OCCURRENCE AND SEASONAL VARIABILITY OF SELECTED PHARMACEUTICALS IN SOUTHERN ONTARIO DRINKING WATER SUPPLIES

By

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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ABSTRACT

Occurrence and seasonal variability of selected pharmaceuticals in Southern Ontario drinking water supplies

The presence and seasonal variability of human and veterinary pharmaceuticals in surface water (raw water) and treated water samples from two drinking water facilities in Southern Ontario was investigated. Water samples were collected at monthly intervals for one year to characterize the seasonal variability of these contaminants. The presence of these compounds in raw water samples collected from groundwater wells, which were potentially under the influence of surface water, was also examined. All samples were extracted by solid phase extraction (SPE) techniques and analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-ESI-MS/MS). The compounds detected represented different therapeutic classes, including antibiotics, lipid regulating agents and anti-inflammatory drugs. The concentrations detected for most compounds were in the low ng/L range, with one compound being detected close to 1 μg/L. In general, human pharmaceuticals (i.e. gemfibrozil, ibuprofen and carbamazepine) were detected in raw and treated water samples, while the antibiotics were not detected after treatment. Seasonal variability was observed in the concentrations and compounds detected, which could be partially explained by changes in surface water hydrology and sources of contamination. The results demonstrate that the application of conventional treatment technologies were not very effective in reducing some of these compounds from a drinking water facility. In contrast, a second drinking water facility using additional treatment technologies, including ozonation and granular activated carbon (GAC) filters, could reduce the concentrations of these contaminants. Although, the presence of these contaminants in surface water represents a potential risk, the results suggest

that appropriate treatment can minimize exposure to at least some of these emerging contaminants.

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CHAPTER 1

General Introduction

In the last 15 years, research investigating environmental impacts of contaminants has begun to focus on a number of "emerging" contaminants. One group of "emerging" contaminants is a widely diverse group of biologically active compounds, which are commonly referred to as pharmaceuticals and active agents in personal care products (PPCPs) (Daughton and Ternes, 1999). This large group consists of non-prescription drugs, prescription drugs, veterinary medicines, growth promoters, diagnostic agents, cosmetics, fragrances, sun screen agents, musks and disinfectants used in industry, households and agricultural practices (Halling-Sørensen et al., 1998; Barceló and Petrović, 2007).

Although, these compounds have only recently been recognized as environmental contaminants, they have gained the attention of the general public and the scientific community. Concern has been raised based on their continuous release into the environment at low concentrations and the possible subtle effects of these compounds on non-target organisms over an extended period of time (Jones et al., 2005; Barceló and Petrović, 2007).

In contrast to other environmental contaminants, pharmaceuticals are designed to be biologically active and have specific modes of action to help prevent, treat or cure health conditions in humans as well as animals (i.e. fish, cattle, swine and poultry) (Derksen et al., 2004; Bendz et al., 2005). The application of these compounds in healthcare is considered to be one of the greatest benefits in modern society, and has improved the health and lifestyle of individuals dealing with a diversity of specific health problems (Roberts and Bersuder, 2006). However, limited attention has been given to the possible impacts these compounds may have on ecosystem health (O'Brien and Dietrich, 2004). Regulations and guidelines for these

compounds are now being explored to control their release and potential impacts in the environment (EMEA, 2006a; EMEA 2006b).

Some of the first reports of pharmaceuticals detected in the environment were in the 1970's when clofibric acid was detected in wastewater samples collected in the United States (Garrison et al., 1976; Jones et al., 2001). However, limited attention was given to the presence of these contaminants in the environment until the early 1990's when clofibric acid was detected in groundwater and tap water samples collected in Germany (Stan and Heberer, 1997; Heberer et al., 1998; Heberer et al, 2002; Jones et al., 2005). Clofibric acid was the first pharmaceutical to be reported in tap water samples with concentrations up to 270 ng/L (Heberer, 2002b; Jones et al., 2005). In recent years, advancements in analytical methods have contributed to the detection of over 100 active agents in pharmaceutical products in different environmental matrices (Richardson and Ternes, 2005; Zwiener, 2007). It is anticipated that this number will continue to grow as more pharmaceutical products are approved for use and advancements in analytical methods continue.

1.1 Development of Analytical Methods

Advancements made in environmental and analytical chemistry with the combination of liquid chromatography and mass spectrometry (LC-MS or LC-MS/MS) has allowed researchers to confidently detect these compounds in complex matrices at low concentrations (Niessen, 1998; Zwiener and Frimmel, 2004). LC-MS with electrospray ionization (ESI) has commonly been applied as a selective and sensitive tool for the detection of PPCPs in biological and environmental matrices because of the polar, thermoliable and non-volatile nature of these contaminants (Hernando et al., 2004; Zwiener and Frimmel, 2004). Niessen (1998) reported that approximately 95% of LC-MS work uses ESI or atmospheric pressure

chemical ionization (APCI) as the ionization source, however ESI is most commonly used in the detection of PPCPs in environmental matrices. The major disadvantage of using LC-MS with ESI is the potential of matrix effects occurring during the ionization of the analytes in the source interface (Petrović et al., 2005). Matrix effects are defined as a change in analyte signal caused by something in the sample other than the analyte (Harris, 1999). During method development, the identification and correction for possible matrix effects must be accomplished to achieve accurate measurements. If matrix effects are not addressed an overestimation or underestimation of the actual environmental concentration can occur, and the accuracy of the method is impacted (Van De Steene et al., 2006).

Matrix effects are observed when the signal intensity of an analyte detected in a field sample is different from the signal intensity detected in a solvent solution (Miao and Metcalfe, 2003; Hernando et al., 2004). Studies have shown that an increase in the amount of matrix present (i.e. organic material) results in signal suppression or enhancement, with higher ion suppression occurring in wastewater influent and effluent samples (Hirsh et al., 1998; Miao and Metcalfe, 2003; Vanderford et al., 2003; Hernando et al., 2004; Vieno et al., 2006). However, the impact that the matrix has on a compound is dependent on the analyte as well as the composition of the matrix.

The best approach to compensate for matrix effects is the use of internal standards (isotopically labeled standards), which elute from the separation column at a similar retention time and undergo the same conditions in the ionization source. Recent studies investigating the presence of pharmaceuticals in complex matrices have incorporated the use of internal standards into their methods to correct for matrix effects. The disadvantage of using these

labeled standards is the cost of purchasing them and the availability of them for use in research.

Matrix effects can also be addressed by using a standard addition approach, improving clean-up and extraction procedures to reduce the amount of matrix entering the instrument, reducing the flow rate of the sample matrix into the ionization source, and decreasing the injection volume (Hernando et al., 2004; Gómez et al., 2006; Kloepfer et al., 2005; Van De Steene et al., 2006). The application of all these approaches have been useful in reducing matrix effects, but all exhibit some limitations, including decreases in sensitivity, and increases in time and labour costs.

Advancements made in this area of environmental chemistry have allowed researchers to determine the occurrence of pharmaceuticals in the environment and apply this information to help assess the impacts of these compounds on environmental and human health.

1.2 Entry into the Environment

Pharmaceuticals are ubiquitous environmental contaminants, and have been detected in raw and treated wastewater, soil, biosolids, sediment, groundwater, surface water and drinking water supplies in North America and Europe (Ternes et al., 2001; Sacher et al., 2001; Heberer et al., 2002; Löffler and Ternes, 2003, Metcalfe et al., 2003a; Ashton et al., 2004; Miao et al., 2005; Vanderford and Snyder, 2006; Gómez et al., 2007). The concentrations detected have been relatively low, with concentrations in the µg/L range for wastewater samples, and in the low ng/L range for drinking water.

Reviews by Halling-Sørensen et al. (1998), Ternes (1998), Heberer (2002b) and Derksen et al. (2004) have illustrated possible exposure routes for the entry of human and veterinary pharmaceuticals into the environment. Human pharmaceuticals enter the

environment mainly through their therapeutic use. The drug is excreted via urine or feces as various combinations of metabolites, the parent compound or conjugated compounds (Halling-Sørensen et al., 1998). In the body, most drugs are metabolized to some extent by phase I and/or phase II reactions before leaving the body and entering the environment. Phase I reactions include oxidation, reduction and hydroxylation reactions, and produce reactive water-soluble compounds which can either add or expose functional groups needed for further reactions (Halling-Sørensen et al., 1998; Josephy and Mannervik, 2006). Phase II reactions, also called conjugation reactions, involve the binding of functional groups to the compound, which increases its water solubility for elimination from the body. Phase II reactions include sulfation, methylation, acetylation, glutathione conjugation and glucuronide conjugation (Josephy and Mannervik, 2006).

The release of human pharmaceuticals and/or its metabolites into the aquatic environment can occur through a number of different exposure pathways, including surface runoff after the application of biosolids, disposal of pharmaceutical production wastes and discharging of hospital wastewater effluents (Halling-Sørensen et al., 1998; Daughton and Ternes, 1999; Gómez et al., 2006). However, the major route by which human pharmaceuticals and their metabolites enter the aquatic environment is through the discharging of these compounds into surface waters from domestic waste after incomplete removal during wastewater treatment (Ternes, 1998; Heberer et al., 2002; Metcalfe et al., 2003b; Bendz et al., 2005; Lishman et al., 2006). This route also provides a significant pathway for how these compounds can contaminate source waters for drinking water production.

The release of veterinary pharmaceuticals into the environment can take place directly when the livestock animals are on pasture or indirectly by run-off or leaching through the soil (Derksen et al., 2004). Veterinary pharmaceuticals can also be released into the environment after the stored manure is applied onto agricultural land during the spring and fall months as a soil amendment. Surface runoff and leaching after the application of manure depends on a number of parameters, including climatological conditions as well as the physical and chemical properties of the compound (Derksen et al., 2004).

In either case, for human and veterinary pharmaceuticals, these compounds will eventually enter surface waters or infiltrate into groundwater aquifers, which can be used for potable water use (Jones et al., 2001). The concern of drinking water supplies contaminated with PPCPs will continue to grow as urbanization and intensification of animal production increases.

1.3 Presence of Pharmaceuticals in the Canadian Environment

In Canada, research has focused on determining the presence of selected pharmaceuticals in wastewater treatment facilities and surface waters (Metcalfe et al., 2003a; Metcalfe et al., 2003b; Brun et al., 2006; Lishman et al., 2006), as well as surface waters exposed to agricultural inputs (Hao et al., 2006; Lissemore et al., 2006). Hao et al. (2006) and Lissemore et al. (2006) investigated the presence of pharmaceuticals (antibiotics, acidic and neutral pharmaceuticals) in surface waters receiving agricultural inputs as well as one sampling location near an urbanized area in a Southern Ontario watershed. The authors reported the presence of selected pharmaceuticals, with lincomycin HCl, trimethoprim, sulfamethazine, carbamazepine and monensin being the most frequently detected.

Brun et al. (2006) investigated the presence of neutral and acidic drugs in wastewater treatment facilities and surface waters in Atlantic Canada. High concentrations of bezafibrate, gemfibrozil, ibuprofen, naproxen and carbamazepine were detected in wastewater effluent samples. The highest median concentrations was for ibuprofen and naproxen with median concentrations over 1000 ng/L during the spring and summer months. Carbamazepine was detected at lower concentrations compared to the acidic drugs, but the concentrations were more consistent over the sampling period. The presence of bezafibrate, gemfibrozil and carbamazepine were sparse, with median concentrations below 20 ng/L. The concentrations of the selected pharmaceuticals decreased when samples were collected further downstream from wastewater effluent discharge location or the receiving environment merged with other water bodies to dilute the percentage of the wastewater effluents in the surface waters (Brun et al., 2006).

A recent study by Verenitch et al. (2006) detected similar results as Brun et al. (2006) in which human pharmaceuticals were detected in wastewater effluents collected on the West Coast of Canada. The concentrations were above 1µg/L in the effluent samples and significantly lower concentrations in the samples collected in the receiving waters.

Lishman et al. (2006) investigated the presence and possible reductions of acidic drugs in the Thames River watershed in Southwestern Ontario. Ibuprofen, gemfibrozil and naproxen were some of the compounds detected in final wastewater effluent samples, with median percent reductions of greater than 90% for ibuprofen and naproxen and 66% for gemfibrozil. The median concentrations detected in the final wastewater effluent samples were 500 ng/L or lower for the acidic pharmaceuticals.

Metcalfe et al. (2003a) detected the presence of acidic and neutral drugs in influent and effluent samples collected from a number of Canadian wastewater treatment facilities. The most frequently detected compounds included salicylic acid, naproxen, ibuprofen, gemfibrozil and carbamazepine. Metcalfe et al. (2003b) investigated the presence of acidic and neutral pharmaceuticals in four wastewater treatment facilities and surface waters located near wastewater treatment facilities in selected regions of Ontario. The compounds detected during the sampling period included carbamazepine, ibuprofen, gemfibrozil, bezafibrate, naproxen and trimethoprim along with other anti-inflammatory agents and clofibric acid.

