of the selected analytes (trimethoprim, nimesulide and mefenamic acid), while a signal increase of 7.92% was observed for carbamazepine. Alteration of peak shape and retention of compounds was also effected by the presence of the surfactant. The retention time of nimesulide was reduced by 2.5 minutes and the retention of mefenamic acid was also reduced by up to 0.8 minutes (Figure 3.5.1, page 90). No effect on retention of carbamazepine was observed. Retention time may have been reduced due to interactions with the surfactants allowing for accelerated transport of compounds through the column. Suppressive effects may be due to the surfactant binding with the compounds masking their presence or neutralising the charge preventing ionisation.

The presence of the surfactant did not cause complete suppression of the analyte signal for mefenamic acid. Therefore, the presence of the surfactant LAS in influent samples would be unlikely to have caused the low detection of mefenamic acid in influent samples. Similarly, the carbamazepine analyte signal was not completely suppressed and the presence of LAS in influent samples would not have prevented the detection of carbamazepine. The response for both nimesulide and trimethoprim was significantly altered. The analyte signal for nimesulide was completely suppressed similar to that observed in the post column infusion investigation for nimesulide (Figure 3.3.1, page 63). The peak shape of the trimethoprim standard in the presence of LAS was completely altered so that quantification would not be possible.

4.3 Monthly Sampling

The results of the monthly sampling programme are presented in Section 3.6. Overall, low concentrations (μg/L) of fifteen compounds were detected in wastewater streams. The individual concentrations present in effluent streams are mostly below that which may impart any toxic effect to aquatic organisms (Table 1.5.1). Nevertheless, there is one cause for possible concern, the concentration of mefenamic acid in effluent streams. Using predicted no effect concentrations and the measured effluent concentration the risk quotients of the effluent streams are >1, indicating a potential ecotoxicological risk with sewage effluent. However, when a commonly employed dilution factor of 10 is taken into account the risk quotient is reduced to below 1.

Removal of pharmaceuticals from a wastewater stream during treatment is affected by numerous factors including: the physio-chemical nature of the compound, the composition of the sewage as well as weather conditions and operational parameters of the treatment process. Consequently, to determine the impact of such parameters, influent and effluent concentrations were plotted against pK_a (acid dissociation constant) and log P (partition coefficient) values and operational values (flowrate, biological oxygen demand (BOD), chemical oxygen demand

(COD), pH and suspended solids (Appendices B, C and D). No relationships were observed. Weather conditions such as temperature and rainfall have been shown to affect the concentration of pharmaceuticals in influent and effluent samples. These parameters were analysed however no relationships were determined. The effects of temperature, rainfall and hours of sunshine have also been shown to correlate with removal rates. However, as a significant number of the analytes under investigation was detected only in effluent samples and not in corresponding influent samples, removal efficiencies could not be determined for the majority of compounds. While these apparent increases in concentration may be due to the emergence of the parent compound following deconjugation of metabolites during wastewater treatment (Ternes, 1998; Lishman *et al.*, 2006), it is more likely to be as a result of suppressed analyte signal during analysis of influent samples as discussed in Section 4.1.

As the analyte signal for carbamazepine was not completely suppressed in either influent or effluent matrices and good linearity was attained for standard additions in influent and effluent samples, removal efficiencies for this analyte could be analysed. In general no removal of carbamazepine was observed in the three treatment plants with the concentration apparently increasing during treatment. For example, Table 4.3.1 highlights the increase (+) or decrease (-) in concentration observed in the Leixlip plant over the twelve months.

	Influent	Effluent	Change
July 07	<LOD	0.12	+ 0.12
August 07	0.15	0.35	+ 0.20
September 07	0.20	0.52	+ 0.32
October 07	<LOD	<LOQ	0
November 07	<LOD	0.50	+0.50
December 07	0.28	0.32	+0.04
January 08	<LOQ	<LOQ	0
February 08	<LOQ	0.58	+0.58
March 08	0.72	0.70	-0.02
April 08	0.13	1.20	+1.07
May 08	0.70	<LOQ	-0.70
June 08	0.77	1.20	+0.43

Table 4.3.1 Change in carbamazepine concentration (µg/L) in influent and effluent samples at the Leixlip plant.

This behaviour was also observed in previous studies (Clara *et al.*, 2004). However, with an increase in temperature and daylight hours and a reduction in flow to the plants from April to June, removal of carbamazepine from effluent streams at the Ringsend plant was greater than 99% (Figure 4.3.1). In samples from the Swords plant in June over 99% removal was also observed. It can be assumed that the removal of carbamazepine during wastewater treatment is a result of degradation, as sorption to sludge would not be expected due its low K_{ow} (octanol-water partitioning coefficient) of 2.25. Removal, due to adsorption alone, determined in other investigation has been low at < 10% (Clara *et al.*, 2004; Ternes *et al.*, 2005). Microbial activity is temperature dependent and therefore higher removal rates would be expected during the summer (Castiglioni *et al.*, 2006). The complete removal of carbamazepine in April, May and June 2008 seen at the Ringsend plant may also be as a result of photolysis. UV tertiary treatment is used to treat effluent from the plant during the bathing season. Direct photolysis of carbamazepine using an immersed medium-pressure mercury lamp in ideal conditions (Milli-Q water) has shown that carbamazepine is degraded to six intermediates

(Chiron *et al.,* 2006). 100% removal efficiencies were observed during the bathing season, while removal of up to 20% was determined at other times during the year (Ringsend data Figure 4.3.1).

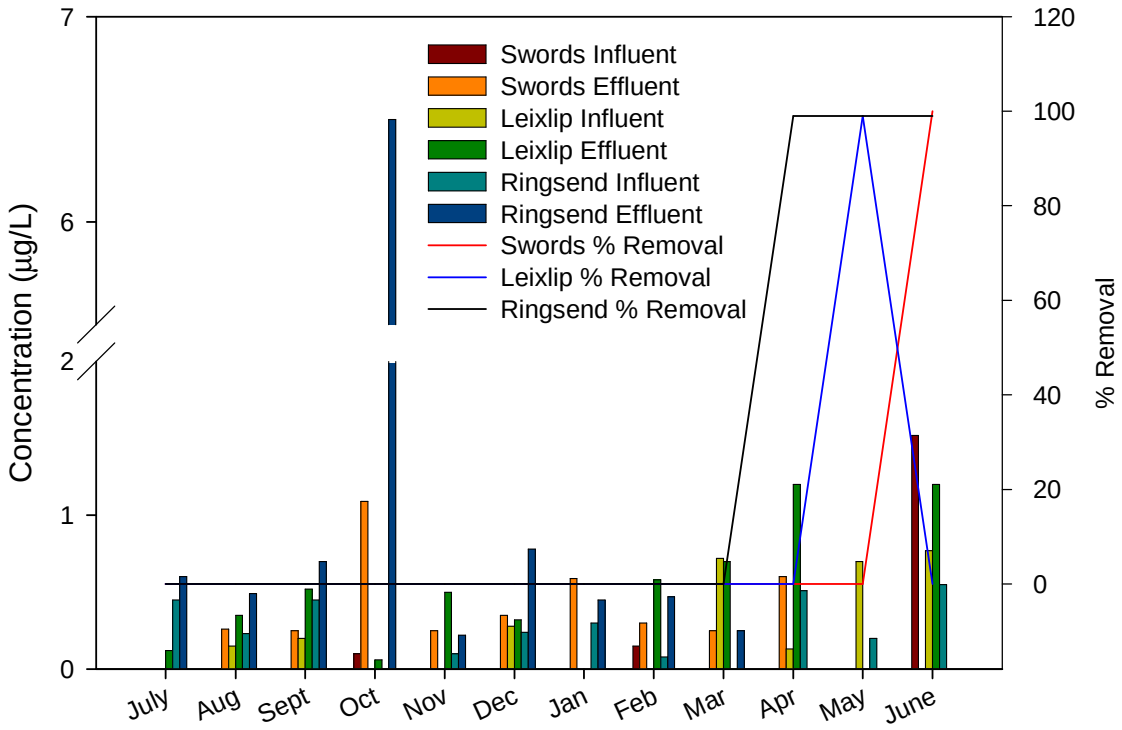


Figure 4.3.1 Influent and effluent concentrations of carbamazepine (July 2007 – June 2008).

Statistical analysis of calculated influent and effluent concentrations was completed. A method of standard additions was used to determine the concentration of each analyte in both influent and effluent samples. This method allowed for a calibration graph and measurement of concentrations relative to the effects of the matrix to be obtained. As a number of concentrations are used to determine the concentration the standard deviation of the extrapolated concentration is calculated using equation (1).

$$S_{x_E} = \frac{S_{y/x}}{b}\left\{\frac{1}{n} + \frac{\bar{y}^2}{b^2\sum_i (x_i - \bar{x})^2}\right\}^{\frac{1}{2}} \qquad (2)$$

where: $$S_{y/x} = \left\{ \frac{\sum_i \left(y_i - \bar{y}_i \right)^2}{n-2} \right\}^{\frac{1}{2}}$$

and b = slope.

The 95% confidence limits of the extrapolated concentrations are equal to the standard deviation of the extrapolated concentration multiplied by the 95% confidence t value.

The calculated confidence limits indicate that the concentrations obtained for each of the analytes are subject to wide variation. This is due in part to the low number of points on each of the calibration curves. Ideally six points would be used for a standard addition experiment as the greater the value of n (number of data points) the more precise the estimated concentration (Miller and Miller, 1993). However, due to the limited sample volume (2 litres) available from each of the sample locations monthly, a maximum of 4 samples could be analysed in this work. That is the raw sample with no addition of a standard and three samples with a standard addition. This reduced value of n means that the degrees of freedom (n-2) used to determine the t value in the calculation is 2. The concentrations obtained in this work are however are deemed to be valid as the calibration curves yielded high regression coefficients.

	Ringsend Nov 07		Swords Nov 07		Leixlip Nov 07		Ringsend April 08		Ringsend April 08		Leixlip April 08	
	Conc	CL	Conc	CL	Conc	CL	Conc	CL	Conc	CL	Conc	CL
Carbamazepine (Inf)	0.10	0.56	-	-	0.22	0.60	0.51	9.78	0.60	5.81	1.30	2.04
Carbamazepine (Eff)	0.22	2.17	0.25	1.40	0.50	1.40	-	-	-	-	1.20	2.57
Clotrimazole (Inf)	0.17	3.22	0.90	1.53	0.05	1.13	-	-	-	-	-	-
Clotrimazole (Eff)	0.61	0.73	1.20	1.48	0.13	1.30	-	-	-	-	0.21	0.70
Nimesulide (Inf)	0.88	7.04	-	-	-	-	0.10	1.30	-	-	-	-
Nimesulide (Eff)	-	-	0.20	0.87	0.35	2.50	-	-	-	-	-	-
Furosemide (Inf)	-	-	-	-	-	-	-	-	0.85	8.60	0.45	0.72
Furosemide (Eff)	1.33	6.73	0.50	9.82	0.68	1.14	-	-	-	-	-	-
Trimethoprim (Eff)	-	-	0.12	0.73	0.57	8.98	-	-	-	-	-	-
Metoprolol (Inf)	1.70	2.10	-	-	-	-	-	-	-	-	-	-
Mefenamic Acid (Eff)	0.30	4.77	-	-	-	-	-	-	-	-	-	-
Propranolol (Eff)	0.26	1.19	-	-	-	--	-	-	-	-	-	-
Diclofenac (Eff)	0.24	0.15	-	-	-	-	-	-	-	-	-	-
Gemfibrozil (Eff)	0.12	0.15	-	-	-	-	-	-	-	-	-	-

Table 4.3.2 Confidence limits for extrapolated concentrations from November 2007 and April 2008 for the three WWTPs. Conc = Concentration (μg/L); CL = Confidence Limit (μg/L).

4.4 Seasonal Trends

As influent concentration data sets are incomplete due to matrix ion suppression effects, data from effluent streams were examined for seasonal trends. The total effluent concentrations for each plant, in μmol, were plotted against flowrates and temperature. The plots are presented in Figures 4.4.1, 4.4.2 and 4.4.3. Lower effluent concentrations were observed with increased flowrate and reduced temperatures (Figure 4.4.1 – see range from November 2007 – March 2008). This may be due to a higher dilution factor because of increased rainfall and also reduced biodegradation at lower temperatures. Reduced flowrates and higher temperatures also resulted in low effluent concentrations in August and September 2007 and April - June 2008 in Figure 4.4.1, which would be consistent with an expected increase in biodegradation at increased temperatures. Similar trends are seen in the Ringsend and Swords plants (Figures 4.4.2 and 4.4.3, page 112 and 113). The trends observed here are generally in line with those seen in previous investigations (Castiglioni *et al.*, 2006; Vieno *et al.*, 2005). The removal of eight compounds was reduced during winter months. For example, sulfamethoxazole had removal rates of 71% in summer which reduced to 17% in winter (Castiglioni *et al.*, 2006). While the total removal levels increased from 61% in March to 88% in August in the Aura WWTP (Vieno *et al.*, 2005).

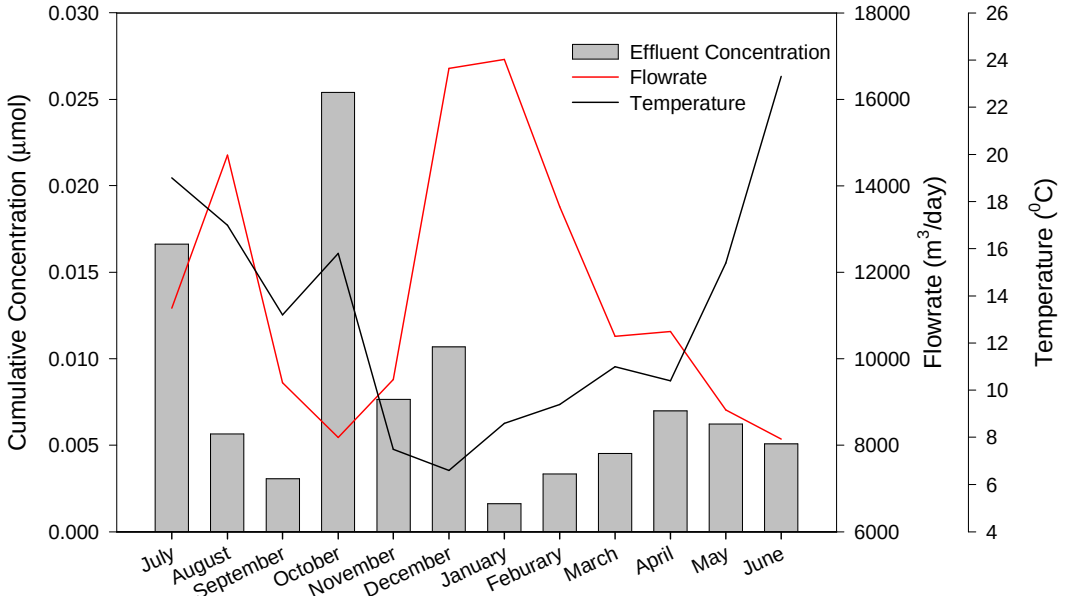


Figure 4.4.1 Cumulative concentration of pharmaceuticals in effluent at Leixlip WWTP and daily temperature and flowrates (July 2007 – June 2008)

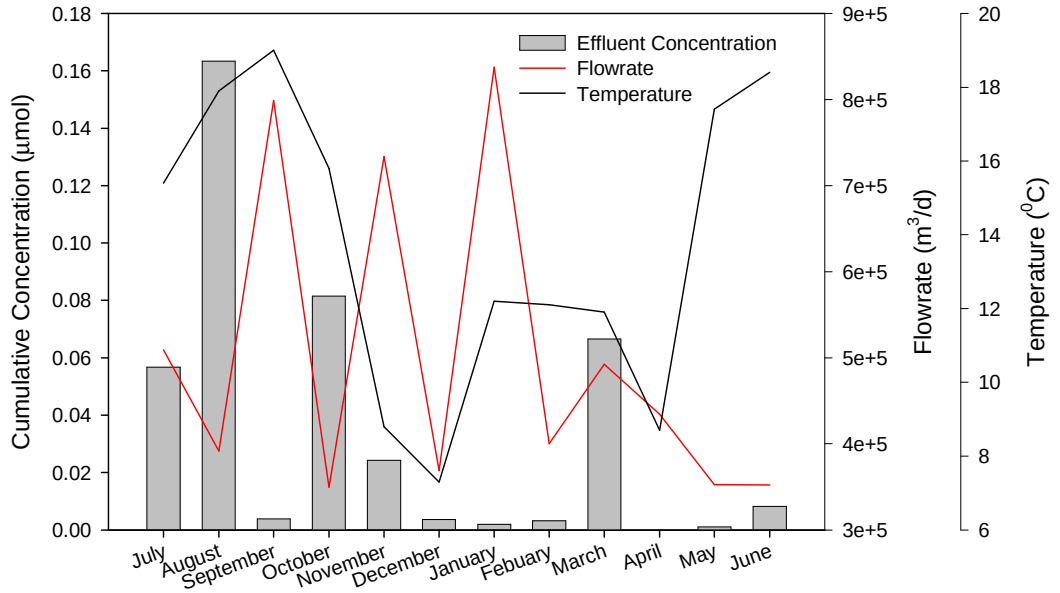


Figure 4.4.2 Cumulative concentration of pharmaceuticals in effluent at Ringsend WWTP and daily temperature and flowrates (July 2007 – June 2008)

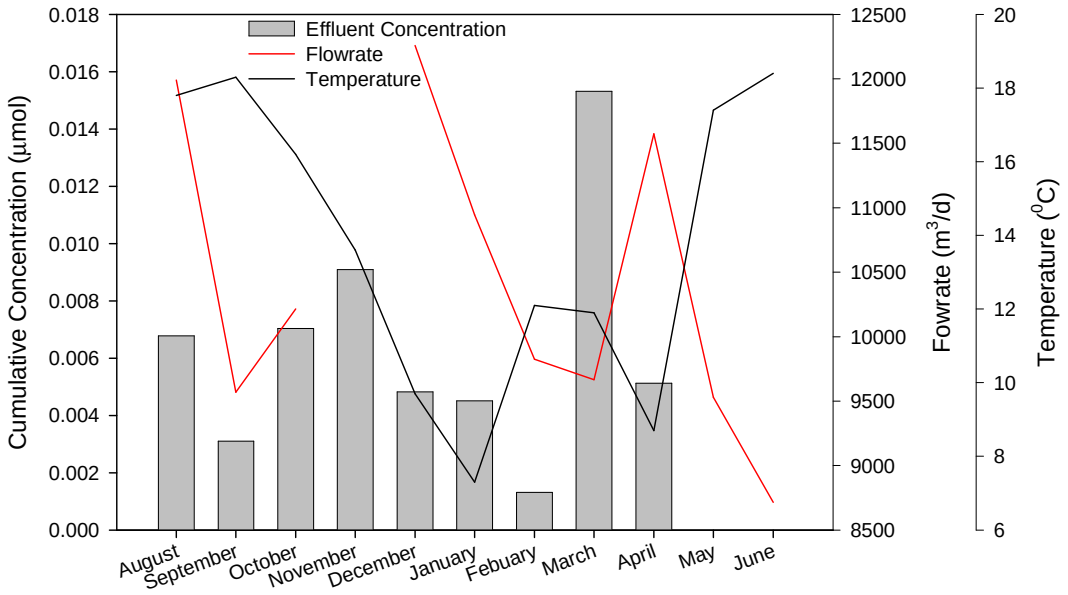


Figure 4.4.3 Cumulative concentration of pharmaceuticals in effluent at Swords WWTP and daily temperature and flowrates (August 2007 – June 2008)

4.5 Analyte occurrence

The occurrence of analytes in influent and effluent streams from the three wastewater treatment facilities will be discussed here by therapeutic class and toxicity will be discussed where data are available.