Hua et al. (2006b) investigated the same compounds as Metcalfe et al. (2003a and 2003b) but concentrated on the presence of these compounds in one wastewater treatment facility in Windsor, Ontario and along the shoreline of two rivers. Ibuprofen, naproxen, bezafibrate, gemfibrozil, carbamazepine and trimethoprim were found at high concentrations. The concentrations of bezafibrate and carbamazepine were relatively consistent over the three sampling months. Higher concentrations of gemfibrozil and trimethoprim were detected in samples collected during March and June compared to September. The concentrations of ibuprofen over the three sampling months were quite random with higher concentrations detected in September and March. Naproxen was detected at relatively high concentrations over each sampling month, with lower concentrations detected in March. The concentrations of these compounds dramatically decreased downstream of wastewater effluent discharge site due to the effluent being diluted by large water bodies, with most concentrations below 100 ng/L where the two rivers joined.

The presence of acidic pharmaceuticals, carbamazepine and its metabolites, and anti-microbials have been detected in a number of wastewater treatment facilities in Canada, with

concentrations in the low µg/L range (Miao et al., 2002; Miao et al., 2004; Miao et al., 2005). Selected statin drugs have also been detected in wastewater influent and effluent samples as well as surface water samples in Ontario, but were found at lower concentrations compared to other pharmaceuticals (Miao and Metcalfe, 2003).

The results from the studies conducted in Canada illustrate that pharmaceuticals are widespread contaminants in Canadian wastewater treatment facilities and surface waters. Research has shown the presence of these compounds in surface waters receiving wastewater effluents, but limited information is known about the presence of these compounds during drinking water treatment when contaminated surface waters are used as source water.

1.4 Presence of Pharmaceuticals in Drinking Water Supplies

Globally, there have been few studies that have investigated the presence of pharmaceuticals in full scale drinking water treatment systems (Zuccato et al., 2000; Redderson et al., 2002; Boyd et al., 2003; Stackelberg et al., 2004; Hernando et al., 2006; Hua et al. 2006a; Hummel et al., 2006; Kim et al., 2006; Vanderford and Snyder, 2006), and few compounds have been reported in drinking water samples.

Zuccato et al. (2000) reported concentrations of tylosin, clofibric acid and diazepam in drinking water samples collected from three water systems in Italy, with the highest concentration reported for diazepam at 23.5 ng/L. Hummel et al. (2006) reported maximum concentrations of 20 ng/L of carbamazepine in drinking water samples collected from three conventional water treatment facilities in Germany. Carbamazepine was also reported in drinking water samples collected in water treatment facilities in Canada (Hua et al., 2006a) and South Korea (Kim et al., 2006). Hua et al. (2006a) reported low concentrations of carbamazepine in finished water collected from a water treatment facility in Windsor, Ontario

with mean concentrations of 2 ng/L or below for water not treated with ozone and non-detectable levels for water treated with ozone.

In the United States, ibuprofen was detected in two out of fifteen finished drinking water samples collected from water treatment facilities in Southern California, with a maximum concentration of ibuprofen at 1.25 µg/L (Loraine and Pettigrove, 2006). Boyd et al. (2003) investigated the presence of a number of pharmaceuticals in drinking water treatment facilities located in Ontario, Canada and Louisiana, USA. Water samples were collected at different locations along the treatment processes at each facility. Naproxen was one of the few compounds detected in the surface water samples and was not detected in any of the finished water samples collected at the water treatment facilities investigated. Stackelberg et al. (2004) investigated the presence of a number of organic contaminants in surface water and treated water samples collected from a conventional water treatment facility in the United States. A total of seventeen contaminants were detected in the finished drinking water samples, and four were prescription and non-prescription drugs. Carbamazepine was one of the compounds detected in the finished drinking water at a maximum concentration of 258 ng/L.

Tauber et al. (2003) investigated the presence of acidic and neutral pharmaceuticals and antibiotics in drinking water treatment facilities in ten Canadian cities. Carbamazepine and gemfibrozil were the only two compounds detected in finished drinking water samples. Carbamazepine was detected in water treatment facilities located in three cities at concentrations of 6.5, 8.4 and 24 ng/L. Gemfibrozil was detected in only water treatment facility at a concentration of 70 ng/L. Although, this report was not peer-reviewed and was conducted by two Canadian media sources, it indicates that current water treatment

technologies being employed in Canadian cities are not effectively reducing these compounds from drinking water supplies.

Servos et al. (2007) investigated the presence of acidic drugs in surface water and treated water samples collected from drinking water facilities in Ontario, Canada. Naproxen, gemfibrozil, and ibuprofen were detected at the highest concentrations in the surface water samples, with concentrations in the low ng/L range. Ibuprofen was one of the few compounds detected in the treated water samples and showed minimal reduction during water treatment.

There have also been very few studies that have monitored the presence of pharmaceuticals in groundwater wells used for drinking water production. Gemfibrozil and ibuprofen were not detected in the groundwater monitoring wells used for water recharge purposes (Drewes et al., 2002). However, carbamazepine was present in two monitoring wells at concentrations of 455 ng/L and 610 ng/L, and naproxen was detected at 20 ng/L in one of the wells (Drewes et al., 2002). Sacher et al. (2001) detected the presence of carbamazepine (maximum concentration of 900 ng/L) and sulfamethoxazole (maximum concentration of 410 ng/L) during a groundwater monitoring study in Germany, in which 105 samples were collected. Carbamazepine was detected in 13 samples and sulfamethoxazole was detected in 11 samples. Carbamazepine and ibuprofen have also been detected in groundwater samples collected in France at concentrations of 13.9 and 43.2 ng/L and 0.2 and 0.6 ng/L, respectively (Rabiet et al., 2006).

Heberer (2002a) summarized results from previous studies that had investigated the presence of pharmaceuticals in different matrices in Germany, including groundwater wells and drinking water samples. Gemfibrozil and ibuprofen were the only compounds detected in groundwater wells at concentrations of 340 ng/L and 200 ng/L, respectively.

1.5 Drinking Water Treatment Processes

The ability to reduce the concentrations of pharmaceuticals during wastewater and water treatment has been the focus of many research efforts (Zwiener, 2007). Numerous studies have shown that current wastewater treatment technologies, and to a lesser extent water treatment processes, are not eliminating these compounds from entering surface waters and drinking water supplies (Ternes, 1998; Thomas and Foster, 2005; Castiglioni et al., 2006). The current conventional technologies (coagulation, flocculation and sedimentation) were not designed to remove emerging contaminants, like PPCPs, therefore more advanced treatments have been developed and applied to see if they are capable of reducing these compounds (Ternes et al., 2002; Ternes et al., 2003; Vieno et al., 2005). The application of ozonation, advanced oxidation processes (AOPs), membrane technologies and filtration devices have proven to be effective in reducing some pharmaceuticals present in wastewater and water (Zwiener and Frimmel, 2000; Ternes et al., 2001; Ternes et al., 2002; Huber et al., 2003; Ternes et al., 2003; Khan et al., 2004; Vogna et al., 2004; Huber et al., 2005a; Huber et al., 2005b; Nghiem et al., 2005; Vieno et al., 2005; Westerhoff et al., 2005; Hua et al., 2006b; Radjenovic et al., 2007).

1.6 Objectives of Thesis

The purpose of this thesis project was to identify and quantify the presence of pharmaceuticals in Canadian drinking water supplies, and to provide information to assess the implications of these compounds on drinking water quality. It has been well established that these compounds are present in wastewater effluents and surface waters in Canada, but minimal information is known about their presence in drinking water supplies and the effectiveness of current water treatment processes in reducing these compounds.

The first objective of this thesis was to investigate the presence of a selected number of human and veterinary pharmaceuticals in water samples collected from two large municipal full scale water treatment facilities and groundwater systems in a susceptible Southern Ontario watershed. The second objective was to investigate the seasonal variability of these compounds in surface water and drinking water, and the third objective was to determine how effective two water treatment facilities were in reducing pharmaceuticals during drinking water production.

Surface water (raw water) and treated water samples were collected from two drinking water systems over a 12 month period to investigate the seasonal changes in the compounds and concentrations detected. The two treatment facilities were selected based on their relative position in the watershed and application of different treatment technologies used. Both facilities used conventional treatment technologies, but one of the selected water treatment facilities had advanced treatment with ozonation, granular activated carbon (GAC) filters and ultra-violet irradiation. Comparisons were made in the ability of the facilities to reduce the concentrations of these contaminants to non-detectable levels. All of the water samples collected were extracted using solid phase extraction (SPE) techniques and were analyzed by liquid chromatography with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS).

This thesis supports the international effort to evaluate the risk of these emerging contaminants and supports initiatives to manage and reduce their impact to ecosystem and human health.

CHAPTER 2

The Impact of Matrix Effects on Determining the Presence of Selected Pharmaceuticals in Surface Water and Drinking Water Using Liquid Chromatography Coupled with Electrospray Ionization Tandem Mass Spectrometry

2.1 Abstract

This study investigates the use of solid phase extraction (SPE) techniques and liquid chromatography with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) in determining the presence of selected pharmaceuticals in environmental matrices. The analytical method was applied to surface water (raw water) and treated water samples collected from two drinking water treatment facilities in Southern Ontario over a 12 month period, and raw water samples collected from eight groundwater wells. Three different quantification approaches were applied, and the estimated final concentrations were compared to determine the extent of how matrix components can impact the signal response. The results illustrate that compounds analyzed in negative ionization mode are impacted by the matrix to a greater extent compared to compounds analyzed in positive ionization mode. However, seasonal trends remained similar among the different quantification methods. The compounds detected in the water samples, using the analytical method, represent a variety of therapeutic classes including human and veterinary antibiotics, anti-inflammatory agents, and lipid regulating drugs. The concentration range varied from below 6 ng/L for gemfibrozil to close to 1 μg/L for carbamazepine. Failure to recognize the impact of matrix effects on the analysis can lead to major errors in quantification. These effects are dependent on the matrix, the methodologies, and the properties of the analyte. This study illustrates the importance of addressing matrix effects in order to report reliable data to be used in assessing the potential risks of these environmental contaminants on environmental and human health.

2.2 Introduction

In recent years, the use of liquid chromatography and mass spectrometry has become routine instrumentation for determining the presence of a wide variety of pharmaceuticals in

the environment (Zwiener and Frimmel, 2004; Richardson and Ternes, 2005). The development of ionization interfaces, which act as a connection between liquid chromatography and mass spectrometry, has allowed for the direct introduction of analytes from the separation column into the high vacuum mass spectrometer (Rossi and Sinz, 2002). Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are commonly applied ionization sources used in helping to determine the presence of pharmaceuticals and personal care products in environment matrices. One of the disadvantages of using ESI, and to a lesser extent APCI, is the susceptible to matrix effects. It has been suggested that the actual ionization mechanism of how ions are created in solution phase of the ESI interface is responsible for matrix effects observed (Hernando et al., 2004; Bos et al., 2006). The exact mechanism of how the co-eluting matrix components interfere during the ESI process is not clear, but involves the mechanisms of how ESI produces gas phase ions (Kloepfer et al., 2005). Signal suppression or enhancement are attributed to matrix components that co-elute from the LC column at the same time as the analytes, and as a result enter the ionization source at the same time, where ionization efficiency of the analyte is impacted by the presence of matrix components (Kloepfer et al., 2005; Gómez et al., 2006; Hummel et al., 2006; Van De Steene et al., 2006; Vieno et al., 2006). This situation creates the possibility of the analyte and matrix interacting with each other during ionization, and in most cases ionization of the analyte being suppressed. The end result is inaccurate final concentrations being reported because matrix effects have not been addressed during method development. There have been a number of recent published studies that have used only a limited number of internal standards to quantify the analytes without careful consideration of

potential matrix effects (Hirsch et al., 1998; Castiglioni et al., 2004; Bendz et al., 2005; Castiglioni et al., 2005; Hao et al., 2006; Lissemore et al., 2006).

Research has started to investigate and summarize possible solutions for addressing matrix effects, when using LC-ESI-MS/MS instrumentation (Hernando et al., 2004; Gómez et al., 2006; Hua et al., 2006b; Hummel et al., 2006). Possible solutions have included improvements in sample extraction and clean-up procedures (Zrostlíková et al., 2002; Hernando et al., 2004; Kloepfer et al., 2005; Stoob et al., 2005; Van De Steene et al., 2006); decreasing the injection volume into the LC, in which a reduced amount of matrix enters the LC column at one time (Hernando et al., 2004; Hua et al., 2006b), changing operational parameters of the instrument (i.e. decreasing flow rates into the ionization source) (Choi et al., 2001; Zrostlíková et al., 2002; Kloepfer et al., 2005; Van De Steene et al., 2006) or applying alternate quantification methods (i.e. standard addition and internal standard/surrogate standard calibration methods), which makes use of standards being added during sample extraction and analysis, to correctly quantify compounds in environmental matrices (Richardson and Ternes, 2005).

The standard addition approach has been used extensively to quantify concentrations of pharmaceuticals in different matrices and correct for potential matrix effects (Lindsey et al., 2001; Zrostlíková et al., 2002; Metcalfe et al., 2003a; Gómez et al., 2006; Hernando et al., 2006). The main disadvantage of this calibration approach is it tends to be labour intensive and time consuming because different calibration curves have to be prepared for each matrix type (i.e. soil, sediment, surface water, sewage effluent) and for different compositions within each matrix type (Miao and Metcalfe, 2003; Van De Steene et al., 2006).