Analgesics/Anti-inflammatories

Of the twenty compounds selected for this investigation six (diclofenac, flurbiprofen, ibuprofen, indomethcin, mefenamic acid and nimesulide) are anti-inflammatories. Salicylic acid, a metabolite of the analgesic acetylsalicylic acid, will also be included under this heading. Concentrations of flurbiprofen, ibuprofen and indomethcin were below limits of detection in all influent and effluent samples during this study. Significant analyte signal suppression was observed for ibuprofen (>70%) in the addition post extraction study (Table 3.3.1). This indicates that the effects of signal suppression may account for the absence of ibuprofen

from influent and effluent streams. Standard additions in influent and effluent samples confirmed that the absence of ibuprofen was due to signal suppression, as no signal was identified for ibuprofen in either matrix. Significant suppression of flurbiprofen and indomethcin was also observed (Table 3.3.1) with suppression values of up to 60.5% and 44.2% exhibited respectively. Again suppression of the analyte signal affected the analysis of these compounds with standard additions confirming suppression of the signal with complete suppression being observed in influent matrices.

Salicylic acid was detected only in influent samples over the sampling period. Removal of salicylic acid is expected as it is readily biodegradable and has a high removal rate during wastewater treatment (Daughton and Ternes, 1999). Residues of salicylic acid in environmental samples are not necessarily from acetylsalicylic acid. Other sources include its use in food preservation or in acne medications (Heberer, 2002). Theoretical toxicity data obtained using ECOSAR indicate that EC_{50} values for salicylic acid are in mg/L quantities (Sanderson *et al.*, 2003). ECOSAR is an ecological structure activity relationships model that estimates the toxicity of chemicals to aquatic organisms based on similarities in molecular structure to chemicals for which toxicity data are available. The absence of salicylic acid in effluent streams indicates that there is no threat to the environment.

Diclofenac was present in a limited number of effluent samples from the three plants during the sampling period (July 2007 – June 2008). In corresponding influent samples the concentration of diclofenac was below detection limits. Results from initial sample analysis by LC-MS also showed an increase in analyte concentration during wastewater treatment (Appendix A). Poor removal rates were observed in previous studies (Buser *et al.*, 1998, Stumpf *et al.*, 1999, Zwiener *et al.*, 2000). Suggested reasons for this emergence pattern include deconjugation of conjugated metabolites over the treatment process as the primary metabolites of diclofenac are glucuronides and sulphate conjugates which may be cleaved during wastewater treatment and release the parent compound. On the other hand ion suppression in influent matrices may result in a reduction in intensity of the signal and mask the presence of the

compound. Ion suppression was quantified at 23.7% in influent samples (Table 3.3.1). Complete suppression of standard additions was observed in influent samples. Concentrations observed in effluent samples (0.24μg/L – 2.95μg/L) in this study are in line with those previously reported (Table 1.2.1).

Mefenamic acid was detected in approximately 35% of effluent samples while corresponding influent samples did not contain mefenamic acid at a quantifiable level. Similar trends of occurrence in effluent samples were observed in early investigations with LC-MS analysis (Appendix A). This is most likely due to the suppression of compound signal in influent samples (Figure 3.3.2). The results obtained in the post column infusion investigation and subsequent standard additions show that mefenamic acid could not be determined in influent samples due to the complete suppression of analyte signal caused by the influent matrix. As a result removal rates for mefenamic acid could not be determined. However, it is clear from the results obtained that the removal of mefenamic acid from wastewater streams is incomplete in investigated plants. Ecotoxicity data are not available for mefenamic acid, however a predicted no effect concentration (PNEC) of 0.428μg/L was established previously using ECOSAR (Jones *et al.*, 2002). Here effluent concentrations of mefenamic acid, at both the Leixlip and Swords plants, exceeded this value. The maximum risk quotient, MEC/PNEC (measured environmental concentration (effluent concentration)/predicted no effect concentration), for mefenamic acid in effluent streams at the three plants were 0.70 (Ringsend), 4.04 (Leixlip) and 1.33 (Swords). This indicates a potential ecotoxicological risk associated with the concentrations being released to the environment at two plants, Leixlip and Swords. If a commonly used dilution factor of 10 is employed (Halling-Sørensen *et al.*, 2000; Jones *et al.*, 2002) the risk quotient is significantly reduced to below 1 which implies that there may be no risk associated with this analyte. Experimental toxicity data for mefenamic acid are currently unavailable and would be required to accurately determine no effect concentrations and also to ascertain specific toxicological effects.

Nimesulide was suspended from the Irish market in May 2007. Following a review of the safety of nimesulide by the European Medicines Evaluation Agency licences for systemic formulations of nimesulide were revoked in December 2007. However, 3% gel formulations are still licensed for use (Nimesulide, IMB). Monitoring of influent and effluent streams for this compound showed a decline in the concentration present at the Ringsend site from July to September 2007 (Figure 4.5.1). This decline is in keeping with the decrease in usage of the compound. Levels at the other two WWTPs were less than 30% of the concentration recorded at Ringsend in July. Residual levels present in wastewater streams are likely due to the usage of gel formulations still available on the market.

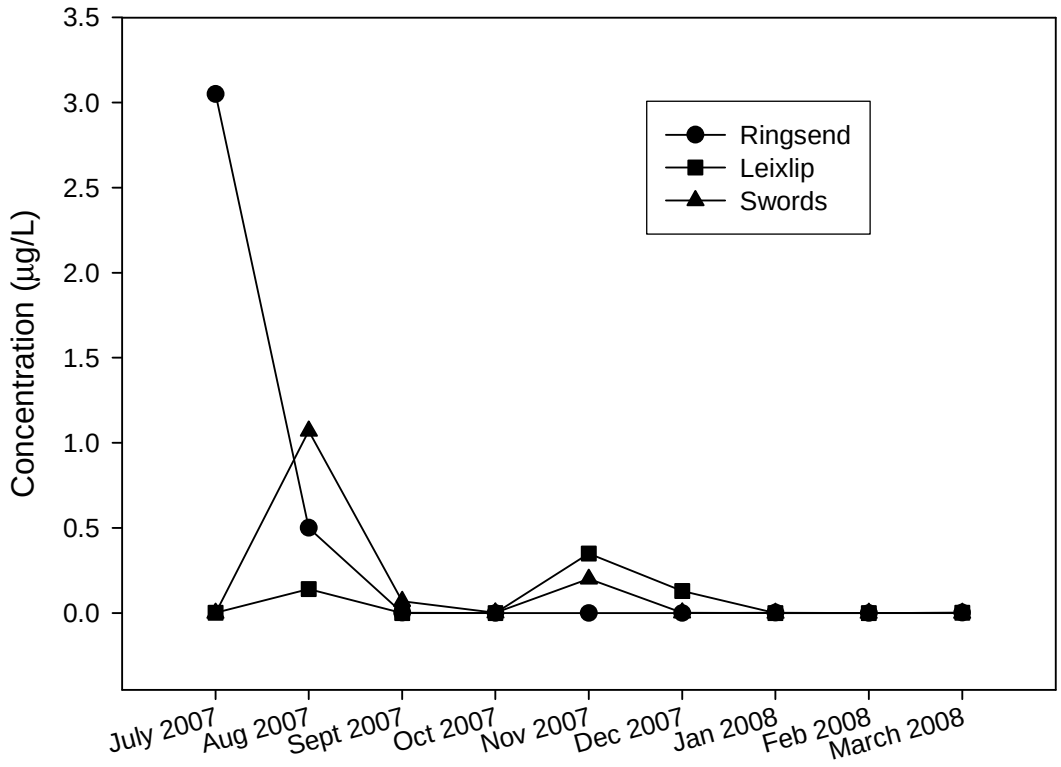


Figure 4.5.1 Decline in effluent concentrations from the three investigated wastewater treatment plants

Anti-convulsant

Carbamazepine was detected every month in the three wastewater treatment plants. It is an anti-convulsant used primarily in the treatment of

epilepsy as well as bipolar depression and trigeminal neuralgia. Concentrations ranging from 0.10-1.52 μg/L were detected in the three WWTPs with the exception of the Ringsend effluent sample in October 2007 which contained 6.5 μg/L of carbamazepine. These concentrations are in line with other studies which found levels of 3.7 μg/L (Ternes, 1998) and 0.3-1.85 μg/L (Clara *et al.*, 2004) in sewage influent and effluent samples. Here an increase in effluent concentration compared to influent concentration was observed at the three WWTPs in more than 90% of samples (Table 3.6.1, 3.6.2 and 3.6.3). On average 72% of ingested carbamazepine is eliminated from the body in the urine of which 2-3% is in the form of the parent compound. The remainder is excreted as an array of metabolites including glucuronide conjugates (Maggs *et al.*, 1997; Ternes, 1998). Deconjugation may occur during wastewater treatment, which would account for the increase in the final effluent concentration. As complete suppression of carbamazepine did not occur and the compound could be quantified in influent samples matrix suppression is not responsible for this increase in concentration during treatment. Carbamazepine would be expected to have low sorption potential with a log K_{ow} of 2.25 (Scheytt *et al.*, 2005a; Jones-Lepp and Stevens, 2007). Minimal or no biodegradation or adsorption of carbamazepine during wastewater treatment was reported previously. Removal rates from conventional activated sludge treatment and during infiltration of treated wastewater to groundwater ranged from 0 - 8% (Castiglioni *et al.*, 2006; Clara *et al.*, 2004; Heberer, 2002; Ternes, 1998). Consequently carbamazepine has been suggested as a good indicator of wastewater contamination in the aquatic environment (Clara *et al.*, 2004). Preliminary analysis on river water approximelety 1.5km downstream of the Leixlip plant discharge point identified carbamazepine. A concentration of 0.22μg/L was determined using standard additions with an R^2 value of 0.98. This also indicates that carbamazepine may be a good marker of effluent contamination. Recent studies on the toxicity of carbamazepine to the cnidarian, *Hydra attenuate,* show carbamazepine has an EC_{50} of 3.76mg/L (Quinn *et al.*, 2008), which is below the effluent concentration

found in this work. This suggests that there is no ecotoxicological risk associated with the quantities of carbamazepine being released from the three plants.

Lipid Modifying Agents

Bezafibrate, a fibrate drug, is used for the treatment of hyperlipidemia. Bezafibrate was not detected in the majority of samples analysed in this study. Only two samples from Swords, one influent and one effluent, and one effluent sample from Ringsend contained bezafibrate. There was no occurrence of bezafibrate in Leixlip. The addition post extraction investigation results indicated that the analysis of bezafibrate was not affected by matrix suppression with <5 % recorded in both influent and effluent (Table 3.2.2). Due to the infrequent occurrence of bezafibrate in the three samples no judgment on the performance of the wastewater treatment process could be made.

Clofibric acid is a metabolite of clofibrate and is commonly detected in environmental samples in European countries (Table 1.2.1). Clofibric acid was not detected in any sample analysed during this study. Detection of clofibric acid would not be expected in this study as clofibrate is no longer available on prescription in Ireland. Clofibric acid was included in this study due to its ubiquitous nature in other European countries, and also to show that the occurrence of residual pharmaceuticals and metabolites can be used to indicate the use of specific compounds. The matrix suppression quantified in the addition post extraction experiment was less than 30%. Standard additions showed that complete suppression of the analyte occurred in influent samples from the three WWTPs while standard additions in effluent samples gave good correlations (>0.9).

Propranolol and metoprolol are two β-blockers used primarily for the treatment of hypertension. In 100% of the occurrences of metoprolol and propranolol at the Leixlip and Ringsend plant the concentration apparently increased during treatment. In the August sample from Swords treatment plant metoprolol was detected in the influent sample while the

concentration was below detection limits in the corresponding effluent sample. Propranolol is metabolized in the liver and one of the metabolites is a glucuronide of the parent compound (Mehvar and Brocks, 2001). Deconjugation to the parent compound during treatment may account for the increase in concentration. As significant suppression was observed for propranolol and metoprolol, 88.7% and 52.8% respectively, masking of the compound in influent samples is the more likely reason. Standard additions of propranolol in the influent sample from Swords in November 2007 show the absence of signal for the compound at ~11 minutes, and confirmed that suppression is the more likely reason for an absence of propranolol in the influent.

Gemfibrozil is a member of the fibrate group of drugs and is used to lower lipid levels in the body. Gemfibrozil was detected only in samples taken from the plant at Leixlip. Concentrations ranged from LOQ–0.15 μg/L in effluent samples. Again gemfibrozil was most frequently detected in effluent samples. Suppression of the analyte signal may account for the apparent absence of gemfibrozil in influent streams as 45.6% suppression was determined in influent streams. The peak areas for standard additions in influent samples were ~70% less than the equivalent in effluent samples.

Pravastatin is one of the naturally occurring statins. It is a lipid-lowering agent used in the treatment of cardiovascular disease. Literature on the occurrence and removal of pravastatin in the environment is limited. Reported concentrations of pravastatin in influent and effluent samples from a Canadian WWTP were 117ng/L and 59ng/L respectively, showing a removal of 50% (Miao and Metcalfe, 2003). In this study pravastatin was detected in a limited number (eight) of samples. Where influent and effluent concentrations were quantified removal rates ranging from 88 – 100% were observed (Table 3.6.1, 3.6.2 and 3.6.3). While pravastatin was quantified in influent and effluent samples complete suppression of the analyte signal was occurred in other samples. This highlights the high degree of sample to sample variability in matrix suppression.

Anti-fungals/Antibiotics

Clotrimazole, an anti-fungal agent, was detected in influent and effluent samples from the three WWTP at concentrations ranging from LOQ-0.65 and LOQ-1.2µg/L with the exception of 8.65µg/L in August 2007 at Ringsend. Clotrimazole has a log K_{ow} of 4.1 and so would be expected to exhibit medium to high sorption potential (OSPAR Commission, 2005; Jones-Lepp and Stevens, 2007). In this study clotrimazole concentrations increased in effluent over corresponding influent in the majority of cases (~70%). As linear standard additions were achieved for the quantitation of clotrimazole in both sample types, it is evident that the increase in concentration is not due to matrix effects. Limited occurrence or toxicity data are available on the level of clotrimazole in the environment. Clotrimazole has been quantified in a WWTP effluent and the river Tyne with median concentrations of 17ng/L and 21ng/L respectively. Despite the lack of information available clotrimazole is included on the list of chemicals for priority action (OSPAR commission, 2007).

The occurrence of two antibiotics (trimethoprim and sulfamethoxazole) was also investigated. These two compounds are commonly prescribed in combination. Sulfamethoxazole was not detected in any samples. The absence of sulfamethoxazole is due to complete suppression of the analyte signal in influent matrices from the three plants as highlighted by the standard additions. Trimethoprim was present in either influent or effluent or both samples in more than forty percent of samples. In some samples trimethoprim was detected in effluent samples and absent from corresponding influent samples. However, when quantifiable in both streams 95-100% removal was observed in samples from Swords in March 2008 and the Ringsend and Leixlip plants in May 2008. Maximum concentrations of up to 0.85µg/L were observed in effluent streams. The presence of antibiotics in wastewater treatment plants and effluent streams is of great concern to public health due to the potential development of antibiotic resistance among strains of bacteria. The toxicity of trimethoprim to aquatic organisms has been investigated. Chronic exposure studies have established that trimethoprim has an effective concentration (EC_{10}) of

1.0mg/L on the duckweed, *Lemna gibba* (Crane *et al.*, 2006). In a 48h test on *Daphnia magna* trimethoprim had an EC_{50} of 123mg/L. Calculated PEC/PNEC ratios were also <1 (Halling-Sørensen *et al.*, 2000). Thus trimethoprim is thought to pose minimal threat to aquatic species at the current usage level (Crane *et al.*, 2006). While the concentrations observed in this study and in other studies (Tables 1.2.1, 3.6.1, 3.6.2 and 3.6.3) are below determined effective concentrations there is still the potential development of antibiotic resistance.

Others

Caffeine is considered as a central nervous stimulant. In general it was readily removed from the WWTPs. As the log K_{ow} of caffeine is -0.1, sorption to sludge is unlikely so the main mechanism of removal is assumed to be biodegradation (Jones-Lepp and Stevens, 2007; Weigel *et al.*, 2004). The occurrence of caffeine in a variety of environmental matrices has been widely reported (Halling-Sørensen *et al.*, 1998; Ternes *et al.*, 2001; Heberer, 2002; Koplin *et al.*, 2002; Weigel *et al.*, 2002; Koplin *et al.*, 2004; Weigel *et al.*, 2004; Bendez *et al.*, 2005). Concentrations determined in influent samples in this study are consistent with ng-µg/L concentrations previously reported.

Furosemide is a loop diuretic. Maximum concentrations observed in effluent streams in this study were 2.6µg/L. The lowest reported EC_{50} were 2.4 mg/L in the crustacean, *Ceriodaphnia dubia* and 2.5 mg/L in the zooplankton *Brachionus calyciflorus* (Isidori *et al.*, 2006). Accordingly the concentrations found in the present effluent samples would not be expected to impart any toxic effect in the environment.

Salbutamol is a β_2 agonist used for the treatment of respiratory diseases such as asthma. Salbutamol was included in this study because it is one of the top ten prescribed compounds in Ireland. However it was not detected in the analysed wastewater samples. This may be because it is predominantly prescribed as an inhaler and therefore does not enter wastewater streams. The addition post extraction experiment indicated that

there was minimal suppression of the analyte signal in influent samples and greater suppression (77%) in effluent samples. The use of standard additions showed that suppression in influent samples was significant with no analyte signal observed in influent samples. Therefore, the absence of salbutamol from analysed samples may also be due to suppression of the signal.

5.0 Artificial Neural Networks

The fate of pharmaceuticals in wastewater treatment processes is complex and not well known or understood. Relationships between effluent concentration and weather data including rainfall, hours of sunshine and temperature and plant operation data (BOD, COD, flow, pH and suspended solids) were investigated however no trends were observed. As a result, the application of an artificial intelligence method was attempted. Artificial neural networks (ANN) were examined to predict effluent concentrations from WWTPs as a function of various weather conditions and plant input parameters. ANNs have been successfully used for prediction and forecasting in a number of fields including water resources, power generation and medicine. The design of a neural network is similar to that of the network of neurons in the brain and central nervous system. The networks are arranged in a series of layers including an input layer, one or more hidden layers and an output layer (Figure 5.0.1). The data from each node in the input layer pass to the hidden layer and are multiplied by the connection weight. In each hidden layer node the weighted values are summed and a threshold value is added. The output of each node is then determined by a non-linear transfer function of the summed input value. These outputs are the inputs of the next layer in the network. The final output is then compared to the known actual output for the training set and the connection weights applied are adjusted to reduce the prediction error (Maier and Dandy, 1998; Gurney, 2003). This form of ANN is called multilayer perceptron.

Data collected from the three wastewater treatment plants were used to construct a model to predict effluent concentrations using neural networks. Carbamazepine occurred most frequently in the treatment plants and therefore the model was constructed to predict carbamazepine concentrations (Table 5.0.1). The network structure was optimised using a trial and error approach. The data were divided into three sections for training, validation and testing. Each network was trained using back propagation until the root mean squared error (RMS) value of the

validation data set began to increase. Back propagation is a useful and frequently used method for environmental models (Maier and Dandy, 1998). Validation data were used to prevent overtraining of the network by performing cross validation. Networks consisting of one and two hidden layers were analysed and the results are shown in Figures 5.0.2 and 5.0.3. One hidden layer with 5 neurons was found to be the best configuration as it produces the lowest RMS error and was therefore chosen for further analysis.

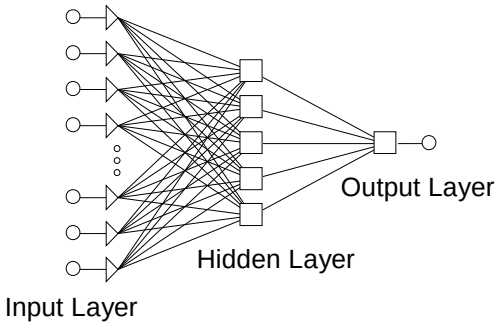


Figure 5.0.1 9-5-1 Network construction

Plant	CarbIn	Rain	Temp	Daylight	Flow	COD	BOD	SS	CarbOut
1	**0.45**	**5.3**	**15.4**	**4.9**	**509245**	**409**	**191**	**240**	**0.6**
1	**0.23**	**0**	**17.9**	**2.4**	**391576**	**426**	**186**	**176**	**0.49**
1	**0.45**	**0**	**19**	**8.4**	**799043**	**843**	**323**	**396**	**0.7**
1	**0.1**	**8.9**	**8.8**	**0.1**	**733549**	**615**	**227**	**276**	**0.22**
1	**0.24**	**0**	**7.3**	**6.9**	**368793**	**530**	**232**	**241**	**0.78**
1	**0.3**	**4.5**	**12.2**	**1.9**	**837565**	**159**	**70**	**70**	**0.45**
1	**0.08**	**0**	**12.1**	**8.2**	**399936**	**543**	**278**	**278**	**0.47**
1	**0.08**	**3.2**	**11.9**	**3.7**	**492370**	**352**	**183**	**220**	**0.25**
1	**0.51**	**0.1**	**8.7**	**1.7**	**434566**	**438**	**220**	**274**	**0.08**
1	**0.2**	**0**	**17.4**	**9.7**	**352482**	**1030**	**565**	**346**	**0.08**
1	**0.55**	**0**	**18.4**	**14.4**	**352043**	**570**	**248**	**272**	**0.08**
2	0.15	16.6	17	4.5	13287	125	61	42	0.35
2	0	1.3	15.8	0.7	8998	408	198	374	0.06
2	0	0.4	7.5	4.7	9468	295	101	116	0.5
2	0.28	0	6.6	6.2	15415	253	97	163	0.32
2	0.002	1.8	8.6	0.1	16920	398	114	182	0.002
2	0.002	1	9.4	5.1	13518	414	200	104	0.58
2	0.72	0.7	4.7	3.4	10518	343	102	102	0.7
2	0.7	0	19.9	9.4	8812	650	206	452	0.002
2	0.77	0	23.3	9.7	8140	447	299	190	1.2
3	0	1.3	17.8	4.8	11991	545	300	401	0.26
3	0	0	18.3	8.8	9570	712	330	247	0.25
3	0.1	0	16.2	5	10216	927	350	407	1.09
3	0	7.8	9.7	3.6	12259	562	270	253	0.35
3	0	4.2	7.3	1.7	10945	1220	420	475	0.59
3	0.15	0	12.1	8.2	9826	1011	350	331	0.3
3	0	3.2	11.9	3.7	9666	482	450	446	0.25
3	0	0	17.4	9.7	9529	543	390	335	0
3	1.52	0	18.4	14.4	8714	1087	410	489	0

Table 5.0.1 Data set for training and testing the 9-5-1 neural network

Plant: Plant 1 - Ringsend, Plant 2 - Leixlip and Plant 3 - Swords, CarbIn: carbamazepine influent concentration (μg/L), Rain: daily rainfall (mm), Temperature: maximum daily temperature (^{0}C), Daylight: hours of sunlight per day (h), Flow: flowrate into the plant (m^3/d), BOD: influent BOD (mg/L), COD: influent COD (mg/L), SS: influent suspended solids (mg/L) and CarbOut: carbamazepine effluent concentration.

The results of the 9-5-1 configured network are presented in Figure 5.4. RMS values for the network were: Training RMS = 6.268 x 10^{-3}, Verification RMS = 1.339 and Testing RMS = 0.3521. The training data show good correlation while the prediction of testing data is less accurate. This may be due to the limited data available for training and testing of the network. A larger database would be required to produce a more accurate model. The relative effect of the nine inputs were determined using the Garson equation (3) where v is the relative effect of the input on the output, n_v the number of input variables, n_h the number of neurons in the hidden layer, w_{kj} the absolute value of the weight from the kth input to the jth neuron and O_j is the absolute value of the weight from the jth neuron (N□ Mhurch□ & Foley, 2006). The results indicate that most of the parameters have a similar effect (~10%) on the effluent concentration (see Figure 5.0.5). However, the quantity of suspended solids in the influent stream was shown to have a greater effect (~17%) on effluent concentration.

$$v = \frac{\sum_{j=1}^{n_h} \left[\left(w_{vj} / \sum_{k=1}^{n_v} w_{kj} \right) O_j \right]}{\sum_{i=1}^{n_v} \left[\sum_{j=1}^{n_h} \left[\left(w_{vj} / \sum_{k=1}^{n_v} w_{kj} \right) O_j \right] \right]} \qquad (3)$$

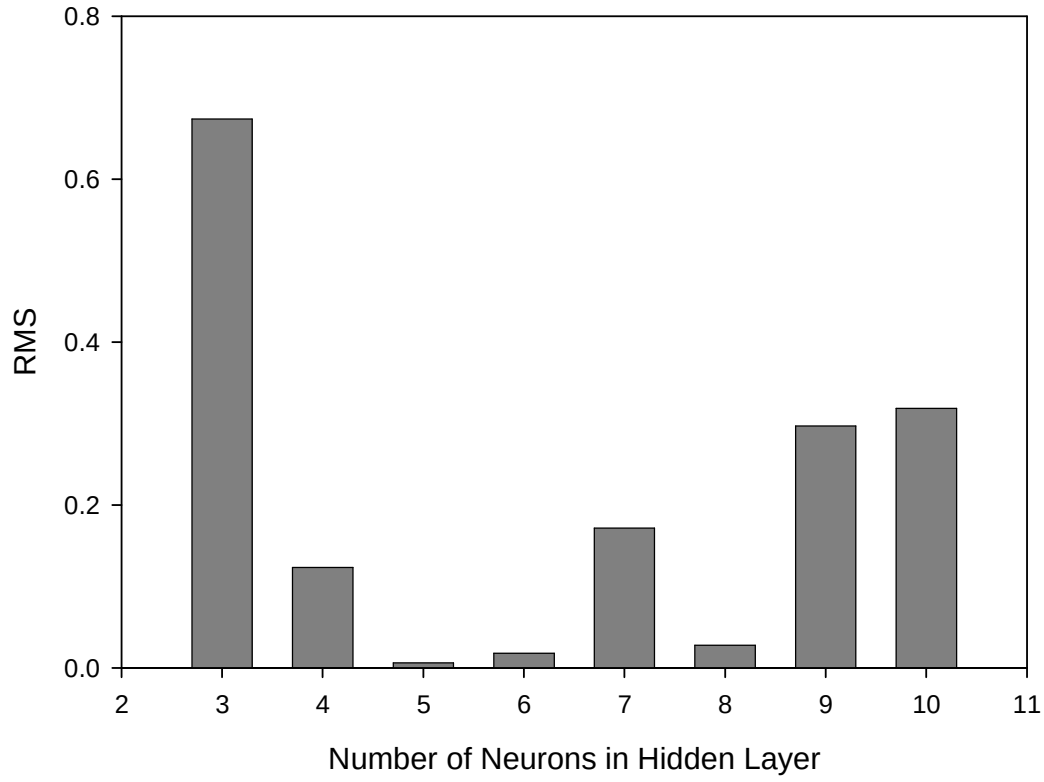


Figure 5.0.2 Optimisation of network structure with one hidden layer

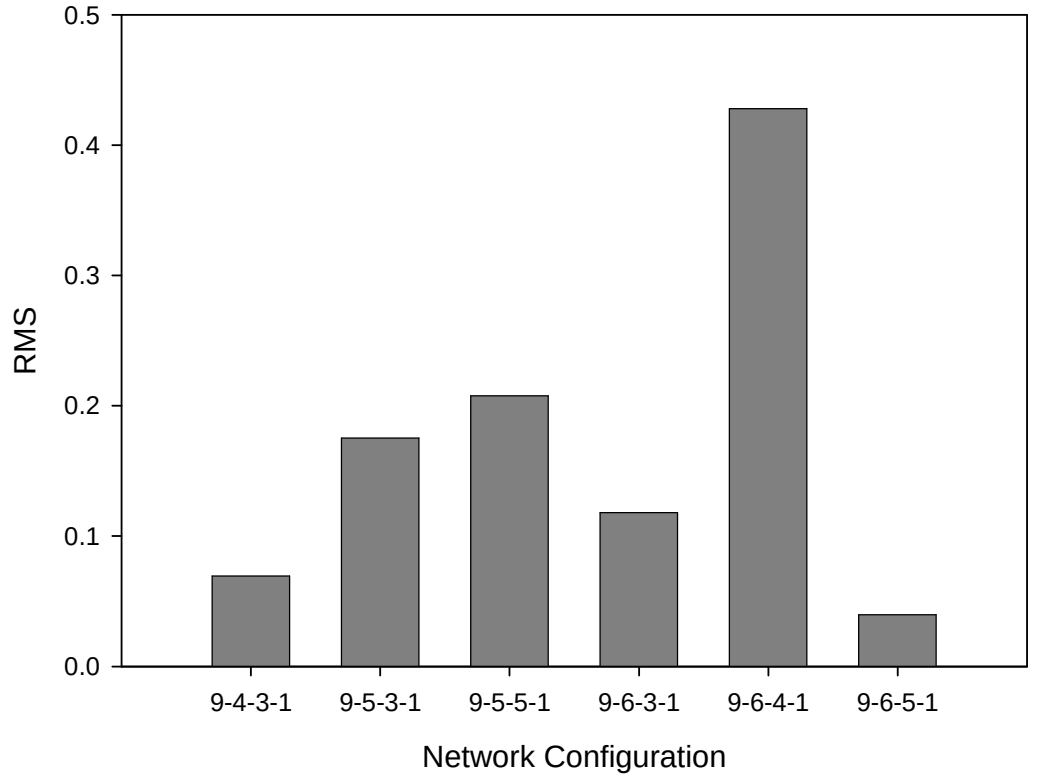


Figure 5.0.3 Optimisation of network structure with two hidden layers

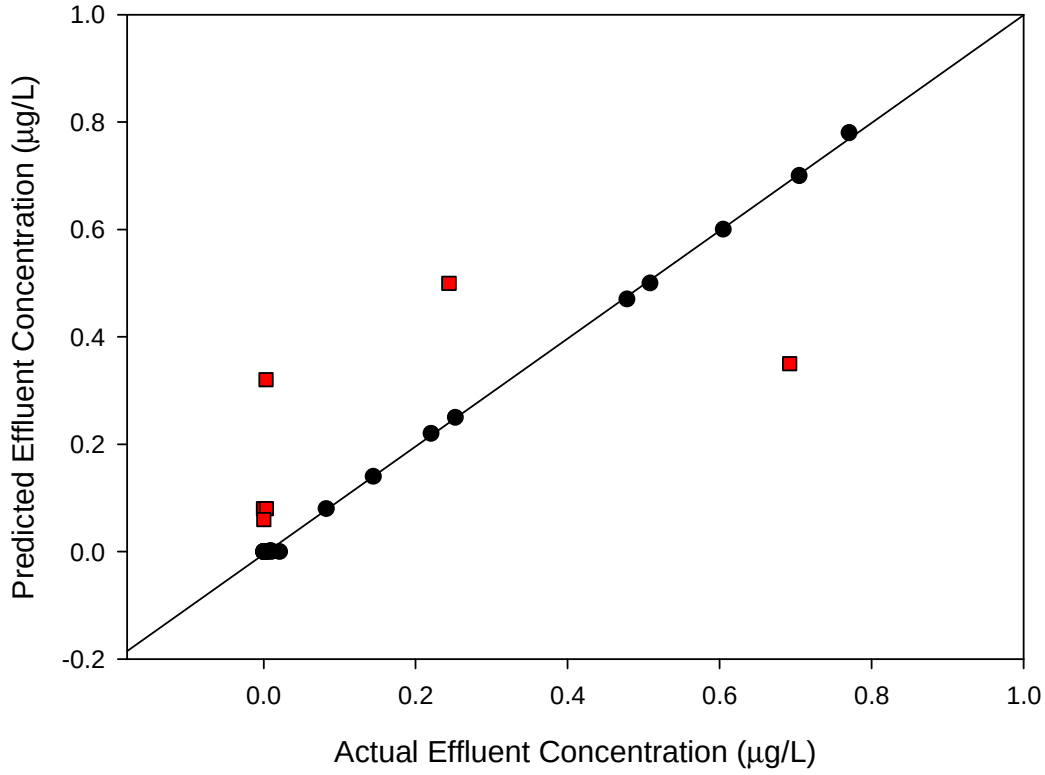


Figure 5.0.4 Results of training (black) and testing (red) data from the 9-5-1 network

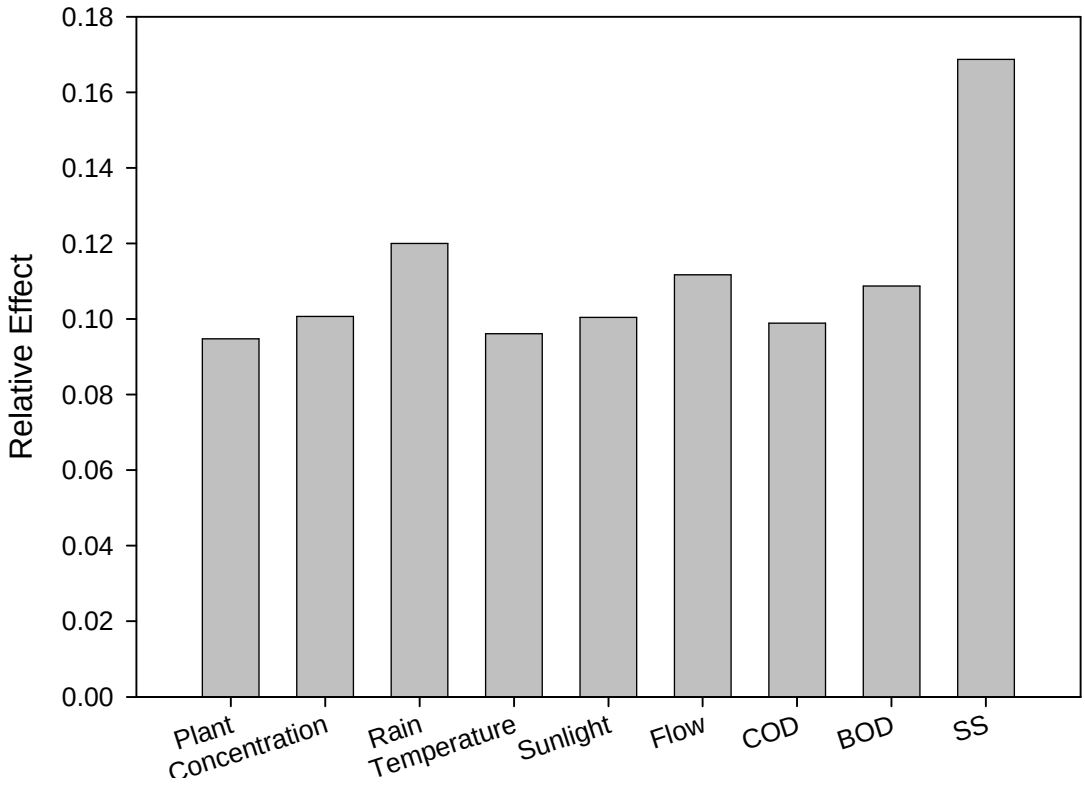


Figure 5.0.5 Relative effect of the nine inputs on the predicted effluent concentration in the 9-5-1 network configuration

A separate network was constructed for the data from Ringsend WWTP (ie. the data in bold in Table 5.0.1). Five neurons in the hidden layer was identified as the best configuration (Figure 5.0.6). However, the RMS error was better for this network when the data from only one plant were used (Training RMS = 4.42 x 10^{-4}; Verification RMS = 5.83x 10^{-8}; Testing RMS = 0.5776). The resulting training and testing graph is shown in Figure 5.7. The relative effect of each of the input variables was calculated for this network and it was found that the inputs had approximately equal effect on effluent concentrations (Figure 5.0.8). The flow into the plant was found to have the least effect on the effluent concentration.