Standard addition or the matrix-matched calibration approach has been commonly used by researchers looking at the presence of acidic and neutral pharmaceuticals, statin drugs and various antibiotics in surface water samples and wastewater samples collected in Canada (Miao et al., 2002; Metcalfe et al., 2003a; Metcalfe et al., 2003b; Miao et al., 2004; Miao et al., 2005). The standard addition approach has also been conducted in determining the presence of selected pharmaceuticals in environmental samples collected in United States and Europe (Sacher et al., 2001; Kolpin et al., 2002; Löffler and Ternes, 2003; Hernando et al., 2004; Hernando et al., 2006).

The internal standard calibration method is another common approach for quantifying the presence of pharmaceuticals and other organic compounds in environmental matrices, and at the same time addressing potential matrix effects (Hua et al., 2006b; Hummel et al., 2006; Van De Steene et al., 2006; Vieno et al., 2006). The use of internal standards, either isotopically labeled internal standards (deuterated surrogates or ^{13}C-labeled standards) or structural analogues, have been used to investigate the presence of a number of human and veterinary medicines in environmental matrices (Hilton and Thomas, 2003; Löffler and Ternes., 2003; Vanderford et al. 2003; Cahill et al., 2004; Lindberg et al., 2004; Stoob et al., 2005; Hernando et al., 2006; Hummel et al., 2006).

The best internal standard is an isotopically labeled standard (^{13}C-labeled compounds or deuterated compounds). These standards have similar evaporation properties and would go through the same degree of signal suppression or enhancement in the matrix as the analyte of interest (Hernando et al., 2006; Van De Steene et al., 2006). Recently, the use of isotopically labeled standards have been used for quantification of selected pharmaceuticals in drinking water, surface water and wastewater samples collected in Canada (Hua et al., 2006a; Hua et

al., 2006b), the United States (Vanderford and Snyder, 2006) and Germany (Hummel et al., 2006).

One of the disadvantages of using an internal standard calibration approach is that these internal standards, isotopically labeled surrogates, are sometimes not available commercially and can be expensive. In addition, if structural analogues are used as internal standards, it is difficult to find a compound that is structurally similar to the analytes of interest and not already present in environmental matrices (Van De Steene et al., 2006).

This study critically assesses a LC-ESI-MS/MS analytical method for determining the presence of selected pharmaceuticals in water samples used in previous studies (Hao et al., 2006; Lissemore et al., 2006). Three quantification methods were applied in this study which include an external solvent calibration approach, and two internal standard calibration approaches, one involving the use of deuterated surrogates (isotopically labeled standards) and the other using a ^{13}C-labeled compound ($^{13}C_6$-sulfamethazine phenyl). The analytical method was applied to surface water (raw water) and treated water samples collected from two full scale drinking water systems in a Southern Ontario watershed. The field samples were used to determine the effectiveness of the analytical method and the limitations associated with using different quantification approaches for addressing potential matrix effects.

2.3 Materials and Methods

2.3.1 Chemicals and Standards

Most pharmaceutical standards were purchased from Sigma-Aldrich (St. Louis, MO, USA), along with ammonium hydroxide (ACS reagent grade), heptafluorobutyric acid (HFBA), and ethylenediaminetetraacetic acid (EDTA) disodium salt dehydrate (ACS reagent

grade). Clofibric acid and 4-acetamidophenol were purchased from Aldrich Chemical Company (Milwaukee, WI, USA), lasalocid A sodium salt was purchased from Riedel-de Haën (Seelze, Germany), and ammonium acetate (above 99% purity) was purchased from Fluka Chemika (Mississauga, ON, Canada). Sulfamethazine-phenyl-$^{13}C_6$ (^{13}C-90%) was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). The deuterated surrogates, carbamazepine-D_{10} (98.2 atom %D), gemfibrozil-D_6 (2,2-dimethyl-D6, 99.7 atom %D), diclofenac-D_4 (phenyl-D_4, 92.5 atom%D), (+)-ibuprofen-D_3 (α-methyl-D_3, 99.4 atom %D) and N-(4-hydroxy-2,3,5,6-D_4) acetamide (4-acetamidophenol-D_4, 99.4 atom %D), were purchased from CDN Isotopes (Pointe-Claire, PQ, Canada). Methanol (distilled in glass), acetone (HPLC grade), acetronitrile (HPLC grade), water (HPLC grade) and sulphuric acid were purchased from Caledon Laboratories Ltd. (Georgetown, ON, Canada), and sodium hydroxide pellets (ACS reagent grade) were purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA) for preparation of a 5% sodium hydroxide solution (w/v) in nanopure water. A Barnstead NANOpure Diamond^{TM} water purification system was used to provide nanopure water for sample preparation and extraction.

2.3.2 Preparation of Solutions

Stock solutions of all analytes, the deuterated surrogates and $^{13}C_6$-sulfamethazine phenyl were prepared by weighing out approximately 10 mg of the powdered neat standard and dissolving the contents with a dilution solvent. Most of the stock solutions were diluted with methanol, however, based on solubility; acetone, mixtures of methanol and water and mixtures of methanol and acetone were used. The concentration of the stock solutions was approximately 1 mg/mL. The stock solutions were used to prepare spiking solutions, calibration standards, control standards, the internal standard solution, and the mixed

surrogate standard solution containing the deuterated surrogates. The control standards were used to determine relative recovery rates for the spiked analytes and consisted of a known volume of the spiking solutions. The calibration standards (6 to 7 calibration points) consisted of known concentrations of the analytes in a solvent solution. The internal standard solution consisted of $^{13}C_6$-sulfamethazine-phenyl in nanopure water, and was used for quantification and to correct for volume injection problems. The mixed surrogate standard solution consisted of the five deuterated surrogates including, D_{10}-carbamazepine, D_6-gemfibrozil, D_4-diclofenac, D_4-4-acetamidophenol and D_3-ibuprofen. The deuterated compounds monitored method performance and extraction efficiency. All standard solutions were stored at -20 °C except the internal standard solution, which was stored at 4°C.

2.3.3 Study Sites

Description of Drinking Water Treatment Facilities

Two large residential municipal drinking water treatment facilities were selected for this project, Facility A and Facility B. These facilities were selected based on previous data collected, differences between treatment processes used, raw water source and location within the watershed. Both of the facilities use river water as their raw water source and are located within the same watershed in Southern Ontario.

This particular watershed is one of the largest in Southern Ontario with a drainage area of 6,965 km^2. This watershed was selected due to its susceptibility of contamination from the 26 municipal wastewater treatment facilities discharging into waterways, and approximately 76% of the land being used for agricultural practices. There are approximately 900,000 people that live in this watershed, with the population steadily increasing. The increase in

population growth and urbanization together with agricultural stressors will put pressure on the finite water resources of this area.

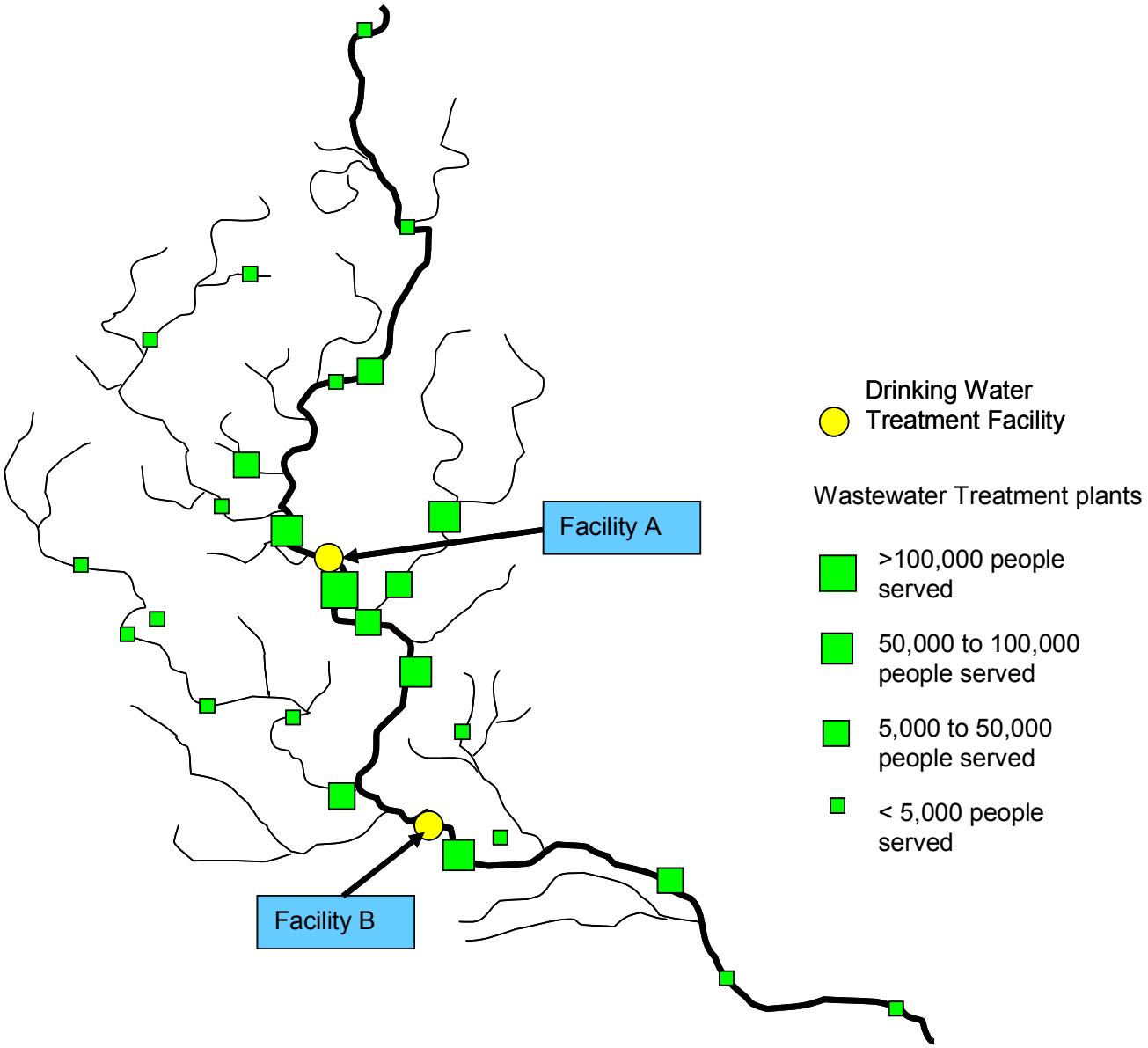
Facility B is located downstream of Facility A, with an additional eleven wastewater treatment facilities located upstream of Facility B. Figure 2.1 shows the locations of the drinking water treatment facilities relative to each other and to the wastewater treatment facilities within the watershed.

Facility A is considered to be a full conventional water treatment facility and has a dual treatment system. In addition to coagulation, flocculation, sedimentation, filtration, chlorination and chloramination processes; ozonation, GAC filters and ultra-violet (UV) irradiation are also present at this facility.

Facility A receives their surface water from a low lift pumping station next to the river. The water from the station is pumped to two reservoirs, where it is stored for a few days. The two reservoirs can hold up to 0.15 million cubic metres of water. The storage of the surface water in the reservoir reduces the turbidity in the water and provides more consistent source water characteristics before it enters the actual treatment facility. The storage reservoir also provides another control mechanism over the water collected from the river. After a few days, water from the reservoir is pumped to surface water terminal storage units, which are located next to the treatment facility. The water from these units can then flow by gravity into the facility and enters the treatment system.

Once the water reaches the treatment facility, it enters rapid mixing tanks, where a coagulant (i.e. polyaluminum chloride) is added. Polyaluminum chloride helps to destabilize or entrap suspended particulate matter to allow the formation of settling aggregates. The mixing tanks evenly distribute the coagulant agents into the surface water, allowing for

Figure 2.1: Schematic diagram of the locations of the two drinking water treatment facilities and wastewater treatment facilities in a Southern Ontario watershed (modified from Conversation Authority).


Facility A
Facility B
Drinking Water Treatment Facility
Wastewater Treatment plants
>100,000 people served
50,000 to 100,000 people served
5,000 to 50,000 people served
< 5,000 people served

destabilization of particles and impurities, and allows for agglomeration. The next step in the treatment process is flocculation. A polyelectrolyte agent (a polymer) is added at this point and helps to agglomerate suspended or very fine particles to form larger particles. Gentle mixing also promotes collisions of the clumps to allow larger flocs to form. The next step is sedimentation, in which floc particles formed in the previous step pass through a series of plates. The particles can settle on the plates and then drop to the bottom of the settling basin. Once the large particles have been removed in the sedimentation process, water enters the ozone contactor tanks. Liquid ozone is added to react and neutralize taste, odour and colour, and can also be used as a disinfecting agent. For this particular facility, ozone is mainly used for taste and odour control. The next step is filtration, in which the water is passed through dual media filters to remove fine suspended particulate and organic matter. This is completed by chemical and physical processes in the filters. The filters used at this facility consist of granular activated carbon (GAC) and sand.

After the water has been filtered, it passes into the ultra-violet (UV) irradiation system. UV irradiation is considered the primary disinfection step at this particular facility. At this facility ozone and UV are not added together and therefore this treatment is not considered an advanced oxidation process.