The results of this ANN analysis show the suitability of artificial intelligence for the prediction of pharmaceutical effluent concentration. Relative effect analysis completed using the Garson equation presented two unexpected results. Firstly, suspended solids were identified as having a higher relative effect on carbamazepine effluent concentration (Figure 5.0.5.) and secondly that flow has less of an effect than the other inputs on the effluent concentration (Figure 5.0.8). Both these findings and the prediction limitations seen with the networks produced in this study are due to the small data set available. A significantly larger data set would be required to construct a robust and accurate network.

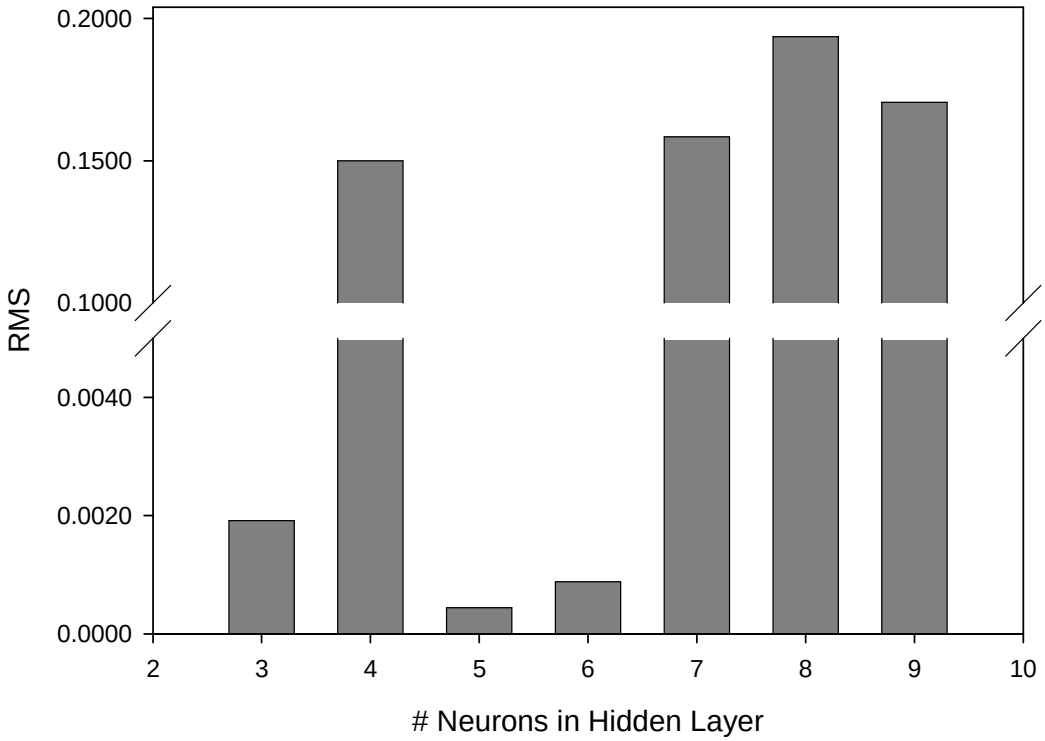


Figure 5.0.6 Optimisation of network structure for one plants data

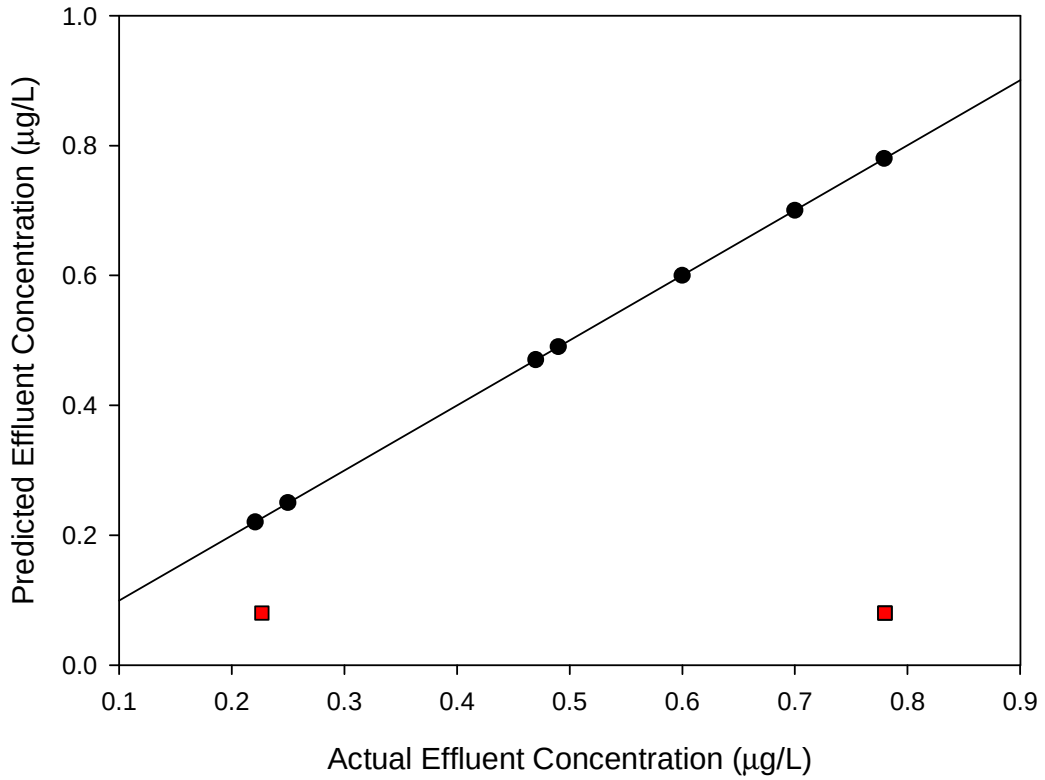


Figure 5.0.7 Results of training (black) and testing (red) data from the 8-5-1 network

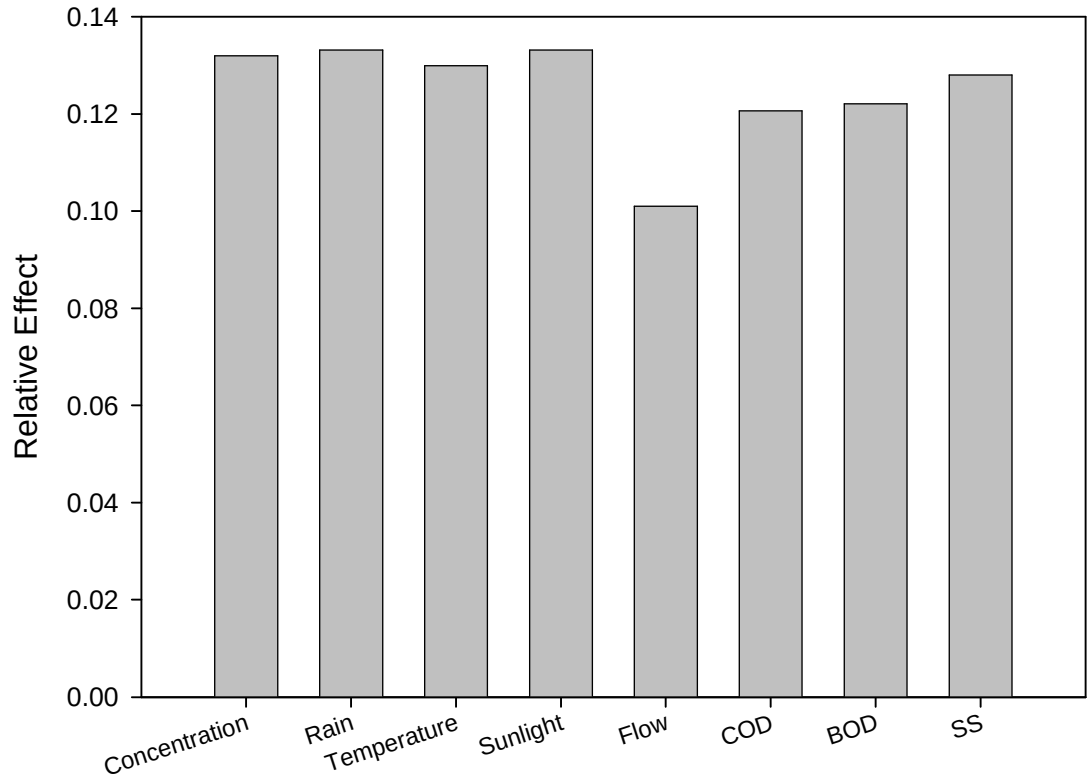


Figure 5.0.8 Relative effect of the eight inputs on the predicted effluent concentration in the 8-5-1 network configuration

6.0 Conclusion and future work

The level of pharmaceutical contamination entering the environment in WWTP effluents is largely unknown in Ireland. The aim of this research was to determine the concentration of twenty compounds released to the environment at three locations in the greater Dublin region. The three WWTPs are located at Leixlip, Swords and Ringsend. A SPE-LC-MS/MS method was developed for the identification and quantitation of the selected analytes in both influent and effluent streams. Twenty-four hour composite influent and effluent samples were collected from the Ringsend, Swords and Leixlip wastewater treatment facilities every month over a twelve month period. The presence of selected compounds in both influent and effluent samples was investigated. Fourteen of the selected compounds were found to be present in samples. The aim of the method developed for this analysis was to have one method to analyse for all compounds. Because the selected analytes are a range of basic, neutral and acidic the development of one method was challenging. While the resulting method was successful in analysing for the twenty analytes there was a loss in sensitivity. Due to the low levels of analyte present in samples a more selective sample preconcentration and detection method for each analyte would reduce the limits of detection and quantitation and improve the analysis. A method of standard additions was used to extrapolate the concentration of each analyte in influent and effluent samples. Due to this the confidence limits of the concentrations were statistically determined. The confidence limits were very large and showed wide variability. This was because two factors. Firstly, a wide variety of analytes were analysed in a very complex inconsistent matrix. For this reason to accurately quantify each analyte and allow for suppression or enhancement of signal intensities a method of standard additions was used as opposed to repeat analysis of a sample with no addition. Secondly, a limited volume of sample was available monthly and there for a maximum of four points could be obtained on any one standard additions graph. Increasing the number of data points improves the precision of the extrapolated concentration and reduces the confidence limits. Despite the

relatively poor confidence limits obtained in this study regression analysis on the standard additions gave R^2 values of >0.98 in most cases. It is therefore thought that the confidence limits in this study are not reflective of the accuracy of the data and that extrapolated values are accurate. The number of data points analysed for a standard addition graph would need to be increased in future work for meaningful confidence limits to be determined.

The concentration of analytes in effluent streams was then compared to ecotoxicity data available in the literature. The data currently available report LC_{50} and EC_{50}, however a smaller concentration may still impart a negative effect on non-target organisms. In general it was found that the determined effluent concentrations were significantly lower than levels reported to cause toxicity. However, a cocktail of the selected compounds is being released to the environment in the effluent and the combined toxic effect of these compounds may be significant. Toxicity data on the effect of multiple compounds are limited but some studies have identified a additive toxic effect (Cleuvers, 2003). Given the toxicity data available it is thought that there is currently no risk to the receiving waters at Ringsend, Leixlip and Swords WWTP.

The concentration of compounds in effluent samples was frequently greater than that determined in corresponding influent samples. A commonly suggested reason for an increase in concentration during wastewater treatment is the deconjugation of metabolites to release the parent compound that is then detectable in effluent samples. Matrix effects have also been identified as a potential reason for this apparent increase in concentration during treatment. The effects of matrix components on the analysis of compounds were investigated. The suppression of analyte signal was determined using two methods 1) Addition post extraction and 2) Post column infusion. Both investigations highlighted that the level of suppression due to influent matrix components was more significant than that observed for the effluent matrix. To further establish the effects of matrix components and allow for accurate quantitation of all analytes a method of standard additions was used in all monthly samples. Complete

suppression of analyte signal in influent samples prevented the analysis of some compounds including flurbiprofen, mefenamic acid, diclofenac, clofibric acid, sulfamethoxazole and ibuprofen. When an analyte was detected in both influent and effluent samples, the analyte response in influent samples for the same standard addition was less than that observed in effluent samples. These results highlight the negative effect of signal suppression on the analysis of influent samples and also call into question the hypothesis that an increase in concentration during treatment is as a result of deconjugation. Clarification of the major components in wastewater samples in future work may improve the analysis and detection limits of the analytical techniques by selectively removing the suppressing components.

In this work metal and surfactants were investigated as potential sources of suppression. Of the twelve metals analysed only five were detected above detection limits and the concentration of those did not reduce significantly during treatment. Consequently metal interferences were not considered as a potential suppressive agent in influent samples. WWTPs have been shown to effectively remove surfactants from wastewater streams. The suppressive effect of the surfactant LAS on the analyte signal of four compounds was therefore investigated. It was determined that LAS completely suppressed the analyte signal for nimesulide. The peak shape for trimethoprim changed in the presence of LAS such that it was unquantifiable. The peak was split into numerous peaks with no baseline separation and the elution occurred over ~ 4 minutes. The signal intensity of both mefenamic acid and carbamazepine was less affected by the presence of LAS. The signal intensity was not significantly affected and while the retention time of the compounds was altered slightly the elution time was not lengthened. Thus detection and quantitation with the aid of standard additions would be possible.

Artificial neural networks were used in an attempt to predict the concentration of pharmaceutical compounds in effluent streams given a set of input parameters. The results indicated that ANNs would be suitable for the prediction of effluent concentrations as the prediction of training

data was very accurate with R^2 values of 1 for plots of predicted against actual effluent concentrations. However, prediction of effluent concentration in the testing data set was poor. This indicates that a larger data set would be required for a full evaluation of the suitability of ANNs.

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8.0 Appendices

Appendix A

	Sali	Gemf	Diclo	Ibup	Parac	Mefe	Furo	Nime	Sulf	Indo	Beza	Keto	Carb
July 06													
Influent	7.19	<0.03	0.37	5.33	nd	0.66	nd	nd	nd	nd	nd	nd	nd
Effluent	nd	0.23	0.33	4.53	nd	0.49	nd	nd	0.33	nd	nd	nd	nd
Nov 06													
Influent	0.16	nd	nd	nd	nd	nd	3.2	nd	nd	nd	nd	nd	nd
Effluent	nd	nd	0.28	nd	0.29	nd	0.6	nd	0.29	nd	nd	nd	nd
Jan 07													
Influent	1.22	nd	0.16	nd	0.04	nd	nd	6.11	nd	nd	nd	nd	nd
Effluent	nd	nd	nd	nd	1.07	nd	nd	nd	nd	nd	nd	nd	nd
Feb 07													
Influent	0.07	nd	0.13	0.1	0.05	0.02	nd	nd	nd	nd	nd	nd	0.29
Effluent	nd	0.15	0.48	1.0	nd	0.27	nd	0.04	0.15	nd	nd	nd	1.14

Table A1 Concentrations determined in wastewater treatment samples fro Leixlip using LC-MS technique. Concentrations are in μg/L

nd: analyte was not detected in the sample

na: analyte was not included in analysis.

Sali - Salicylic Acid; Gemf – Gemfibrozil; Clof - Clofibric Acid; Diclo – Diclofenac; Ibup – Ibuprofen; Para – Paracetamol; Mefe.- Mefenamic Acid; Furo – Furosemide; Nime – Nimesulide; Sulf – Sulfamethoxazole; Indo – Indomethcin; Beza – Bezafibrate; Prav – Pravastatin; Keto – Ketoprofen; Carb- Carbamazepine

	Sali	Gemf	Diclo	Ibup	Parac	Mefe	Furo	Nime	Sulf	Indo	Beza	Prava	Keto	Napr
Sept 06														
Effluent	nd	nd	0.78	nd	nd	1.39	0.39	0.5	0.7	nd	nd	na	nd	nd
Oct 06														
Influent	0.14	nd	<0.24	<0.32	0.1	<0.06	5.4	0.02	nd	0.12	nd	0.14	nd	0.02
Jan 07														
Influent	2.0	nd	nd	nd	1.1	nd	Nd	1.9	nd	nd	nd	nd	na	nd
Effluent	nd	nd	0.51	nd	nd	0.54	Nd	1.5	nd	nd	nd	na	nd	nd

Table A2 Concentrations determined in wastewater treatment samples from Ringsend using LC-MS technique. Concentrations are in μg/L.

nd: analyte was not detected in the sample

na: analyte was not included in analysis.

Sali - Salicylic Acid; Gemf – Gemfibrozil; Clof - Clofibric Acid; Diclo – Diclofenac; Ibup – Ibuprofen; Para – Paracetamol; Mefe - Mefenamic Acid; Furo – Furosemide; Nime – Nimesulide; Sulf – Sulfamethoxazole; Indo – Indomethcin; Beza – Bezafibrate; Prav – Pravastatin; Keto – Ketoprofen; Napr- Naproxen.

	Sali	Gemf	Clof	Diclo	Ibup	Para	Mefe	Furo	Nime	Sulf	Indo	Beza	Prava	Keto	Carb
Dec 06															
Influent	nd	nd	nd	0.32	nd	nd	nd	nd	nd	nd	nd	nd	na	nd	Nd
Effluent	nd	nd	nd	0.37	nd	nd	6.27	nd	nd	nd	nd	nd	na	nd	Nd
Jan 07															
Influent	1.97	nd	nd	0.08	nd	nd	0.97	nd	nd	nd	nd	nd	nd	nd	0.11
Effluent	nd	nd	nd	0.21	nd	0.03	2.46	nd	6.96	nd	nd	nd	nd	nd	Nd

Table A3 Concentrations determined in wastewater treatment samples from Swords using LC-MS technique. Concentrations are in µg/L

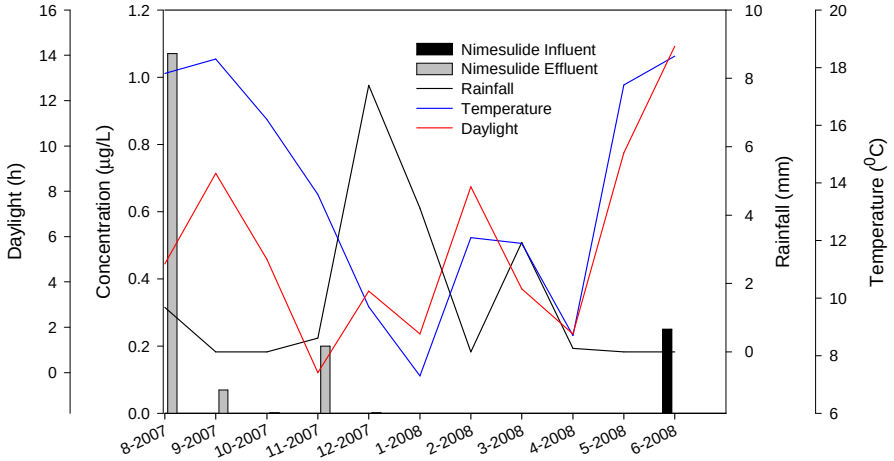
nd; analyte was not detected in the sample

na; analyte was not included in analysis.

Sali.: Salicylic Acid; Gemf – Gemfibrozil; Clof - Clofibric Acid; Diclo – Diclofenac; Ibup – Ibuprofen; Para – Paracetamol; Mefe - Mefenamic Acid; Furo.: Furosemide; Nime – Nimesulide; Sulf – Sulfamethoxazole; Indo – Indomethcin; Beza – Bezafibrate; Prav – Pravastatin; Keto – Ketoprofen; Carb- Carbamazepine.

Appendix B

Swords Data – Investigation of seasonal variability.


Nimesulide Influent
Nimesulide Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
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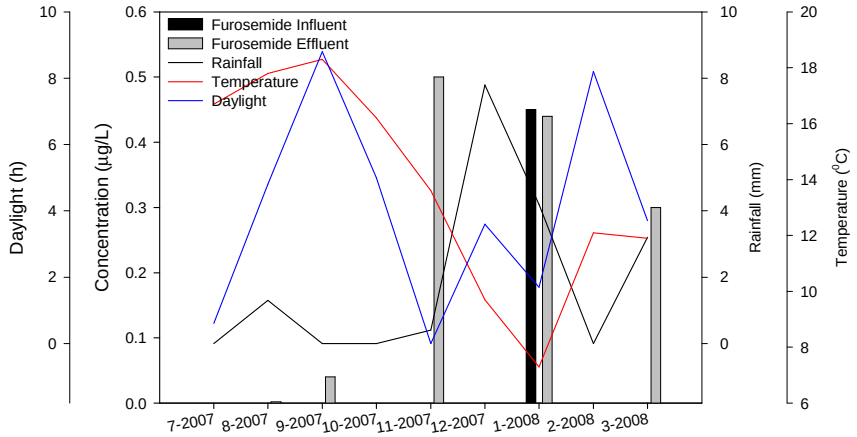
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008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Daylight
Daylight (h)
Concentration (µg/L)
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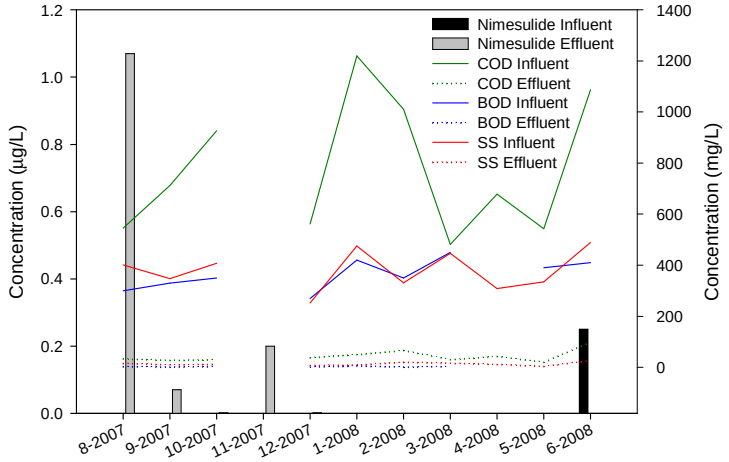
11-2007
12-2007
1-2008
2-2008
3-2008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Nimesulide Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
8-2007
9-2007
10-2007
11-2007
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1-2008
2-2008
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4-2008
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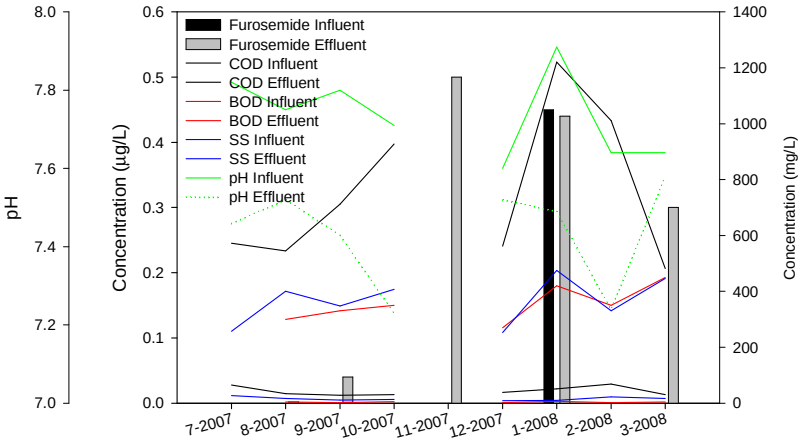
6-2008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Furosemide Effluent
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COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
pH Influent
pH Effluent
pH
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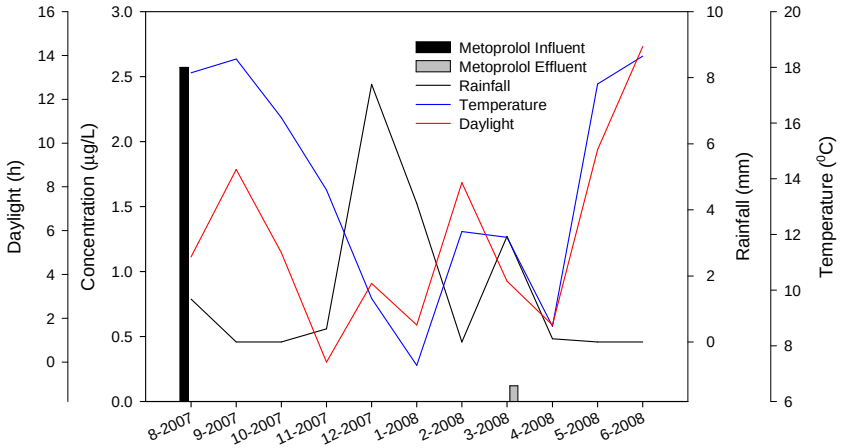
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008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Metoprolol Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
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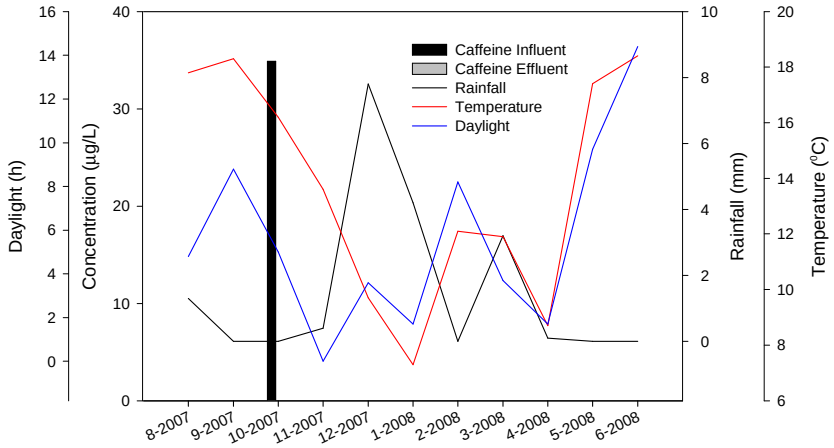
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Caffeine Effluent
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Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
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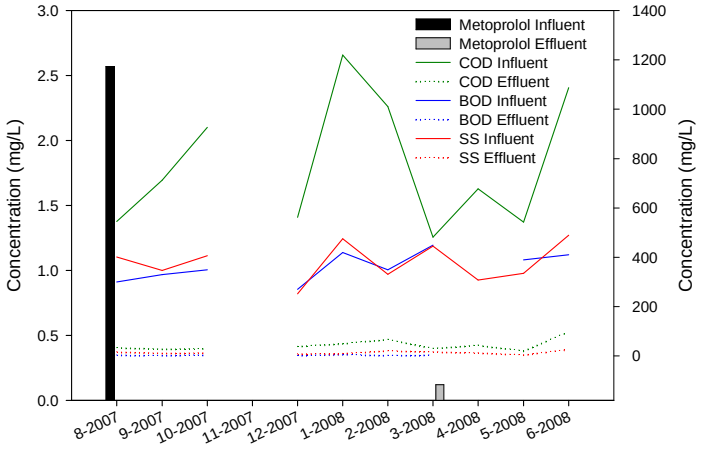
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Metoprolol Effluent
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COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (mg/L)
Concentration (mg/L)
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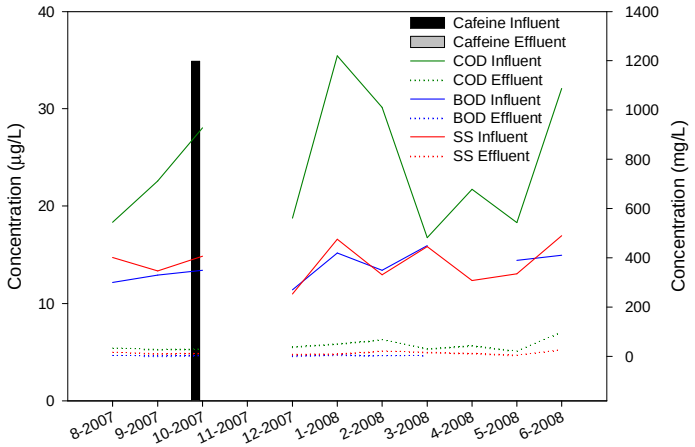
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Caffeine Effluent
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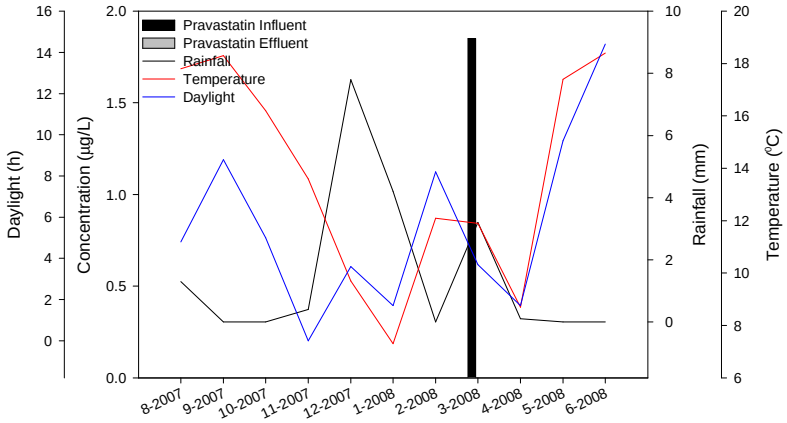
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Pravastatin Effluent
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Rainfall (mm)
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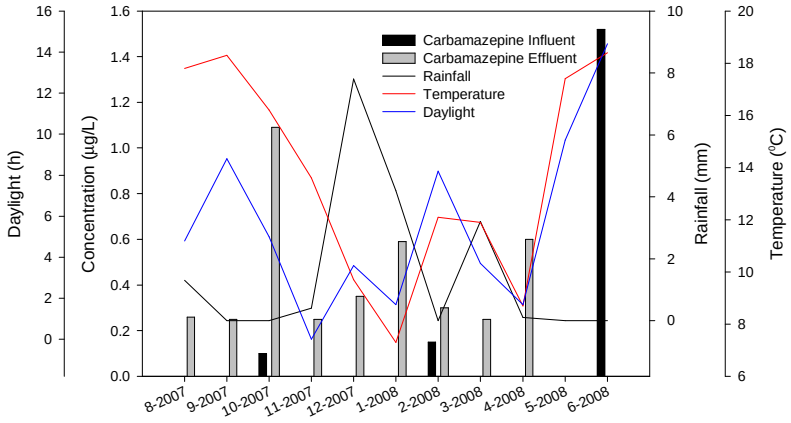
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Carbamazepine Effluent
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Temperature (°C)
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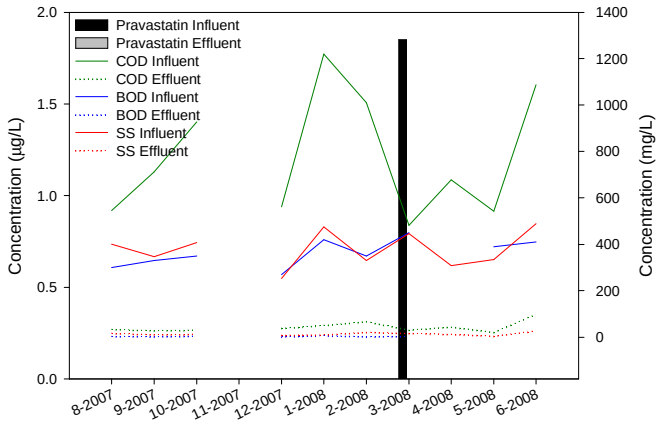
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Concentration (mg/L)
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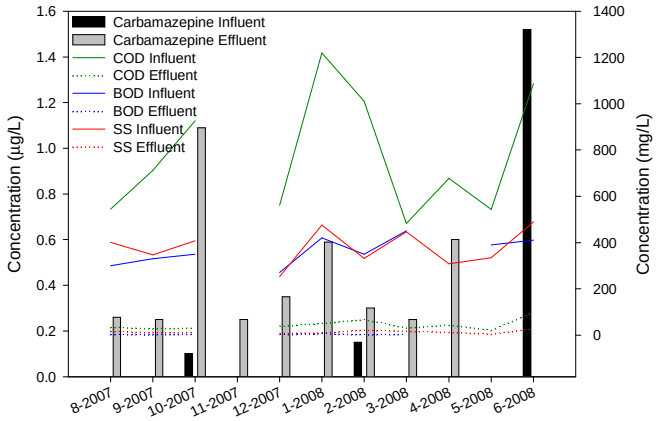
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Concentration (µg/L)
Concentration (mg/L)
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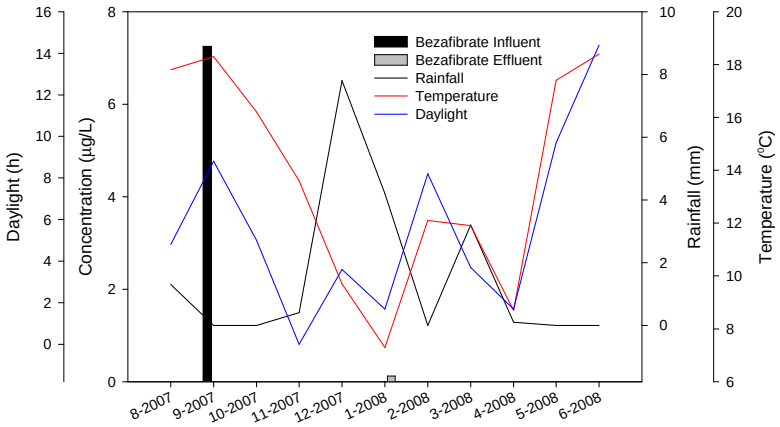
Bezafibrate Influent
Bezafibrate Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
8-2007
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10-2007
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12-2007
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2-2008
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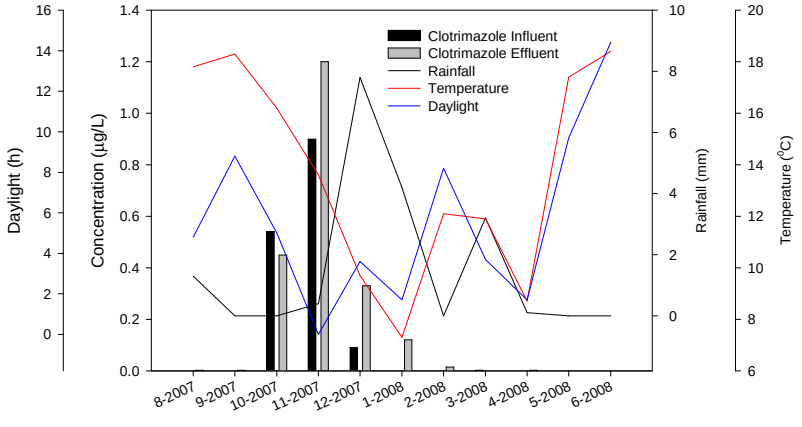
5-2008
6-2008


Clotrimazole Influent
Clotrimazole Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
8-2007
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10-2007
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12-2007
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2-2008
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4-2008
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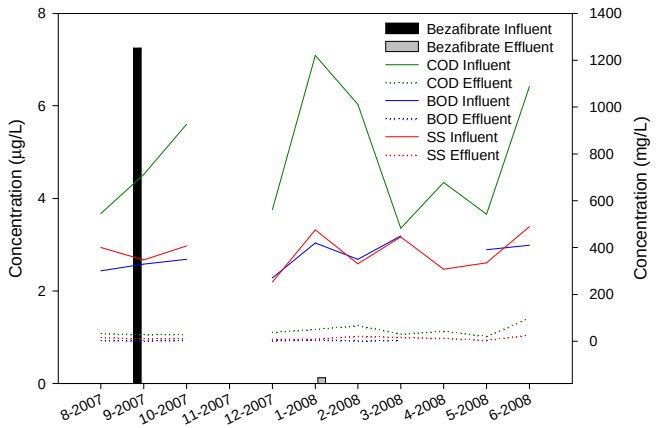
6-2008


Bezafibrate Influent
Bezafibrate Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
8-2007
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10-2007
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12-2007
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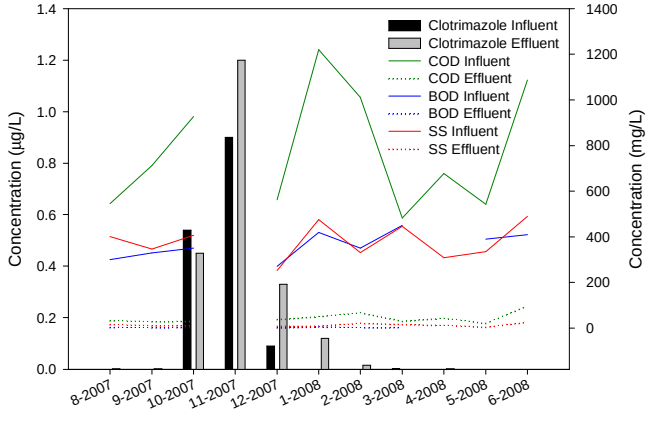
2-2008
3-2008
4-2008
5-2008
6-2008