Once the water leaves the UV system it is chlorinated using chlorine gas. Chlorination occurs before the water is blended with groundwater sources from nearby wells. Chlorine gas and anhydrous ammonia, for chloramination, are both used for disinfection purposes and provide residual levels of disinfection for the distribution system. Anhydrous ammonia is added just before water enters the distribution system (i.e. anhydrous ammonia is added after

the water from the facility is blended with water from the groundwater wells). For back-up purposes, 20% liquid ammonium sulphate can be injected in case the anhydrous ammonia system is not working properly. This facility, along with the nearby groundwater systems serves a population close to 200,000 people. The facility produces about 20% of the potable water in the area, the rest of the potable water comes from groundwater sources.

Facility B is the second facility selected for this project and is considered a full conventional drinking water treatment facility. The treatment processes include screening, coagulation, flocculation (sand ballasted flocculation), sedimentation, chlorination, filtration, chloramination and fluoridation.

The treatment process begins by pumping water from the river into the screening house. After large objects have been removed using coarse screens, the water is pumped to the pretreatment system building. Powdered activated carbon (PAC), certified NSF 61, is the first chemical added in the two pipes that feed the dual/mirror pretreatment system. Approximately one foot downstream of PAC addition is the location of where activated silica enters the treatment process, and where coagulation begins. Activated silica (an inorganic polymer) is produced by the reaction of sodium silicate and sulphuric acid. Activated silica is a coagulant aid that is usually added after the addition of the coagulant. However, this coagulant aid is sometimes added upstream of where the coagulant is added. The reason for this is that during cold water conditions, lower pretreatment turbidities are achieved if the activated silica is added upstream of where the coagulant is added. During the summer months, the location of the activated silica will not have an affect on the performance of the coagulant. At the inlet of the coagulant tank, polyaluminum sulphate hydroxide chloride (SternPac 50) is added which is the coagulant used in this facility. The coagulant and

activated silica are added to help with the destabilization of large particles and the formation of aggregates. The next step is sand ballasted flocculation (Actiflo^{TM}), in which a certain flocculation aid (Ciba's Magnafloc LT 27AG) is added to agglomerate fine particles and form larger particles or flocs. The flocculation aid helps bind the microsand (weighing agent) to the flocs, so the large particles/flocs can settle down and be removed. Two important feature of the sand ballasted flocculation system is that the settling plates in the actual sedimentation tanks increases the surface area for flocs settling out and the sand used can be recycled during treatment by separating it from the sludge with hydrocyclones. If LT 27AG was not added, the sand and flocs would not stick together, and the settling out of particles would be minimal.

Once the water has left the sedimentation tanks, a concentrated chlorine solution enters the two chlorine contact chambers. In other words, chlorine gas is not bubbled in the chlorine contact chambers. The two chambers are designed to provide a T_{10} (time required for 10% of the water to pass through the chlorine contact chambers) of 23 minutes at a flow rate of 100 ML/day. The concentrated chlorine solution is prepared by mixing chlorine gas and water in the chlorinators. After chlorination, a concentrated ammonia solution is added at the outlet of the chlorine contact chambers for secondary disinfection. Ammoniators are used to mix ammonia gas and water to make a concentrated ammonia solution. In cold water conditions, the concentrated ammonia solution is added at the inlet of the high lift (after the treated water reservoirs). This increases the free chlorine contact time (hours at 100ML/day) and maintains the require chlorine residual at the point of entry into the distribution system.

After disinfection, the water is filtered by 5 filters, in which 3 of them are paired. The filters consist of 0.45 metre of sand and 0.45 metre of anthracite coal. Once the water has passed through the filters, fluoridation takes place. Sodium silicofluoride is added to the

water before it enters one of two on-site storage reservoirs for holding treated water. Fluoride is added to the treated water to help prevent tooth decay for the population it serves. This facility supplies drinking water to a city with a population of 93,000 and a smaller community which has a population of 5,000. The facility is designed to treat 100ML/day, but is restricted by the filters to treat no more than 80ML/day.

At both facilities, chloramination (addition of ammonia) takes place to convert free chlorine to the combined form, which is a more stable disinfectant for maintaining chlorine residuals throughout the distribution system.

Water Sample Collection at Drinking Water Treatment Facilities

Two sampling locations were selected at each facility, a raw water location and a treated water location. All raw water samples were collected before any treatment processes had occurred, and all treated water samples were collected prior to the point of entry into the distribution system. It should be stated that the collection of the raw and treated water samples did not account for retention time within the treatment facilities.

Water samples were collected for pharmaceutical analysis, as well as water chemistry parameters. Due to the analytical and sample collection requirements for these analyses, separate water samples had to be collected for each analysis at all sampling locations.

For Facility A, the raw water samples were collected at low lift pumping station, which is located next to the river. This sampling location is situated before the water enters the storage reservoir and is the one of the sites used by the Ontario Ministry of the Environment for their monitoring programs. The treated water samples were collected after chlorination but prior to the water being blended with the groundwater and anhydrous ammonia being added. This sampling site was selected because it represented the water

produced from the treatment processes located at Facility A. This sampling site was not a sampling location used by the Ontario Ministry of the Environment for their programs, but met the criteria for a representative sampling location.

For Facility B, the raw water samples could not be collected at the screening house because there was no appropriate sampling site. The surface water intake pipe where the raw samples are usually collected is located a bit further down the river from the screening house. This should not be a concern considering this part of the river tends to have a long retention time and studies completed by the facility have shown similar source water characteristics are present at the screening house compared to the surface water intake pipe at the raw water sampling location. The treated water samples were collected just before the water entered the distribution system. Both sampling locations at this facility are used by the Ontario Ministry of the Environment for their monitoring programs.

Sample Collection

Water samples were collected at each sampling location at both facilities on the first Tuesday of each month from April 2005 to March 2006 for water chemistry parameters and pharmaceutical analysis. The only exception was November 2005, in which samples were collected on Tuesday November 15th 2005 from both facilities. In addition, water samples collected in January, February and March 2006 for water chemistry parameters were not analyzed due to the laboratories being renovated.

Water samples were collected at Facility A between 10:00 a.m. to 12:00 p.m. and water samples were collected at Facility B between 1:00 p.m. and 2:30 p.m. on sample collection days.

Duplicate grab water samples were collected for pharmaceutical analysis in one litre pre-cleaned amber glass bottles with Teflon-lined caps (ProClean glass bottles) from Systems Plus (Baden, ON, Canada). These glass bottles were precleaned with laboratory-grade phosphate-free detergent wash, rinsed with acid, rinsed three times with reverse osmosis water, oven dried at 300°C for three hours, capped with a Teflon-lined lid and packed under quality controlled conditions. These bottles were not rinsed and were filled to the shoulder of the bottle. Approximately, 30 to 45 drops of a preservative, sodium thiosulphate 25% (w/v), was added to all water samples. The bottle was shaken to mix the contents. The preservative was added to quench the chlorine present in the sample. Previous preliminary data showed that the present of chlorine in the samples was impacting the signal response of certain analytes. Although no chlorine would be present in the raw water samples, sodium thiosulphate was added to maintain consistency between all samples collected for this type of analysis. The bottles were place in a cooler with ice packs to keep the temperature of the samples between 4 to 10°C during transportation. The samples were place into a fridge with a temperature of approximately 4 to 8°C until transported to the laboratory for sample preparation, extraction and analysis.

For general water chemistry parameters, water samples were collected in 500 mL clear plastic bottles with white lids, with only one sample from each site being extracted and analyzed. The lids and bottles were rinsed three times and then filled to the top. For these samples, no preservatives were added. The bottles were placed in the same cooler as the samples collected for pharmaceutical analysis. A total of 164 water samples were extracted and analyzed for pharmaceuticals and 43 water samples were analyzed for water chemistry parameters.

On sample collection days, general monitoring data was recorded, including daily flow rates, retention time and chemical dosages. Temperature, pH and turbidity were also recorded for the water samples as well as free chlorine, combined chlorine and total chlorine levels for the treated water samples.

Description of the GUDI Well Locations

In addition to water samples being collected from two drinking water treatment facilities, water samples were also collected from groundwater wells. Two deep groundwater reference wells (Well C and Well D) and six susceptible groundwater wells under the direct influence of surface water (referred to as GUDI) (Well A, Well B, Well E to H) were investigated for this study. The susceptible GUDI wells were selected based on monitoring data collected by the Municipality (i.e. the presence of *E. coli* bacteria and fecal coliforms), potential sources of contamination and proximity to surface water. According to the Safe Drinking Water Act, 2002, Ontario Regulation 170/03 (Drinking-Water Systems), GUDI systems (groundwater under the direct influence of surface water) are defined in Section 2 (1-4). The susceptible GUDI wells for this project were selected based on the definitions outlined in Section 2.2.2 in which a drinking water system obtains water from an infiltration gallery and Section 2.2.6 in which the drinking water system exhibits evidence of contamination by surface water (Government of Ontario, 2003).

All eight wells were located in the same watershed as the two drinking water treatment facilities, and each well was visited prior to sample collection to ensure the sampling location met the criteria for a representative sampling location. These wells had on-line monitoring analyzers for turbidity, pH, temperature and free chlorine levels. For the most part, UV irradiation, chlorination and chloramination were used for disinfection purposes.

Most of the susceptible GUDI wells were located in rural areas, with the exception of Well G, which was located in an urban area. Well A and Well B were both located in small rural communities and found near surface waters. Well E and Well F were located close to each other and to a small creek. Both of these wells were partly surrounded by agricultural fields. Well G was located in an undeveloped flood plain of the river and close to a new housing development. Well H was located in a small rural community and consisted of 4 submersed wells which formed an infiltration gallery and fed a common header. Well H was also located downstream of a seasonal trailer park (i.e. source of potential contamination), and about 30 feet from the river bed (i.e. in the flood plain of the river). Three of the four wells were only 3 metres deep and the other one was 2.6 metres deep.

Water Sample Collection at GUDI Well Locations

In contrast to the water samples collected at the two drinking water treatment facilities, only raw water samples were collected from each well. All raw water samples were collected before any treatment processes had occurred at the well locations.

Water samples were collected for pharmaceutical analysis, as well as water chemistry parameters. Due to the analytical and sampling collection requirements for these analyses outlined by the Ontario Ministry of the Environment, separate water samples had to be collected for each type of analysis at all sampling locations.

Sample Collection

Duplicate grab raw water samples were collected from each well location on Tuesday September 27th 2005 and again on Tuesday October 11th 2005 for pharmaceutical and water chemistry analysis. One of the reasons for taking samples during the fall season was to capture time periods when these susceptible wells would be under the greatest impact of

potential contamination, with the spreading of livestock manure and biosolids to agricultural land, and changes in water quality and water quantity parameters. Raw water samples were collected at all eight well locations between 9:00 a.m. and 2:30 p.m. on sample collection day. A total of 32 raw water samples were collected and analyzed for pharmaceuticals, and 16 samples were analyzed for water chemistry parameters.

The raw water samples for the GUDI study were collected in a similar way as described for the collection of water samples at the drinking water treatment facilities.

2.3.4 Sample Analysis – Water Chemistry Parameters

Water samples collected for general water chemistry parameters were transported in coolers at approximately 4°C to 10°C, and extracted within 10 days of being submitted to the licensed Ontario Ministry of the Environment Laboratory in Etobicoke, Ontario. A number of different analytical methods were used to determine the general water chemistry parameters (Ontario Ministry of the Environment Laboratory Services Branch, 2005). Colourimetry techniques were used to determine the presence of chloride, true colour, ammonium nitrogen, nitrite nitrogen, nitrite plus nitrate nitrogen, reactive phosphate, total kjeldahl nitrogen, total phosphate, molybdate reactive silicates and dissolved carbon. Sulphates and fluoride concentrations were determined by automated ion chromatography, and the amount of solids present was determined by gravimetry techniques. Atomic absorption spectrophotometry was used to determine the presence of cations in the water samples, and conductivity, pH, and alkalinity were determined by potentiometry techniques. The turbidity of the water was determined by nephelometry under robotic control.

2.3.5 Sample Analysis – Pharmaceuticals

Sample Preparation

The water samples collected for pharmaceutical analysis were transported in coolers at temperatures between 4°C and 10°C to the laboratory. The samples were extracted within 24 to 36 hours of being collected.

Before sample preparation began, the water samples were allowed to warm up to room temperature. A sample volume of 400 mL was measured out and transferred into a 1 L precleaned glass amber bottle with a Teflon-lined cap. In addition to the field water samples being prepared, three 1 L precleaned glass amber bottles with 400 mL of nanopure water were also prepared. One of the three bottles was used as a blank sample and the other two bottles were used for spiked nanopure water samples for quality control and quality assurance (QC/QA). Sodium thiosulphate 25% (w/v) was added as a preservative to both the blank and spiked samples. This was done to maintain the consistency of how the field samples were prepared. Approximately 15 to 23 drops of sodium thiosulphate was added to the blank and spiked samples. For the field water samples, no further sodium thiosulphate was added as the preservative was already added at the time of collection.

For all field water samples and the blank sample, 0.5 mL of the mixed surrogate standard solution was added to each bottle. For the two spiked samples, 0.5 mL of spiking solutions and 0.5 mL of the mixed surrogate standard solution was added. The spiking solutions consisted of known concentrations of all analytes. Approximately, 2 g of EDTA, a chelating agent, was added to the sample bottles. The samples were placed on a roller for 10 to 15 minutes to help dissolve EDTA. After EDTA was dissolved, 10 mL of 0.25 M ammonium acetate solution was added to each bottle as a buffer. The bottles were shaken and

the pH of the all samples was adjusted to 6.95 ± 0.05. The pH of the sample was adjusted using a solution of 5% (w/v) sodium hydroxide in nanopure water to increase the pH or a 10% (v/v) solution of sulphuric acid (H_2SO_4) was added to lower the pH. The final pH for each sample was recorded.

Sample Extraction

The water samples were extracted using solid phase techniques. The goal of this technique was to clean-up and pre-concentrate the water samples for liquid chromatography and mass spectrometry analysis. For each extraction set, a 12-port Visiprep™ vacuum manifold manufactured by Supelco Inc. (Bellefonte, PA, USA) and Waters Oasis® HLB cartridges (6cc, 200mg) (Milford, MA, USA) were used for extracting the samples. The sorbent in the SPE cartridges consisted of a copolymer designed to have hydrophilic and hydrophobic properties, and therefore could be used to extract a board spectrum of compounds with different chemical and physical properties. The sorbent consisted of a copolymer of divinylbenzene and vinylpyrrolidone (Richardson and Ternes, 2005).

Before the samples were extracted, the cartridges were preconditioned with 5 mL of nanopure water, followed by 5 mL of methanol and 5 mL of 5% (v/v) of ammonium hydroxide in methanol. After the cartridges were conditioned, 5 mL of nanopure water was added to each cartridge to prevent the sorbent from drying. During the conditioning process, the flow rate was approximately 5 mL/min.

Teflon tubing with dimensions of 3 mm inner diameter and 4 mm outer diameter and a length of 60 mm was precleaned in a mixture of methanol and nanopure water. The Teflon tubing and adapters were purchased from Supelco Inc. (Bellefonte, PA, USA) and were used to introduce the sample into the SPE cartridges. The free end (with the stainless steel weight)

of each Teflon tube was placed into a sample bottle. The other end of the tube with the adapter was placed onto the top of the SPE cartridge. Once the tubing was connected, the valves of the manifold were opened and the vacuum was turned on. The individual valves of the vacuum manifold were adjusted to maintain a steady drip from each cartridge, with a flow rate of approximately 5 to 10 mL/min and a vacuum pressure not exceeding -20" Hg. After the sample had gone through the cartridge, the cartridge was dried by applying the vacuum for additional 5 to 10 min.

A wash solution of 5% methanol in nanopure water (a volume of 5 mL) was added to each cartridge. The washing solution was used to remove weakly bound impurities from the sorbent of the SPE cartridge. The valves of the manifold were opened and the vacuum was applied to allow the wash solution to go through the cartridges. In order to dry the cartridge as much as possible, the vacuum was left on for about 15 min.

Sample Elution

Labeled 15 mL polypropylene sterile culture tubes were placed in the appropriate slots of the Visiprep^{TM} collection rack. The rack was placed into the manifold and the cover was placed on top.

The contents bound to the sorbent of the cartridge were eluted with 5 mL of methanol. The vacuum was turn on to start the elution process, in which 1 mL was slowly eluted. After 1 mL had been eluted, the vacuum was turned off and methanol was allowed to sit in the cartridges for approximately 1 min. The remaining methanol passed through the cartridge by gravity. This elution process took approximately 15 minutes. Once there were no more drops coming from the bottom of the cartridge, the vacuum was turned on slowly to aspirate the remaining methanol out the cartridges. The manifold cover was lifted and the collection rack

containing the tubes with the final extracts was removed. Pasteur pipettes were used to mix the contents in each tube and 1 mL of each final extract was transferred to a clean 2 mL clear glass vial with PTFE/Sil/PTFE lined cap purchased from Life Science (Peterborough, ON, Canada).

Sample Evaporation

The 1 mL extracts were evaporated using a gentle stream of nitrogen gas. Nitrogen gas was produced by a nitrogen generator from Whatman Canada (Mississauga, ON, Canada), and was controlled by a regulator. For the samples collected from April 2005 to September 2005, an in-house nitrogen gas evaporator apparatus was used to evaporate the samples to dryness. For the remaining sampling months, a solvent evaporation system was used. The SE 500 system from Dionex Canada Inc. (Oakville, ON, Canada) decreased the amount of time needed for evaporating the sample extracts.

In addition to the sample extracts being evaporated, calibration standards and control standards had to be evaporated for analysis. The control standards were prepared by adding 0.3 mL of the spiking solutions plus 0.3 mL of the spiking deuterated surrogate solution into a 2 mL vial. The standards were evaporated to dryness using nitrogen gas. For the preparation of the calibration standards for analysis, 0.2 mL of each calibration standard along with 0.2 mL of the spiking deuterated surrogate solution was added to each vial. The contents were evaporated to dryness in a similar way as the sample extracts and control standards.

The final step before instrumentation analysis was reconstituting the evaporated samples and standards with the internal standard solution. The amount of internal standard solution added to the vial was in portion to the volume that was evaporated. For the sample extracts, 100 µL of the working internal standard solution was added to the vial, and for the

calibration standards 200 µL of the internal standard solution was added. The purpose of the internal standard solution was to monitor and correct for possible sample injection problems into the instrument and to quantify the unknown concentrations in the field samples.

The evaporated contents in the vial were mixed with the internal standard solution using a Pasteur pipette and then the solution was placed into a glass vial insert (Life Science, Peterborough, ON, Canada) and recapped. The vials were placed into the freezer until analysis. The samples were usually analyzed within one week of being extracted.

2.3.6 LC-ESI-MS/MS Instrumentation

All samples were analyzed using a liquid chromatography with electrospray ionization tandem mass spectrometer (LC-ESI-MS/MS). The analytes were separated by an Agilent Hewlett Packer 1100 Series liquid chromatograph system (Mississauga, ON, Canada), which consisted of a vacuum degasser, binary pump, column compartment and an autosampler. The Agilent autosampler was replaced with a CTC Analytics HTC PAL autosampler (LEAP Technologies, Carrboro, NC, USA) in May of 2005. The new autosampler was used for the samples collected from May 2005 to March 2006. The injection volume for April 2005 was 15 µL, but was increased to 20 µL for the remaining sampling months when the new autosampler was installed. A ThermHyperSil Gold RP-C_{18} (2.0 mm × 100 mm, particle size of 3 µm) separation column was used for all analyses and maintained at room temperature.

The analytes were detected using an Applied Biosystems/MDS Sciex 4000 Q Trap™ mass spectrometer with an electrospray ionization source interface (TurboIonSpray™). The 4000 Q Trap™ mass spectrometer is a hybrid triple quadrupole and linear ion trap (LIT) instrument, in which the second mass analyzer or Q3 region can be operated as a standard quadrupole mass spectrometer or have the capability of being used as linear ion trap mass

spectrometer. For this project, the Q3 region was operated as a quadrupole mass analyzer, and multiple reaction monitoring (MRM) was selected as the scan mode to quantify compounds present in the water samples. The mass spectrometer was operated with unit resolution for both mass analyzers.

The target pharmaceuticals investigated for the project were grouped according to analytical method used, and are summarized in Table 2.1.

Analysis of Group A Pharmaceuticals

The pharmaceuticals in Group A consisted of five tetracyclines, three fluoroquinolones, three macrolides, seven sulfonamides, two anti-inflammatory agents (ketoprofen and naproxen), carbamazepine, monensin sodium salt, penicillin G sodium salt, trimethoprim and tylosin tartrate. The compounds in Group A were analyzed with the electrospray ionization source operated in positive ionization mode. The flow rate was 0.2 mL/min and 0.03% heptafluorobutryic acid (HFBA) (mobile phase A) and 100% HPLC grade acetronitrile (mobile phase B) was used for the binary gradient elution. The mobile phase gradient elution was as follows: at 0 min A/B was 85:15, at 13 min and 15 min A/B was 0:100, at 17 min A/B was 85:15 and remained at this gradient until the end of the 28 min run. Details on the MRM ion transitions, collision energies and dwell times for analytes in Group A are summarized in Table 2.2.

Analysis of Group B Pharmaceuticals

The pharmaceuticals in Group B consist of three anti-inflammatory agents (ibuprofen, diclofenac sodium salt and indomethacin), two lipid regulating agents (bezafibrate and gemfibrozil), a metabolite of clofibrate (clofibric acid), 4-acetamidophenol, carbadox,

Table 2.1: List of pharmaceuticals (including deuterated surrogates and internal standard) evaluated in the water samples collected from two drinking water treatment facilities and groundwater wells over a 12 month period.

Group A*	Group B**
Carbamazepine	**4-Acetamidophenol**
Chlortetracycline HCl	Bezafibrate
Ciprofloxacin	Carbadox
Doxycycline HCl	Chloramphenicol
Enrofloxacin	Clofibric acid
Erythromycin	**Diclofenac sodium salt**
Ketoprofen	Gemfibrozil
Lincomycin HCl	Ibuprofen
Meclocycline sulfosalicylate salt	Indomethacin
Monensin sodium salt	Lasalocid A
Naproxen	Sulfadiazine sodium salt
Norfloxacin	Warfarin, minimum
Oxytetracycline HCl	Virginiamycin M1
Penicillin G sodium salt	D_4-4-Acetamidophenol[a]
Roxithromycin	D_4-Diclofenac[a]
Sulfachloropyridazine	D_6-Gemfibrozil[a]
Sulfamethoxine	D_3-Ibuprofen[a]
Sulfamerazine	$^{13}C_6$-Sulfamethazine phenyl[b]
Sulfamethazine	
Sulfamethizole	
Sulfamethoxazole	
Sulfathiazole	
Tetracycline	
Trimethoprim	
Tylosin tartrate	
D_{10}-Carbamazepine[a]	
$^{13}C_6$-Sulfamethazine phenyl[b]	

*Group of pharmaceuticals analyzed in positive electrospray ionization mode
** Group of pharmaceuticals analyzed in negative electrospray ionization mode
[a]Deuterated surrogates
[b]Internal standard

Table 2.2: Multiple reaction monitoring (MRM) ion transitions, dwell time, and collision energy for each pharmaceutical of Group A.

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Dwell Time (msec)	Collision Energy (eV)
Carbamazepine	237	194	10	20
Erythromycin	734	158	25	32
Ketoprofen	255	105	20	20
Lincomycin hydrochloride	407	126	25	32
Naproxen	231	185	20	20
Penicillin G sodium salt	335	176	25	25
Roxithromycin	838	158	25	40
Sulfachlorpyridazine	285	156	15	20
Sulfadimethoxine	311	156	15	30
Sulfamerazine	265	156	15	23
Sulfamethazine	279	186	15	23
Sulfamethizole	271	156	15	20
Sulfamethoxazole	254	156	15	21
Sulfathiazole	256	156	15	20
Trimethoprim	291	123	10	30
Tylosin tartrate	916	174	20	50
$^{13}C_6$-Sulfamethazine phenyl	285	186	20	25
D_{10}-Carbamazepine	247	204	10	25

chloramphenicol, lasalocid A, a sulfonamide (sulfadiazine sodium salt), an anti-coagulant agent (warfarin) and a macrolide (virginiamycin M1). The compounds in Group B were analyzed with the electrospray ionization source operated in negative ionization mode. The flow rate was 0.18 mL/min and 10 mM ammonium acetate (mobile phase A) and 100% HPLC grade acetronitrile (mobile phase B) was used for the binary gradient elution. The mobile phase gradient elution was as follows: at 0 min A/B was 90:10, at 15 min A/B was 20:80, at 18 min A/B was 90:10 and remained at this gradient until the end of the 28 min run. Details on the MRM ion transitions, collision energies and dwell times for analytes in Group B are summarized in Table 2.3.

The operating parameters for the ionization source and mass spectrometer are summarized in Table 2.4 for both analytical methods and were optimized when tuning the instrument during method development.

2.3.7 Data Analysis

The Analyst® software (version 1.4.1) was used to optimize the source and compound-dependent parameters, control the instrument, and perform data analysis. In addition to the field samples being analyzed, calibration standards, spiked nanopure water samples, blank samples and control samples were also analyzed. The calibration standards and control standards did not go through the extraction process, but were evaporated and reconstituted with the internal standard solution ($^{13}C_6$-sulfamethazine phenyl in nanopure water) before injection. In contrast to other analytical methods, the calibration standards and control standards were prepared by spiking the analytes into a solvent solution, instead of spiking the analytes into a matrix solution.

Table 2.3: Multiple reaction monitoring (MRM) ion transitions, dwell time, and collision energy for each pharmaceutical of Group B.