Clotrimazole Influent
Clotrimazole Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
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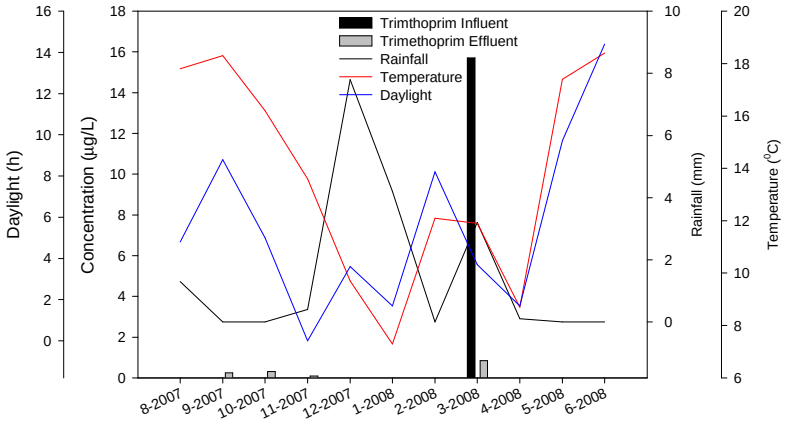
Trimthoprim Influent
Trimethoprim Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
8-2007
9-2007
10-2007
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12-2007
1-2008
2-2008
3-2008
4-2008
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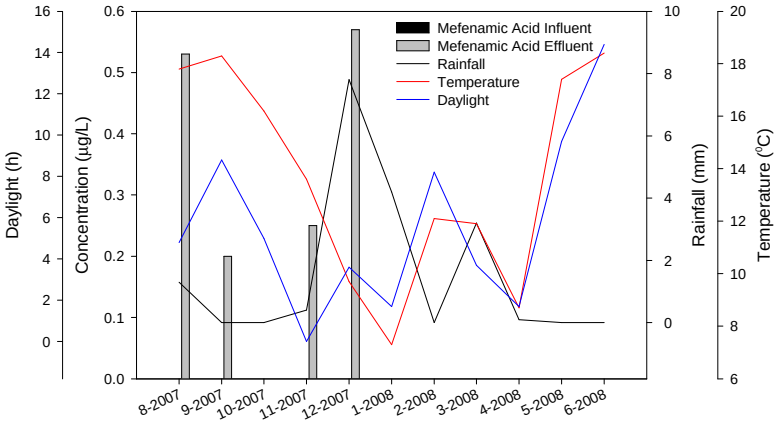
6-2008


Mefenamic Acid Influent
Mefenamic Acid Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
8-2007
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10-2007
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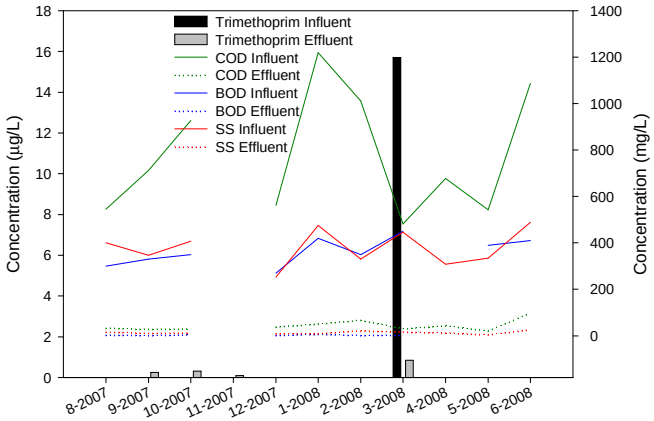
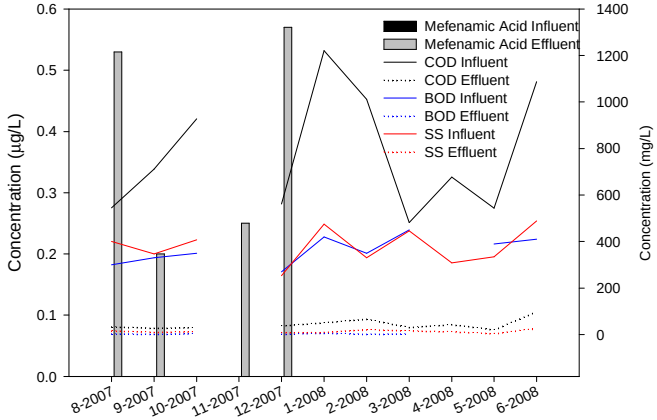
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008


Trimethoprim Influent
Trimethoprim Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008


Mefenamic Acid Influent
Mefenamic Acid Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
8-2007
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10-2007
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1-2008
2-2008
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4-2008
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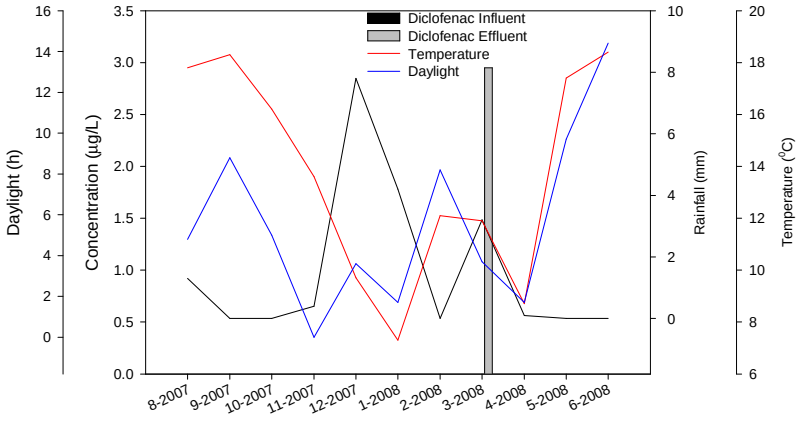
6-2008


Diclofenac Influent
Diclofenac Effluent
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
8-2007
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10-2007
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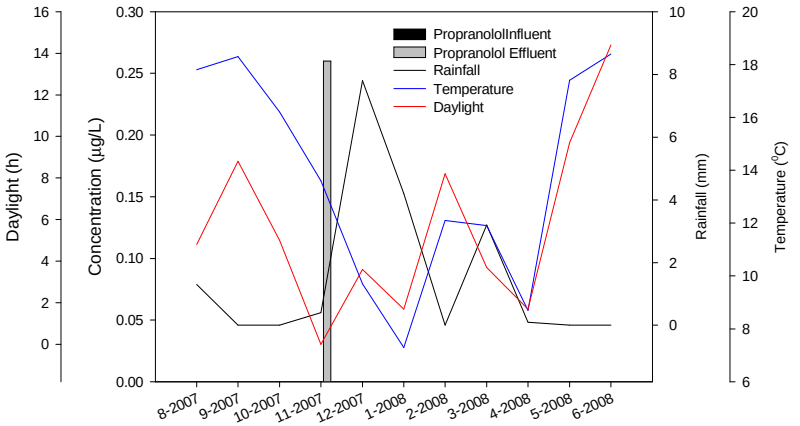
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008


PropranololInfluent
Propranolol Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
8-2007
9-2007
10-2007
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12-2007
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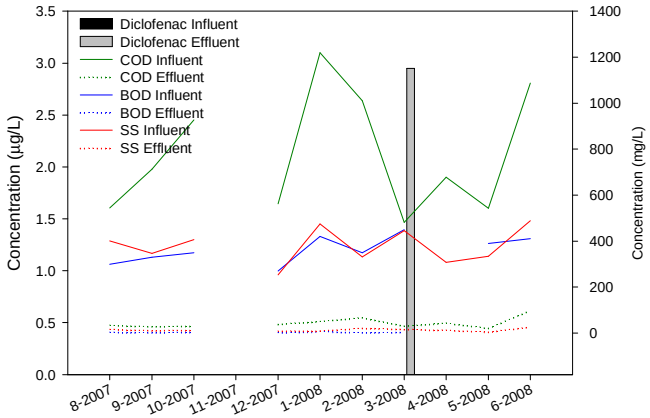
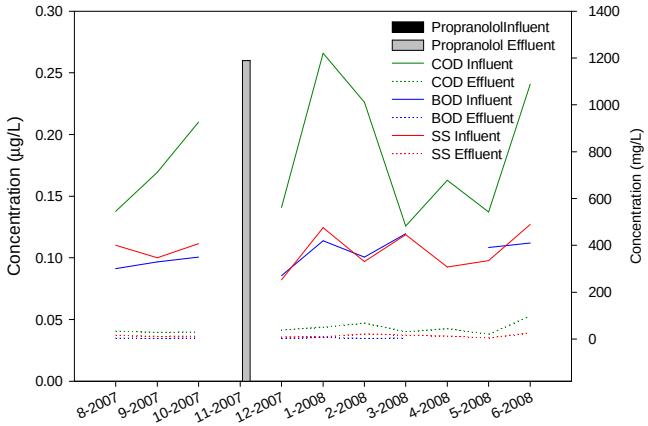
2-2008
3-2008
4-2008
5-2008
6-2008


Diclofenac Influent
Diclofenac Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008


PropranololInfluent
Propranolol Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

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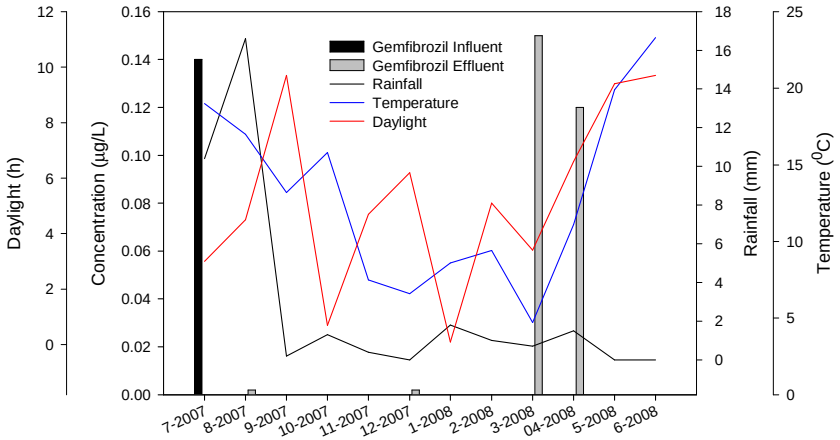
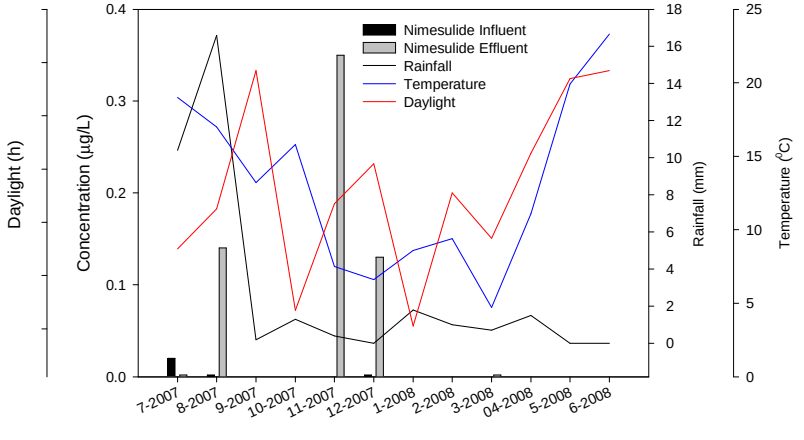
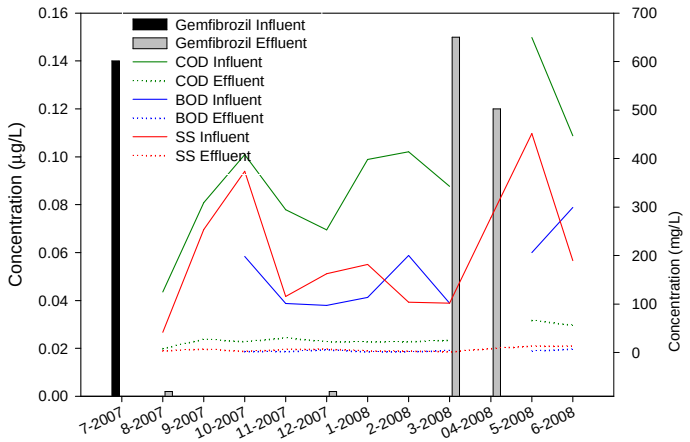
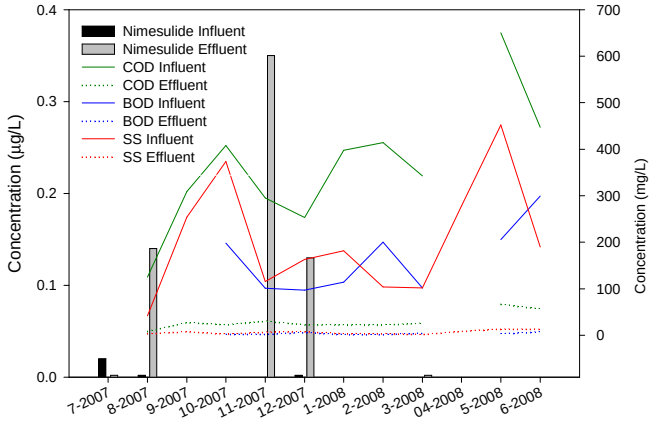
Appendix C

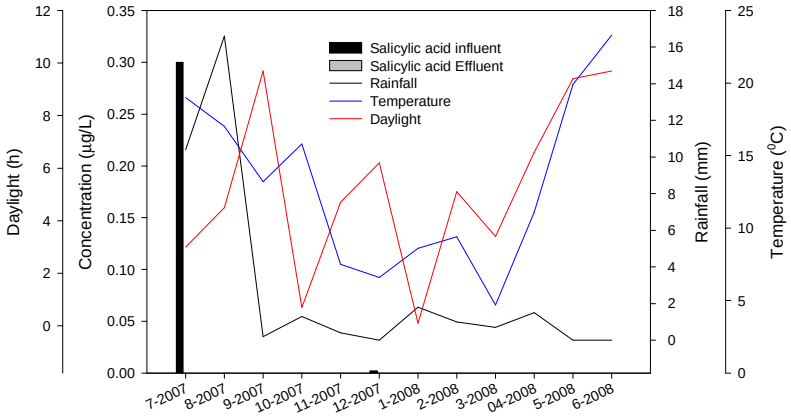
Leixlip Data - Investigation of seasonal variability.

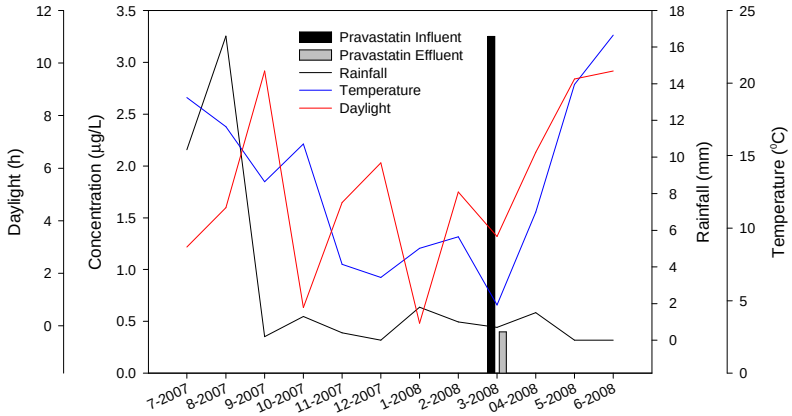

Gemfibrozil Influent
Gemfibrozil Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
04-2008
5-2008
6-2008

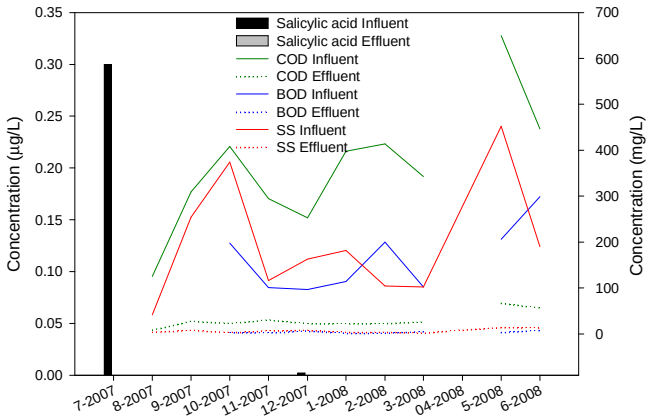

Nimesulide Influent
Nimesulide Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
04-2008
5-2008
6-2008

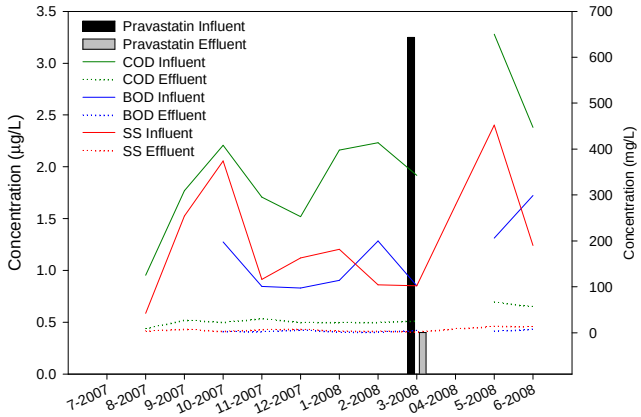

Gemfibrozil Influent
Gemfibrozil Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
04-2008
5-2008
6-2008


Nimesulide Influent
Nimesulide Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
04-2008
5-2008
6-2008


Salicylic acid influent
Salicylic acid Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
04-2008
5-2008
6-2008


Pravastatin Influent
Pravastatin Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
04-2008
5-2008
6-2008


Salicylic acid Influent
Salicylic acid Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
04-2008
5-2008
6-2008


Pravastatin Influent
Pravastatin Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
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12-2007
1-2008
2-2008
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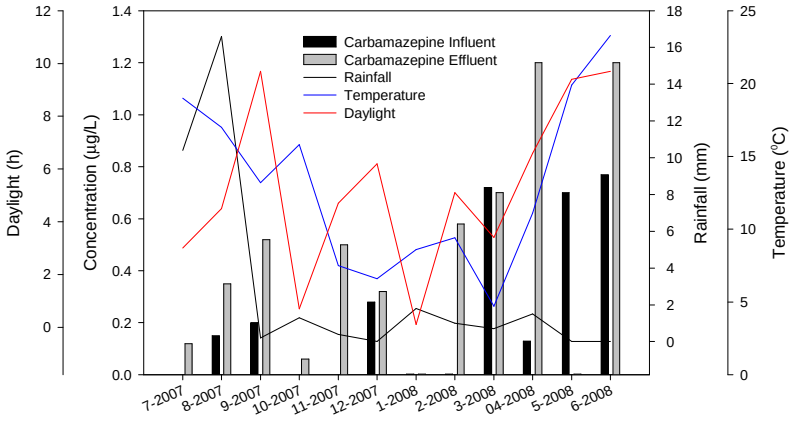
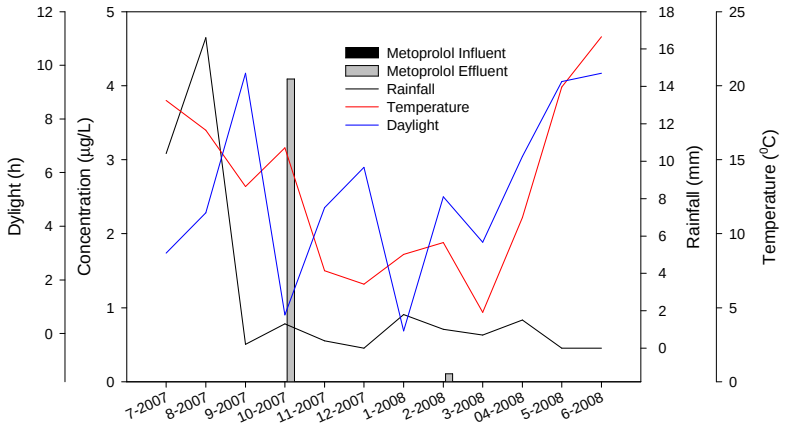
04-2008
5-2008
6-2008


Carbamazepine Influent
Carbamazepine Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
04-2008
5-2008
6-2008


Metoprolol Influent
Metoprolol Effluent
Rainfall
Temperature
Daylight
Dylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
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04-2008
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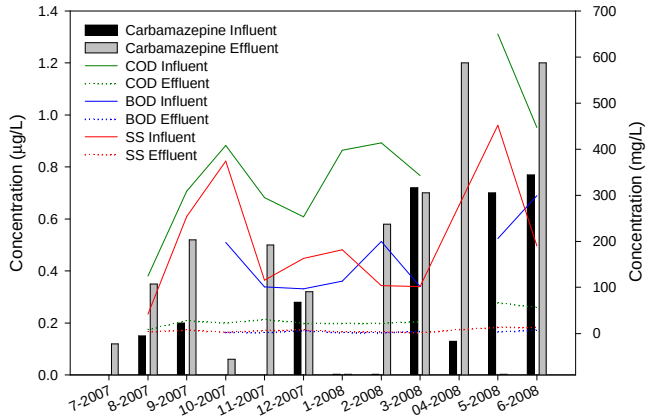
6-2008


Carbamazepine Influent
Carbamazepine Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
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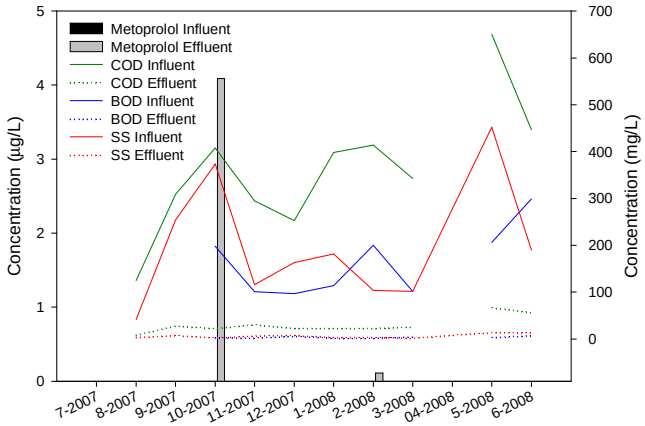
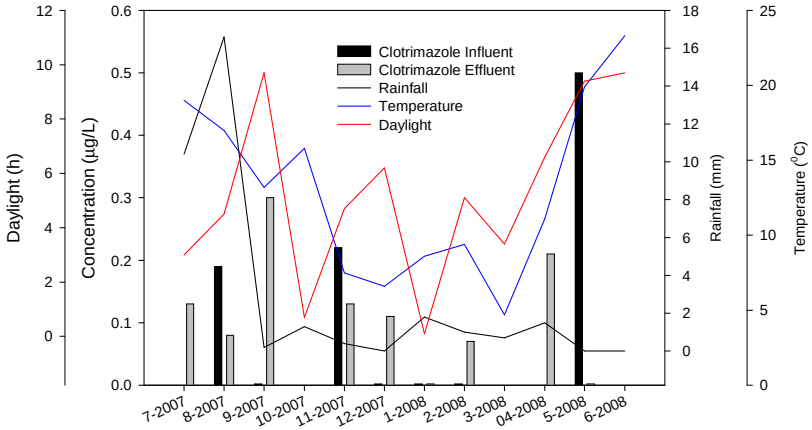
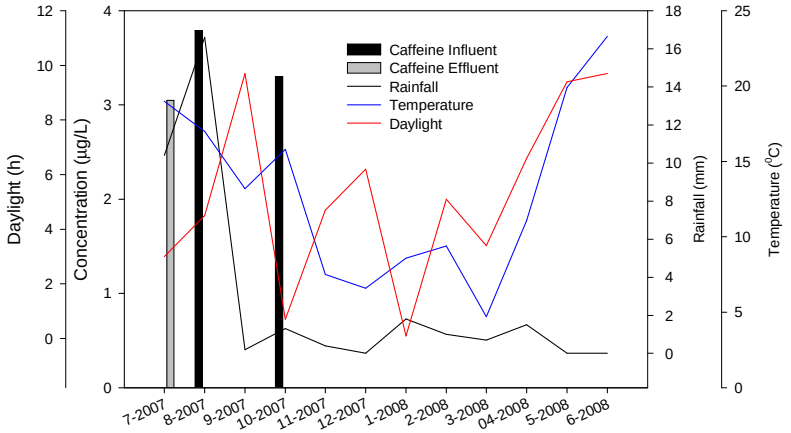

Metoprolol Influent
Metoprolol Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
04-2008
5-2008
6-2008


Clotrimazole Influent
Clotrimazole Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
04-2008
5-2008
6-2008


Caffeine Influent
Caffeine Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
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8-2007
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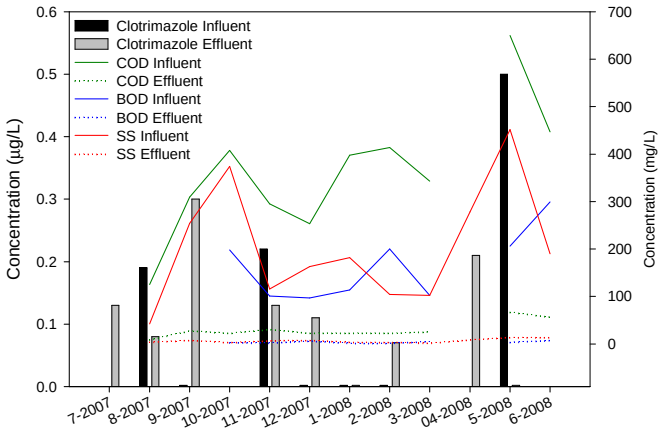
Clotrimazole Influent
Clotrimazole Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
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10-2007
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12-2007
1-2008
2-2008
3-2008
04-2008
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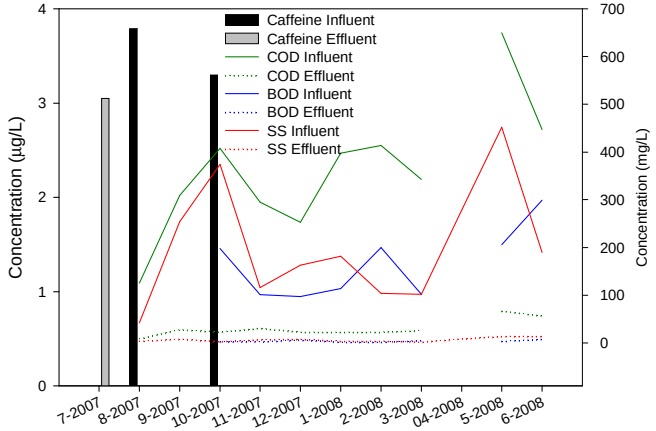
6-2008


Caffeine Influent
Caffeine Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
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2-2008
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04-2008
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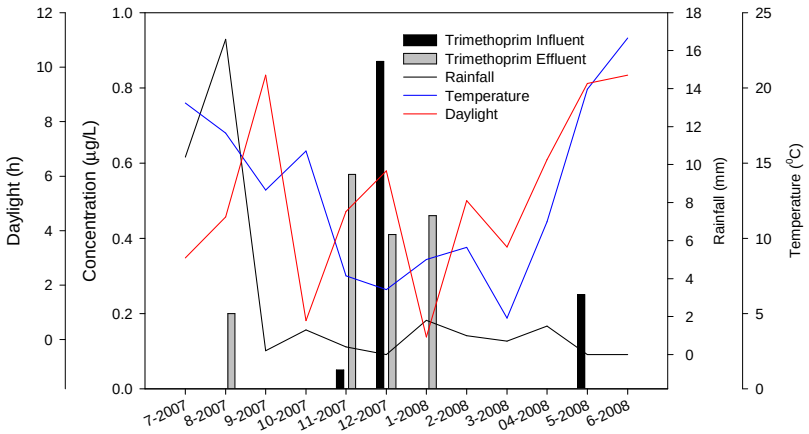
6-2008


Trimethoprim Influent
Trimethoprim Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
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9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
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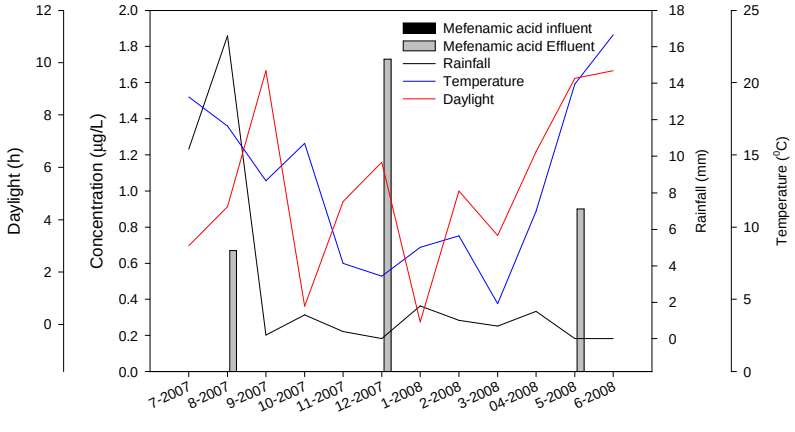
04-2008
5-2008
6-2008


Mefenamic acid influent
Mefenamic acid Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
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1-2008
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04-2008
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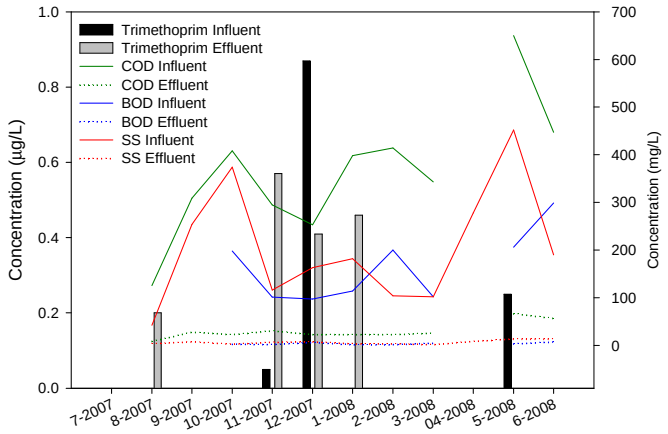
6-2008


Trimethoprim Influent
Trimethoprim Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
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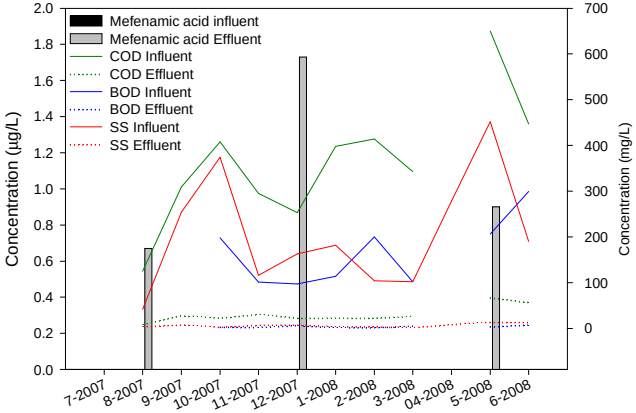
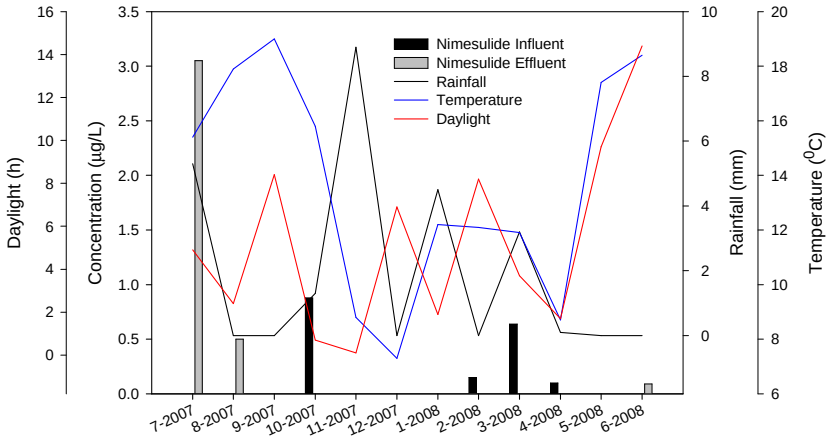
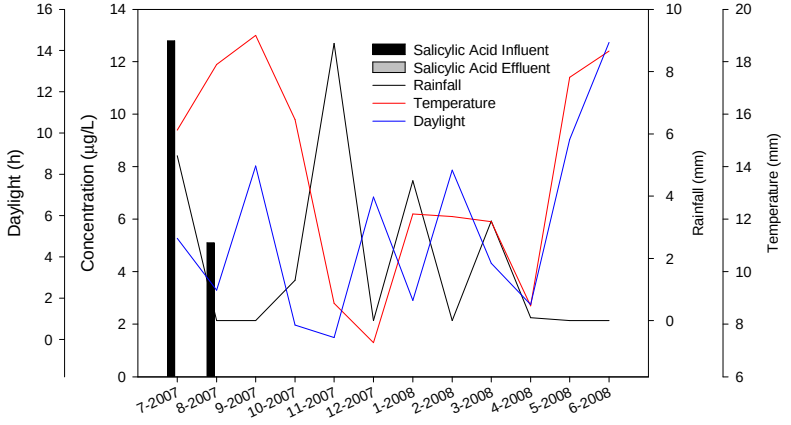
12-2007
1-2008
2-2008
3-2008
04-2008
5-2008
6-2008

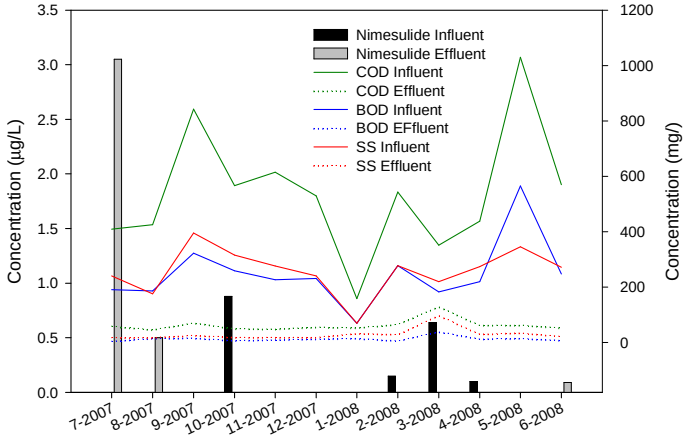

Mefenamic acid influent
Mefenamic acid Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
04-2008
5-2008
6-2008

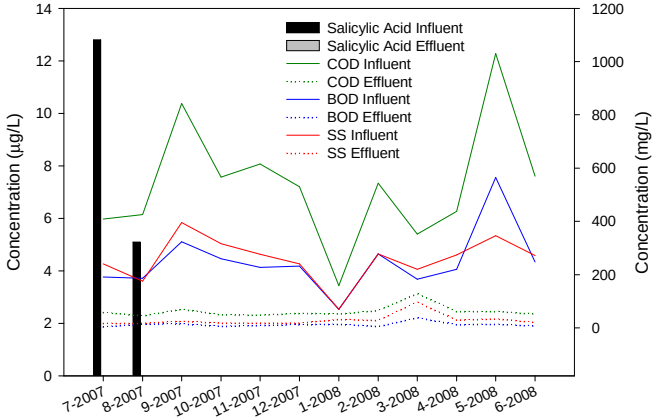
Appendix D

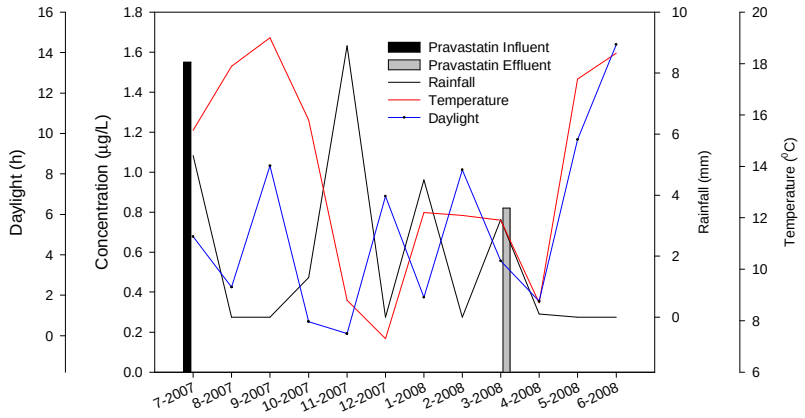
Ringsend Data - Investigation of seasonal variability.