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Dwell Time (msec)	Collision Energy (eV)
Bezafibrate	360	274	25	-25
Carbadox	261	122	35	-25
Chloramphenicol	321	152	25	-25
Clofibric acid	213	127	35	-20
Gemfibrozil	249	121	35	-15
Ibuprofen	205	161	40	-10
Indomethacin	356	312	25	-15
Lasalocid A	589	235	10	-45
Sulfadiazine sodium salt	249	185	15	-25
Warfarin, minimum	307	161	10	-25
Virginiamycin M1	524	245	35	-25
$^{13}C_6$-Sulfamethazine phenyl	283	122	20	-30
D_6-Gemfibrozil	255	121	15	-15
D_3-Ibuprofen	208	164	20	-10

Table 2.4: Summary of electrospray ionization (ESI) source and mass spectrometer parameter values for pharmaceuticals analyzed in positive ionization mode and negative ionization mode.

ESI-MS/MS Parameter	Group A	Group B
Nebulizing Gas	35 psi	35 to 45 psi
Desolvation Gas	45 psi	30 to 45 psi
Source Temperature	500°C	400 to 500°C
Curtain Gas	10 to 12 psi	10 to 12 psi
IonSpray Voltage	5000 to 5200 V	4500 V
Collisionally Activated Dissociation (CAD)	5 to 8 psi	5 to 8 psi
Declustering Potential (DP)	60 V	-80 to -90 V
Entrance Potential (EP)	10 V	-10 V
Collision Cell Exit Potential (CXP)	10 V	-3 to -5 V

Calculation of Recovery Rates

Recovery rates for all analytes were determined by comparing the signal response of the analyte in the spiked nanopure water samples to the average signal response of the analytes in the control standards. The recovery rate of the spiked analytes in the nanopure water samples determined the extraction efficiency of each analyte in water samples.

Recovery rates were also determined for the five deuterated surrogates in the spiked nanopure water samples and field samples. The recovery rates for the deuterated surrogates in the spiked and field water samples were determined the same way as for the analytes in the spiked nanopure water samples.

Quantification

Three quantification approaches were used to determine and compare the concentrations of the selected pharmaceuticals in the water samples. Two different internal standard calibration approaches were used. The original quantification method involved the use of one internal standard, $^{13}C_6$-sulfamethazine phenyl, for determining the unknown concentrations of the analytes in the field samples. A known concentration of the internal standard was added to the field samples, calibration standards and other QA/QC samples, before the samples were injected into the instrument. This internal standard was used in quantifying all analytes in Group A and Group B. Since this internal standard was only added prior to being analyzed, this compound did not correct for extraction efficiencies, but was used as an indicator for instrument sensitivity during the run sequence and corrected for sample injection problems.

A multi-point calibration curve was used to quantify the unknown concentrations of the analytes in the field samples. For each analyte, a separate calibration curve was plotted.

A peak area (signal response) ratio of analyte peak area to $^{13}C_6$-sulfamethazine phenyl peak area (peak $area_{analyte}$/peak $area_{interalstd}$) for each calibration standard (ratio on the x-axis) and a concentration ratio of a known analyte concentration and known $^{13}C_6$-sulfamethazine phenyl concentration for each calibration standard (ratio on the y-axis) were plotted. A linear through zero regression was used because it was the line of best fit for the concentrations in the calibration standards and showed good linearity with correlation coefficients usually greater than 0.98. The unknown concentrations in the field samples were determine from the linear through zero regression line, and appropriate dilution factors were applied to determine the final concentration in the sample.

The second internal calibration approach was similar to the first quantification method, except deuterated surrogates (D_{10}-carbamazepine, D_6-gemfibrozil and D_3-ibuprofen) were used instead of $^{13}C_6$-sulfamethazine phenyl. In addition, this method allowed for extraction efficiency to be addressed, since the deuterated surrogates were added before extraction. A multi-point calibration curve was used to determine the concentrations of carbamazepine, gemfibrozil and ibuprofen in the field samples. A linear through zero regression line was applied to the concentrations and peak areas of the surrogates in the calibration standards. Details of the use of the deuterated surrogates in quantifying the corresponding analytes in the water samples are documented in Chapter 3. Concentrations of diclofenac and 4-acetamidophenol was not quantified using this approach because these compounds were eventually removed from the final list of analytes to investigate (Section 2.4). For the remainder of the analytes, this quantification method could not be applied because deuterated surrogates for these analytes were not commercially available at the time of developing this analytical method.

The last quantification method used in the study was an external solvent calibration approach. For this method, the calibration curve is simplified, in which the analyte concentration and the peak area of the analyte for each calibration standard is plotted to form the calibration curve. Linear through zero regression was used, and the unknown concentrations in the field samples were determined from the calibration curve. Since no standards were added prior to extraction, extraction efficiency was not addressed.

The three quantification methods were applied to each analyte, if applicable, over the sampling period to compare the final concentrations reported for each quantification method. The purpose was to investigate how final concentrations varied depending on the type of quantification method used, and if matrix effects were addressed by these methods.

2.4 Results

2.4.1 Final List of Target Analytes

The original list of analytes to be investigated for this project was 38 analytes (Table 2.1). However, the list of analytes was redefined to a total of 27 based on low recovery rates, missing peaks and carry-over issues observed during sample analysis. The analytes highlighted in Table 2.1 were the analytes that were not quantified and removed from the target analyte list. The analytes removed from the list included the five tetracyclines (chlortetracycline HCl, doxycycline HCl, meclocycline sulfosalicylate salt, oxytetracycline HCl and tetracycline), the three fluoroquinolones (ciprofloxacin, enrofloxacin and norfloxacin), 4-acetamidophenol, diclofenac sodium salt and monensin sodium salt.

For the five tetracyclines and three fluoroquinolones, the peaks in the chromatograms showed excessive tailing and split peaks. In some cases, the recovery rates for these compounds were approaching 200% or as low of 20% in the spiked nanopure water samples.

These compounds were also present in the blank samples and solvent samples, which suggest carry-over issues.

4-Acetamidophenol was also removed from the target list because the recovery rates for this compound were quite low. For 4-acetamidophenol and its deuterated surrogate, the highest recovery rates observed was usually 50%.

Diclofenac sodium salt and monensin sodium salt were not included in the final list of analytes because these compounds were present in the blank and solvent samples during analysis, and variability was observed in the recovery rates between the two spiked nanopure water samples and between duplicate field samples, with recovery rates approaching to 200%.

2.4.2 Recovery Rates

The recovery rates of the 27 analytes in the spiked nanopure water samples averaged between 80% to 120% for most sampling months (data no shown). The recovery rates for the deuterated surrogates and $^{13}C_6$-sulfamethazine phenyl showed similar results. However, the recovery rates of the deuterated surrogates added to the field samples showed a large variability in recovery rates over the sampling period, from 50% to over 200% recovery in some cases (Table 2.5). In general, the recovery rates for the deuterated surrogates in the field samples analyzed in negative ionization mode were more variable compared to D_{10}-carbamazepine, which was analyzed in positive ionization mode. This was observed with the high recovery rates of D_6-gemfibrozil and D_3-ibuprofen in the raw and treated water samples (Table 2.5).

Perhaps, the most interesting results from the recovery rate data was the variability observed for the internal standard, $^{13}C_6$-sulfamethazine-phenyl, in the field samples. Figure

Table 2.5: Range of recovery rate percentages (%) of D_{10}-carbamazepine, D_6-gemfibrozil and D_3-ibuprofen in the raw and treated water field samples for each sampling month

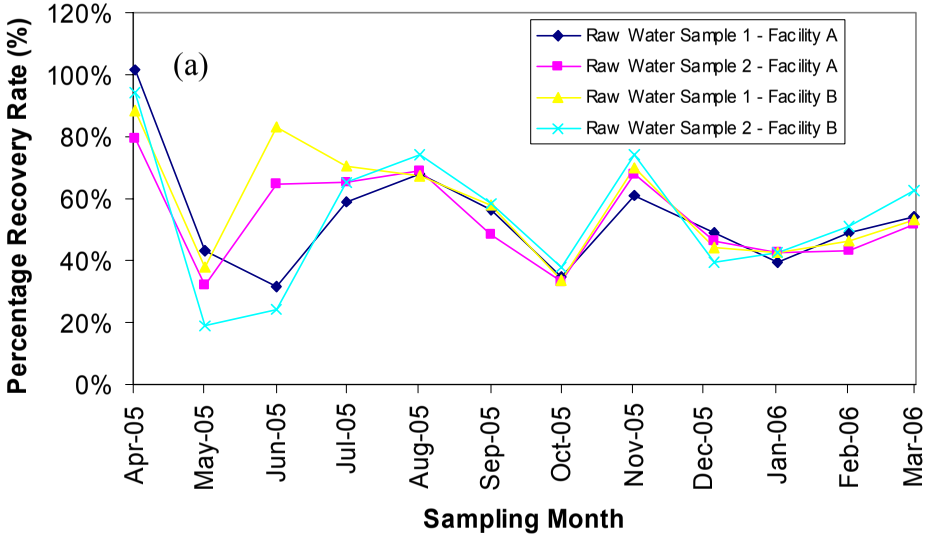
Sampling Month	D_{10}-carbamazepine		D_6-gemfibrozil		D_3-ibuprofen	
	Raw	**Treated**	**Raw**	**Treated**	**Raw**	**Treated**
April 2005	50-56	52-58	92-117	99-108	160-190	187-226
May 2005	70-80	78-85	72-90	91-102	175-198	187-234
June 2005	63-66	37[b]-88	52-58	66-83	83-95	104-139
July 2005	70-80	77-91	60-74	79-104	96-105	129-162
August 2005	83-88	83-98	92-109	89-99	178-198	182-221
September 2005	50-51	52-74	101-136	123-162	193-242	170-267
October 2005	79-81	77-85	108-121	151-158	176-211	238-382
November 2005	74-82	79-93	114-185	151-181	153-189	150-184
December 2005	49-64	63-72	115-146	135-140	177-211	201-256
January 2006	61-66	61-75	93-104	116-136	97-108	120-145
February 2006	71-73	47[b]-82	101-108	107-115	89-123	101-108
March 2006	57-59	57-68	107-114	108-116	122-130	131-149

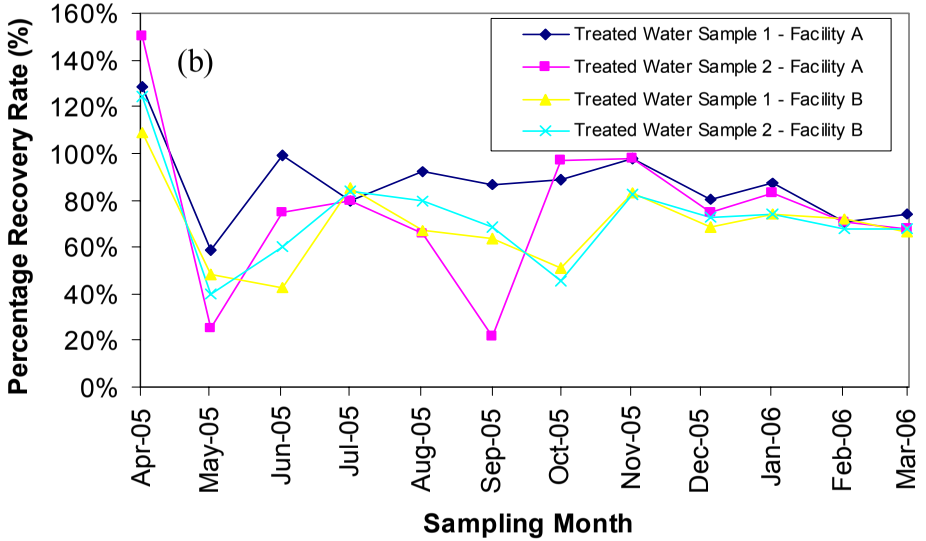
[b]The lowest recovery rate is due to volume injection problems

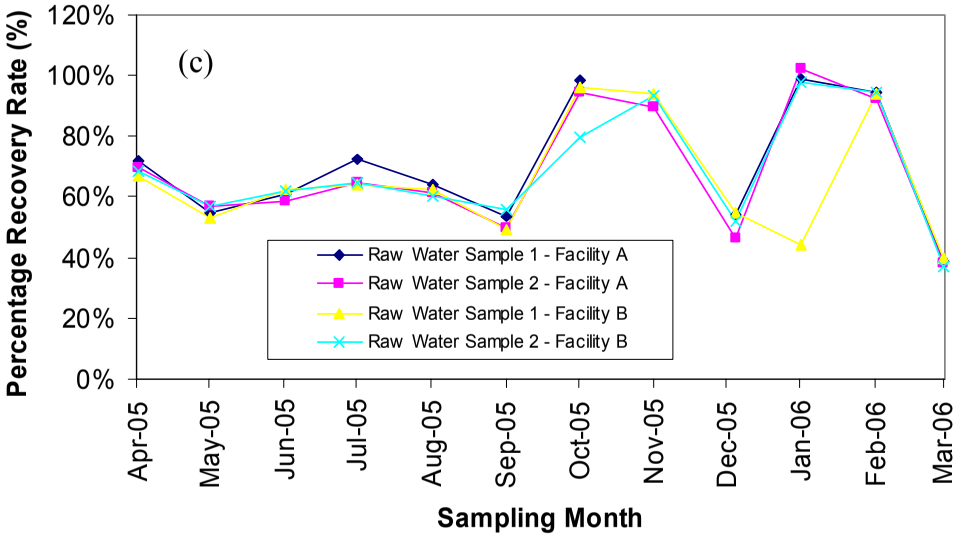
2.2 illustrates the range of recovery rates for the internal standard over the sampling period when 400 mL raw and treated water samples were extracted. The variability was more pronounced when the samples were analyzed in negative ionization mode (Figure 2.2 (a) and (b)), with recovery rates ranging from 20% up to 150%. The range of recovery rates for the internal standard analyzed in positive ionization mode was not as variability with recovery rates between 60% to 100% in the raw and treated water samples (Figure 2.2 (c) and (d)). The graphs in Figure 2.2 also illustrate the consistency of the recovery rates of the internal standard between the four water samples collected each month. This consistency is well displayed in Figure 2.2 (c), where the recovery rates for the internal standard detected in all raw water samples were similar to each other during each month. This suggests that the internal standard was impacted to a similar degree in all raw or treated water samples from both facilities.

The suppression of the internal standard was clearly demonstrated when 800 mL sample volume was extracted. Figure 2.3 show the recovery rates of the internal standard in the 800 mL extracted water samples. The recovery rates in the raw water samples were below 14% when the internal standard was analyzed in negative ionization mode and up to 30% when the internal standard was analyzed in positive ionization mode. Higher recovery rates (up to 50%) of the internal standard were observed in the treated water samples, with higher recovery observed when the internal standard was analyzed in positive ionization mode.

Figure 2.2: Recovery rates (%) of $^{13}C_6$-sulfamethazine phenyl in 400 mL extracted water samples collected from both water treatment facilities from April 2005 to March 2006 when analyzed in negative ionization mode (a) and (b) and positive ionization mode (c) and (d).


(a)
Raw Water Sample 1 - Facility A
Raw Water Sample 2 - Facility A
Raw Water Sample 1 - Facility B
Raw Water Sample 2 - Facility B
Percentage Recovery Rate (%)
120%
100%
80%
60%
40%
20%
0%
Apr-05
May-05
Jun-05
Jul-05
Aug-05
Sep-05
Oct-05
Nov-05
Dec-05
Jan-06
Feb-06
Mar-06
Sampling Month


(b)
Treated Water Sample 1 - Facility A
Treated Water Sample 2 - Facility A
Treated Water Sample 1 - Facility B
Treated Water Sample 2 - Facility B
Percentage Recovery Rate (%)
160%
140%
120%
100%
80%
60%
40%
20%
0%
Apr-05
May-05
Jun-05
Jul-05
Aug-05
Sep-05
Oct-05
Nov-05
Dec-05
Jan-06
Feb-06
Mar-06
Sampling Month


(c)
Raw Water Sample 1 - Facility A
Raw Water Sample 2 - Facility A
Raw Water Sample 1 - Facility B
Raw Water Sample 2 - Facility B
Percentage Recovery Rate (%)
120%
100%
80%
60%
40%
20%
0%
Apr-05
May-05
Jun-05
Jul-05
Aug-05
Sep-05
Oct-05
Nov-05
Dec-05
Jan-06
Feb-06
Mar-06
Sampling Month

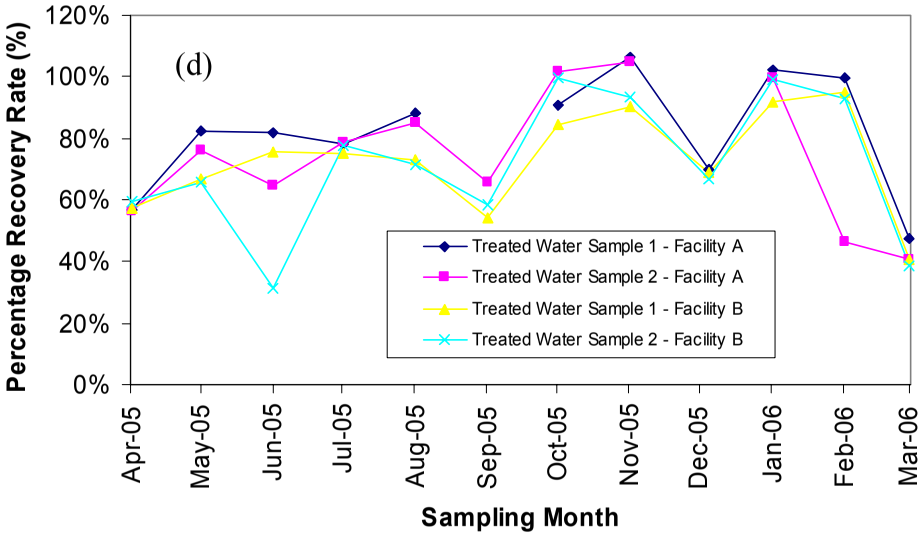
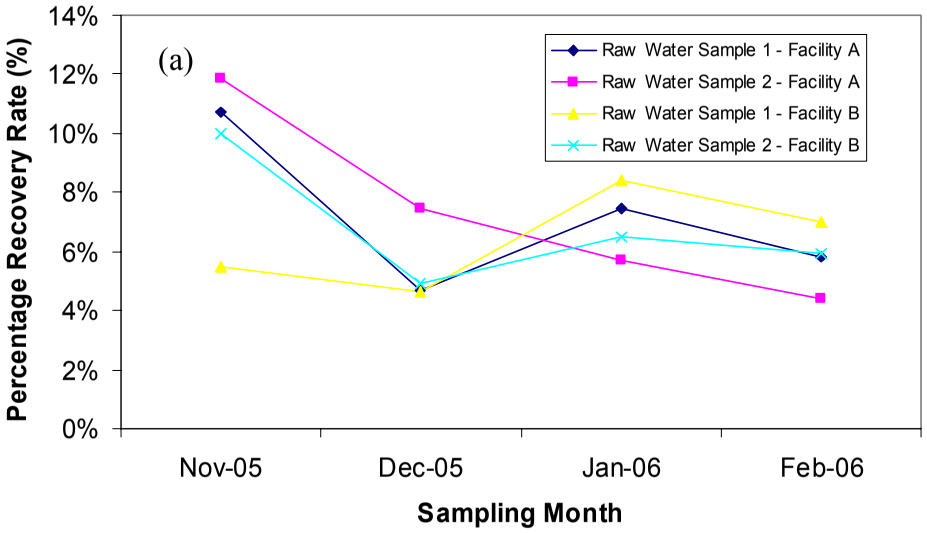
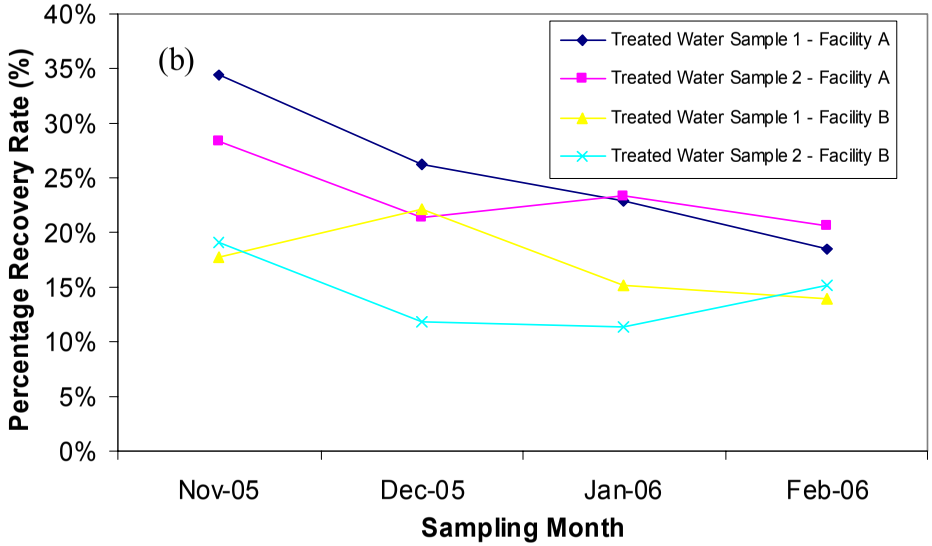
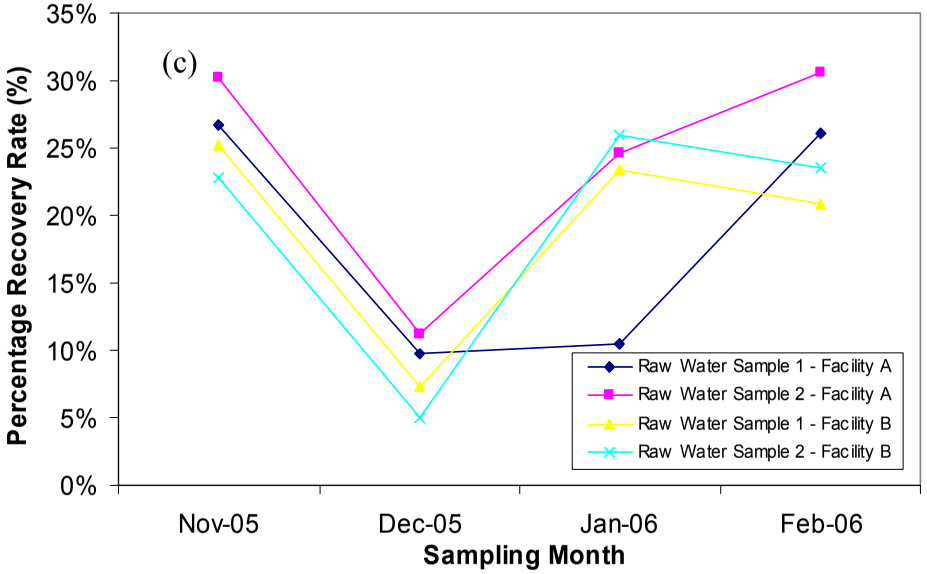
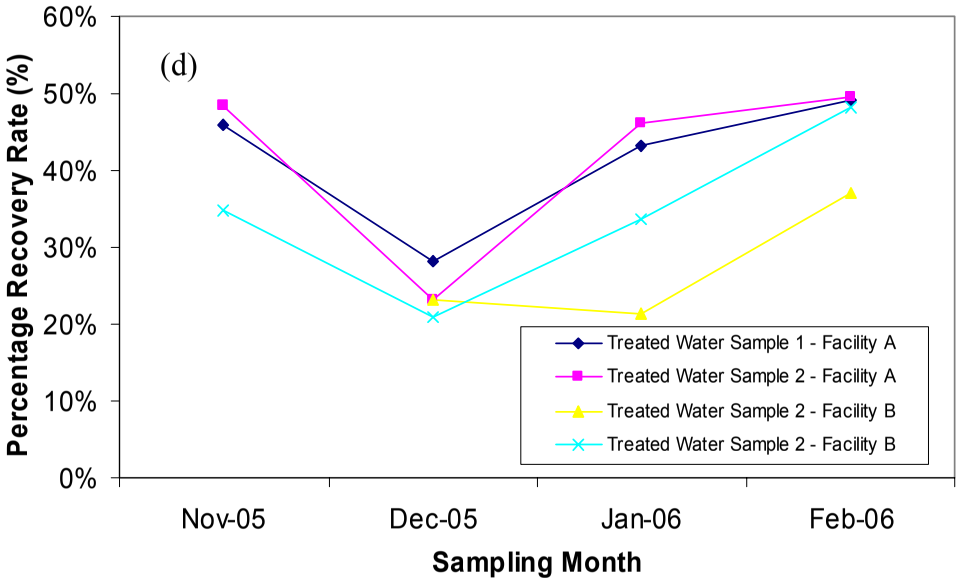

(d)
Treated Water Sample 1 - Facility A
Treated Water Sample 2 - Facility A
Treated Water Sample 1 - Facility B
Treated Water Sample 2 - Facility B
Percentage Recovery Rate (%)
120%
100%
80%
60%
40%
20%
0%
Apr-05
May-05
Jun-05
Jul-05
Aug-05
Sep-05
Oct-05
Nov-05
Dec-05
Jan-06
Feb-06
Mar-06
Sampling Month

Figure 2.3: Recovery rates (%) of $^{13}C_6$-sulfamethazine phenyl in 800 mL extracted water samples collected from both water treatment facilities from November 2005 to February 2006 when analyzed in negative ionization mode (a) and (b) and positive ionization mode (c) and (d).