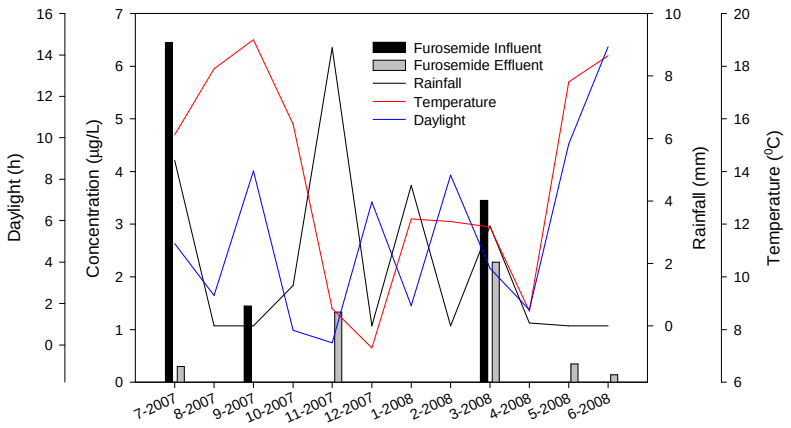

Nimesulide Influent
Nimesulide Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

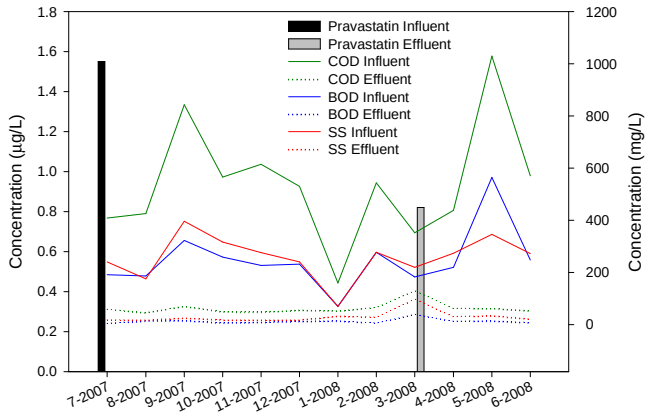

Salicylic Acid Influent
Salicylic Acid Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (mm)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

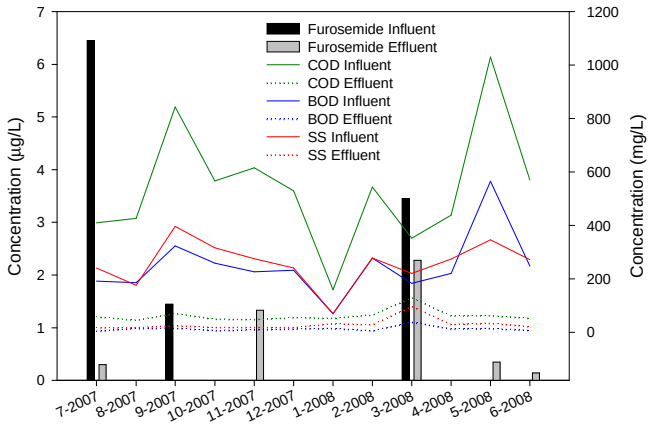

Nimesulide Influent
Nimesulide Effluent
COD Influent
COD Effluent
BOD Influent
BOD EFfluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/l)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

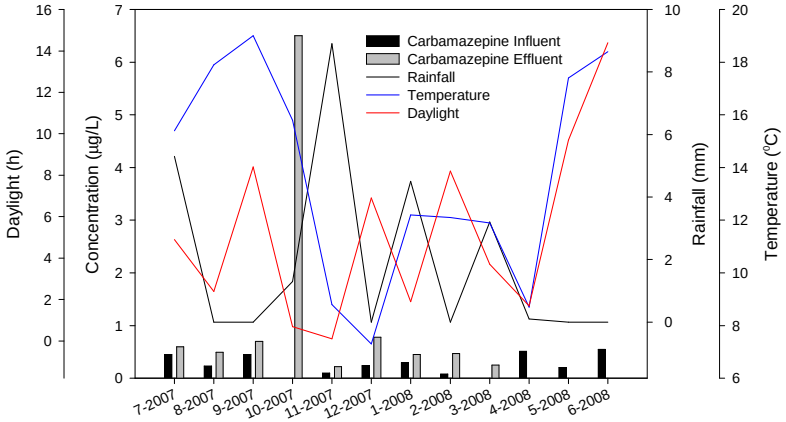

Salicylic Acid Influent
Salicylic Acid Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

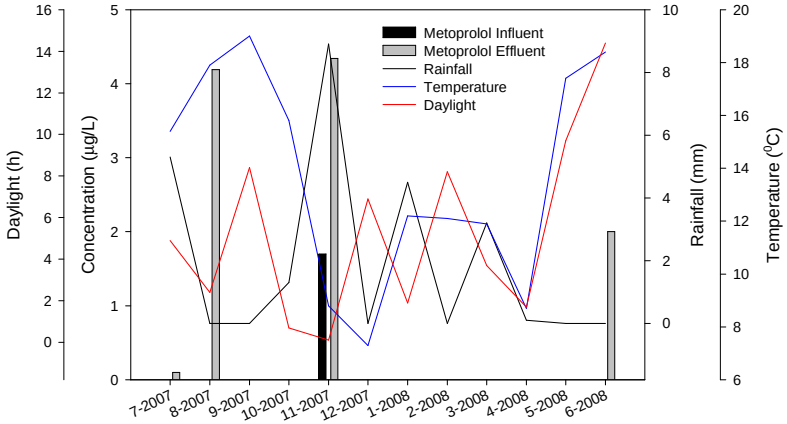

Pravastatin Influent
Pravastatin Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

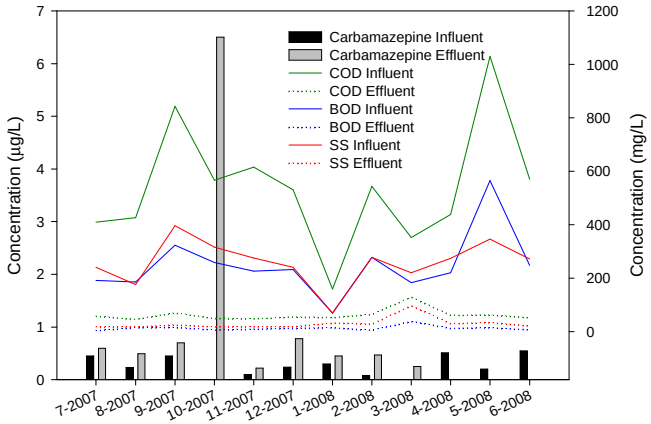

Furosemide Influent
Furosemide Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

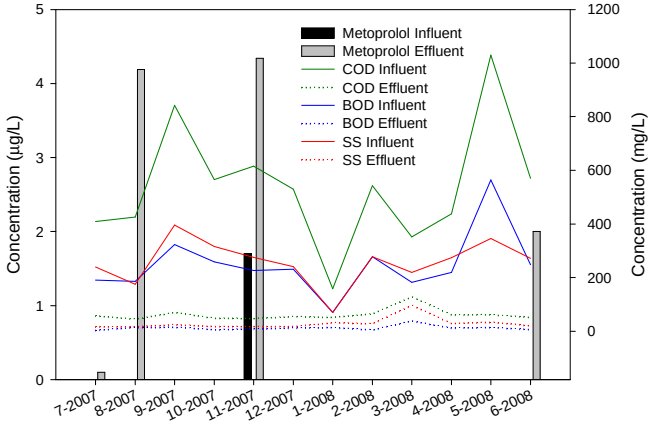

Pravastatin Influent
Pravastatin Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

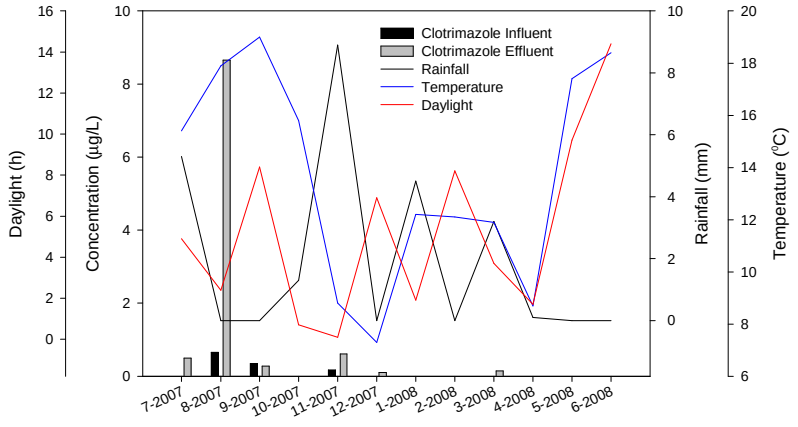

Furosemide Influent
Furosemide Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

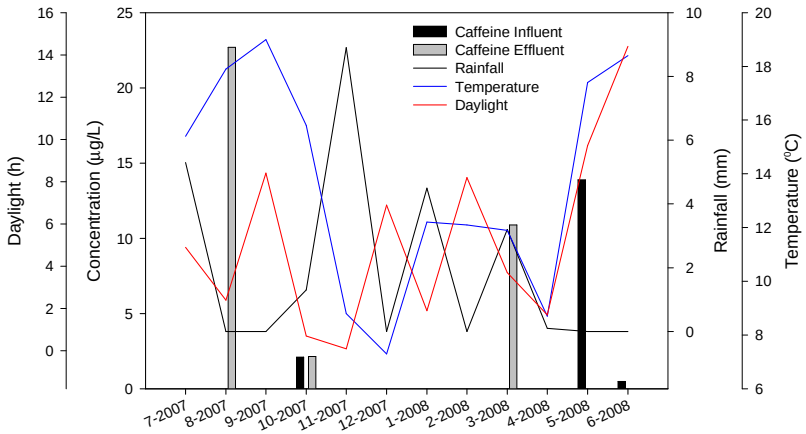

Carbamazepine Influent
Carbamazepine Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

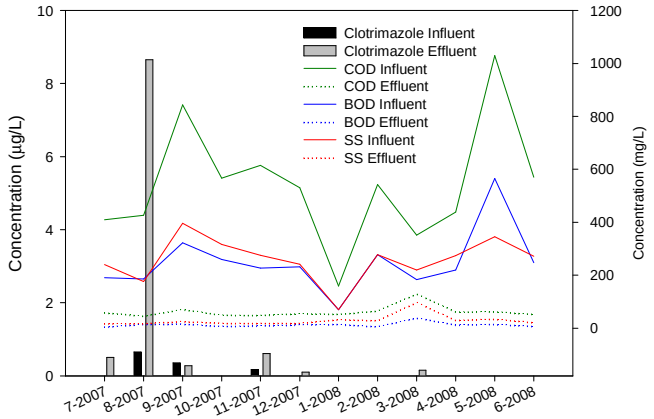

Metoprolol Influent
Metoprolol Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

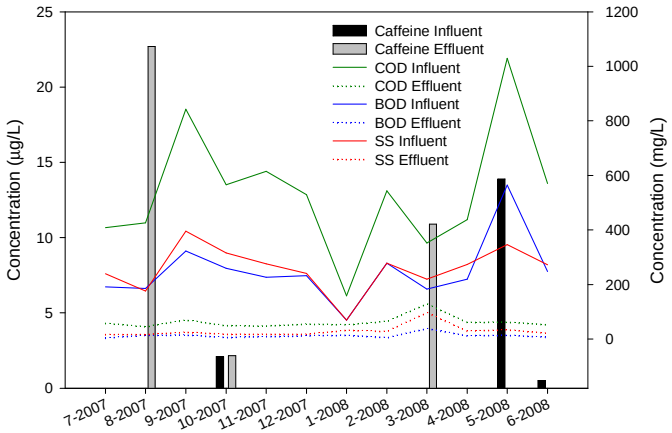

Carbamazepine Influent
Carbamazepine Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

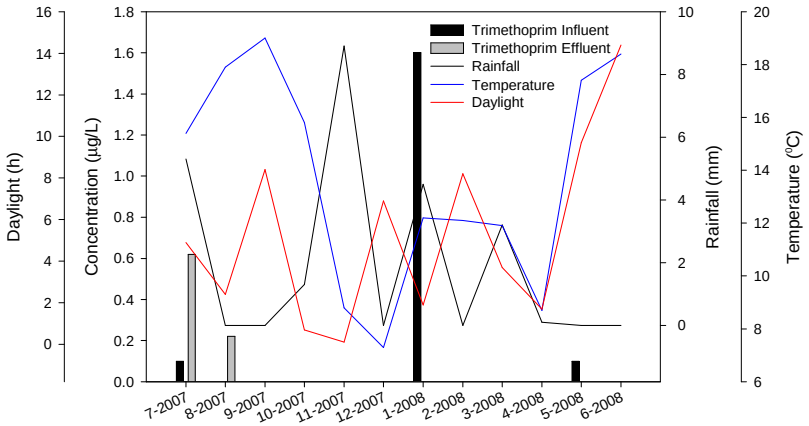

Metoprolol Influent
Metoprolol Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

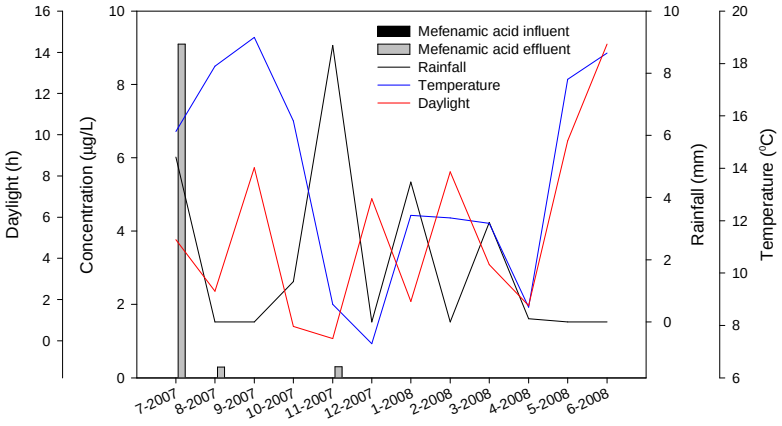

Clotrimazole Influent
Clotrimazole Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

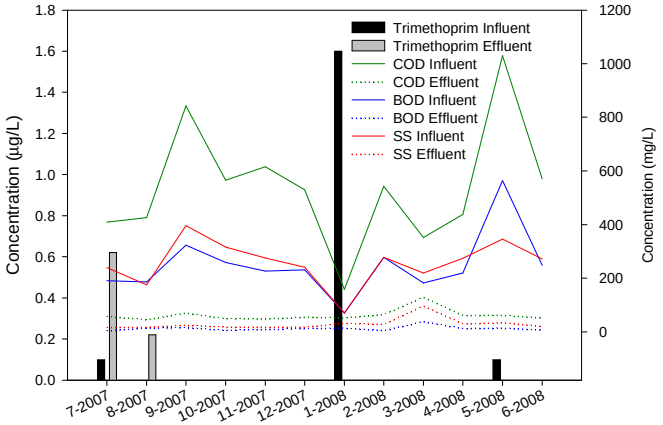

Caffeine Influent
Caffeine Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

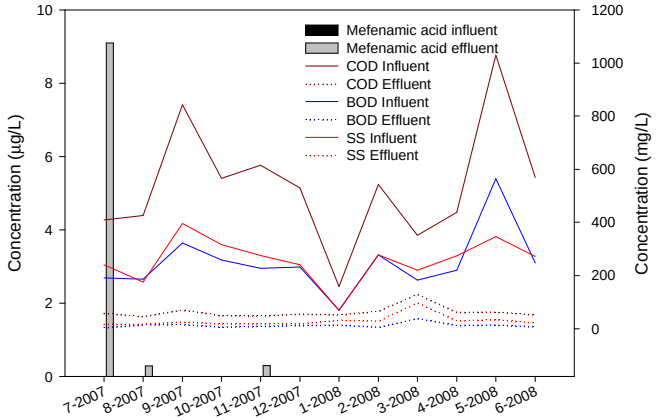

Clotrimazole Influent
Clotrimazole Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

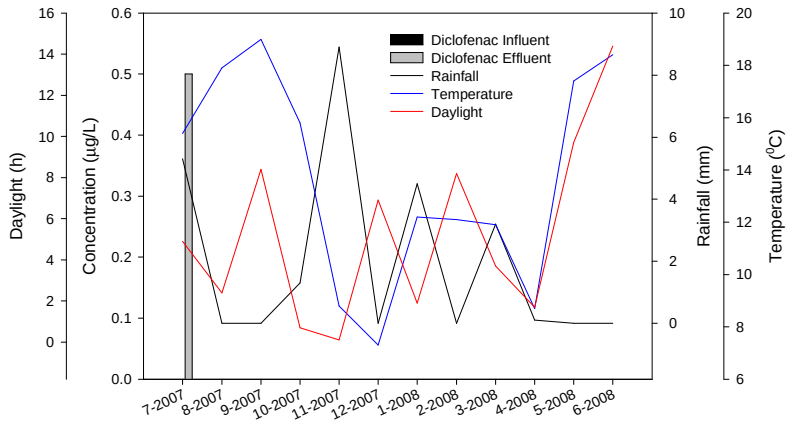

Caffeine Influent
Caffeine Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008

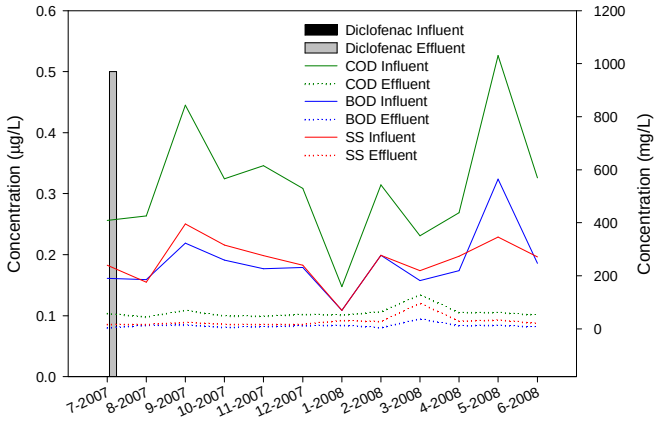

Trimethoprim Influent
Trimethoprim Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008


Mefenamic acid influent
Mefenamic acid effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008


Trimethoprim Influent
Trimethoprim Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008


Mefenamic acid influent
Mefenamic acid effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008


Diclofenac Influent
Diclofenac Effluent
Rainfall
Temperature
Daylight
Daylight (h)
Concentration (µg/L)
Rainfall (mm)
Temperature (°C)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008


Diclofenac Influent
Diclofenac Effluent
COD Influent
COD Effluent
BOD Influent
BOD Effluent
SS Influent
SS Effluent
Concentration (µg/L)
Concentration (mg/L)
7-2007
8-2007
9-2007
10-2007
11-2007
12-2007
1-2008
2-2008
3-2008
4-2008
5-2008
6-2008