(a)
Raw Water Sample 1 - Facility A
Raw Water Sample 2 - Facility A
Raw Water Sample 1 - Facility B
Raw Water Sample 2 - Facility B
Percentage Recovery Rate (%)
14%
12%
10%
8%
6%
4%
2%
0%
Nov-05
Dec-05
Jan-06
Feb-06
Sampling Month


(b)
Treated Water Sample 1 - Facility A
Treated Water Sample 2 - Facility A
Treated Water Sample 1 - Facility B
Treated Water Sample 2 - Facility B
Percentage Recovery Rate (%)
40%
35%
30%
25%
20%
15%
10%
5%
0%
Nov-05
Dec-05
Jan-06
Feb-06
Sampling Month


(c)
Raw Water Sample 1 - Facility A
Raw Water Sample 2 - Facility A
Raw Water Sample 1 - Facility B
Raw Water Sample 2 - Facility B
Percentage Recovery Rate (%)
35%
30%
25%
20%
15%
10%
5%
0%
Nov-05
Dec-05
Jan-06
Feb-06
Sampling Month


(d)
Treated Water Sample 1 - Facility A
Treated Water Sample 2 - Facility A
Treated Water Sample 2 - Facility B
Treated Water Sample 2 - Facility B
Percentage Recovery Rate (%)
60%
50%
40%
30%
20%
10%
0%
Nov-05
Dec-05
Jan-06
Feb-06
Sampling Month

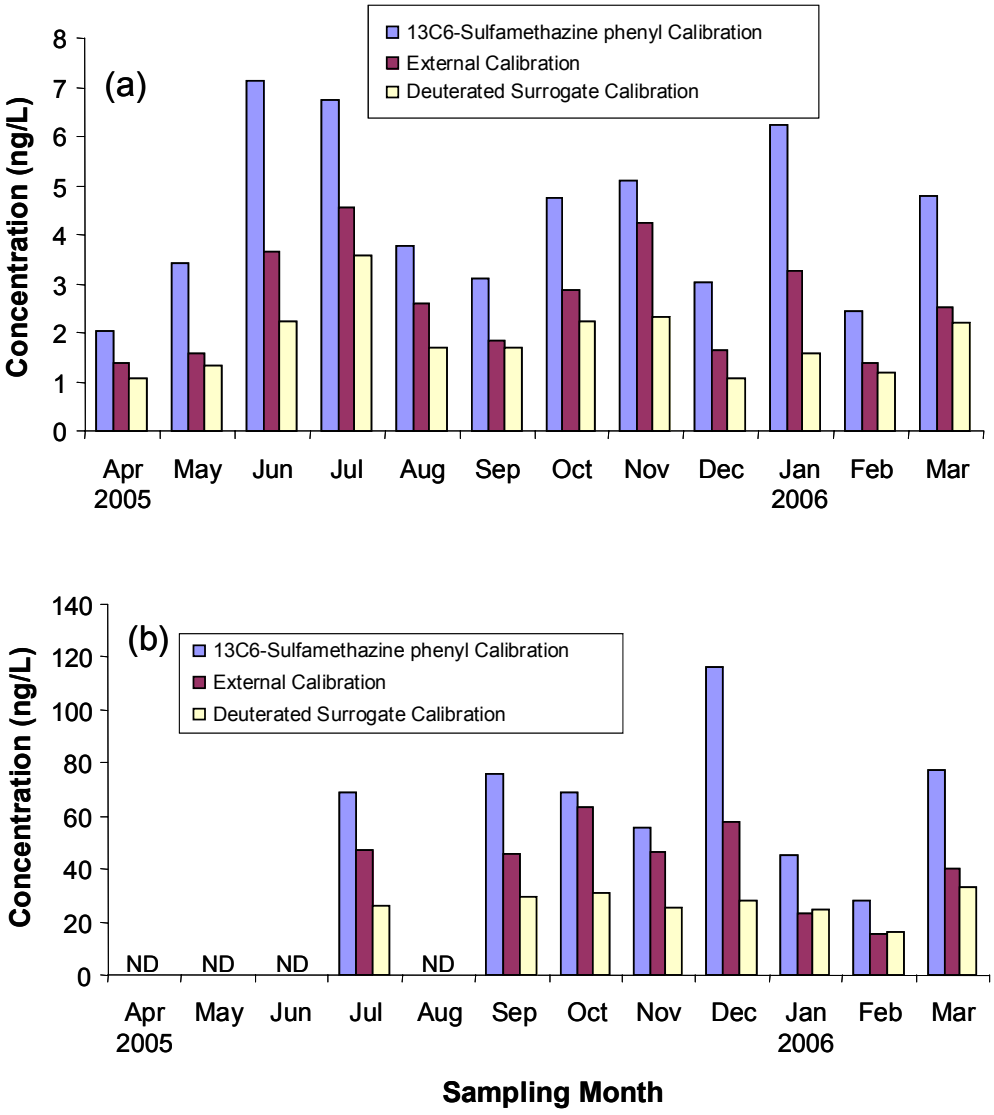
2.4.3 Quantification of Analytes

The final concentrations for all analytes were quantified using different calibration approaches, either an external solvent calibration or an internal standard calibration. For carbamazepine, gemfibrozil and ibuprofen, final concentrations were quantified by an additional method, in which deuterated surrogates were used as internal standards. At the time of method development, few isotopically labeled standards were available. The final concentrations quantified by the use of isotopically labeled standards for the three human pharmaceuticals were compared to the other two methods to determine the variability in final concentrations reported if matrix effects are not addressed and inappropriate calibration approaches are implemented.

Figure 2.4 demonstrates the final mean concentrations detected for gemfibrozil and ibuprofen in raw water samples collected from Facility A when the three different quantification methods were applied. The use of $^{13}C_6$-sulfamethazine-phenyl was found to overestimate the concentrations of gemfibrozil and ibuprofen to the greatest extent compared to the external calibration method and the use of deuterated surrogates as internal standards. The seasonal trends observed, for the most part, appeared to remain the same between the three methods, and therefore seasonal variability was not highly impacted by the quantification method applied.

Similar to the results observed for the recovery rates, the ionization mode played a role in the extent of how the different quantification methods impacted the final concentrations reported. In contrast, to the variability observed in the concentrations of gemfibrozil and ibuprofen with applying the different quantification methods, the concentrations of

Figure 2.4: Mean concentrations (ng/L) of gemfibrozil (a) and ibuprofen (b) in raw water samples collected from Facility A comparing the use of $^{13}C_6$-sulfamethazine-phenyl as the internal standard for quantification, external calibration quantification and using the deuterated surrogates as internal standards for quantification (n=2).

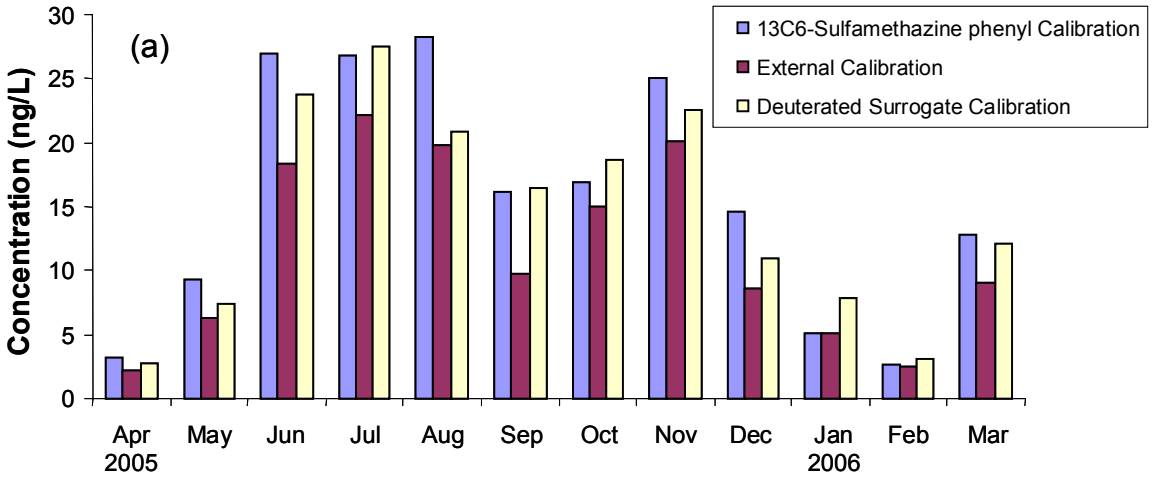

(a)
13C6-Sulfamethazine phenyl Calibration
External Calibration
Deuterated Surrogate Calibration
Concentration (ng/L)
0
1
2
3
4
5
6
7
8
Apr 2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 2006
Feb
Mar
(b)
13C6-Sulfamethazine phenyl Calibration
External Calibration
Deuterated Surrogate Calibration
Concentration (ng/L)
0
20
40
60
80
100
120
140
ND
ND
ND
ND
Apr 2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 2006
Feb
Mar
Sampling Month

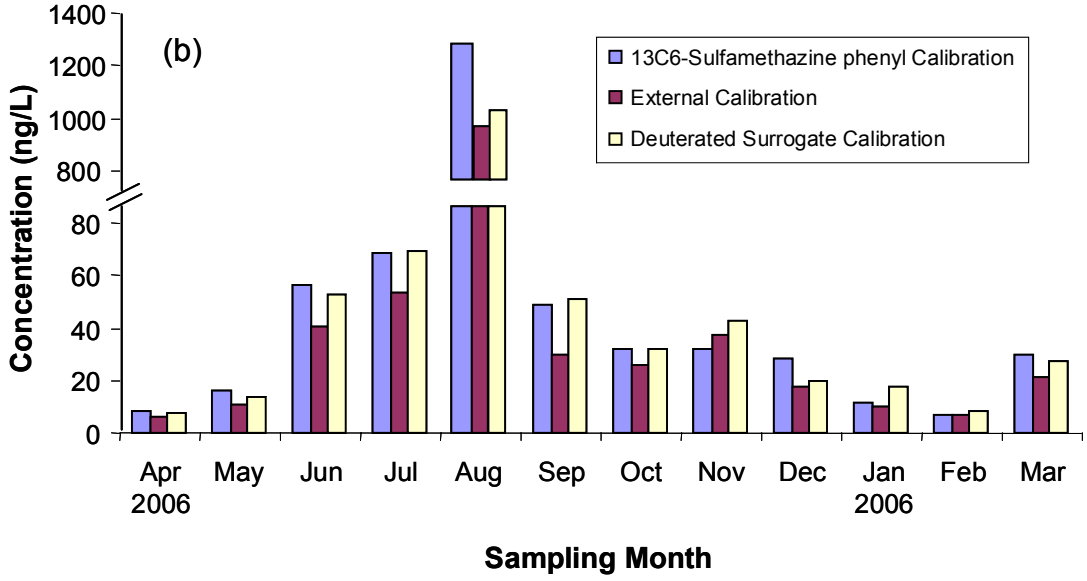
carbamazepine were not as impacted by the choice of quantification method used. Figure 2.5 shows consistency between the concentrations quantified between the three different methods, especially for the two internal standard quantification methods. This suggests that $^{13}C_6$-sulfamethazine-phenyl may be a reasonable internal standard for quantifying concentrations of carbamazepine in the water samples collected. In addition, all three quantification approaches maintain the seasonal trends observed over the sampling period. Higher concentrations were detected by all three methods during the summer months compared to the winter months.

2.4.4 Application of Analytical Method

The analytical method and different quantification approaches were applied to raw and treated water samples collected from two full scale drinking water treatment facilities, and raw water samples collected from groundwater wells in a Southern Ontario watershed. It was found that a selected group of pharmaceuticals were consistently found over the one year sampling period, however, most of the concentrations detected were close to their method detection limit (MDL). In addition to carbamazepine, gemfibrozil and ibuprofen, the most frequently detected compounds included, bezafibrate, naproxen, sulfamethoxazole, trimethoprim and lincomycin HCl. The concentrations detected in the water samples for carbamazepine, gemfibrozil and ibuprofen were quantified using three different quantification approaches including the use of deuterated surrogates (isotopically labeled standards). The remaining compounds were quantified using an external calibration approach due to the absence of isotopically labeled standards available for the remaining analytes.

Figure 2.5: Mean concentrations (ng/L) of carbamazepine in raw water samples collected from Facility A (a) and Facility B (b) comparing the use of $^{13}C_6$-sulfamethazine-phenyl as the internal standard, external calibration quantification and using D_{10}-carbamazepine as an internal standard for quantification (n=2).

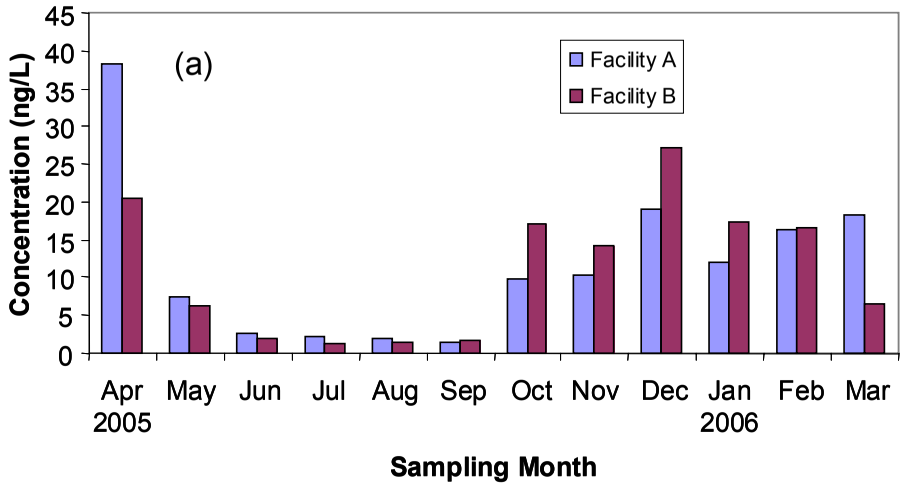

(a)
Concentration (ng/L)
30
25
20
15
10
5
0
13C6-Sulfamethazine phenyl Calibration
External Calibration
Deuterated Surrogate Calibration
Apr 2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 2006
Feb
Mar

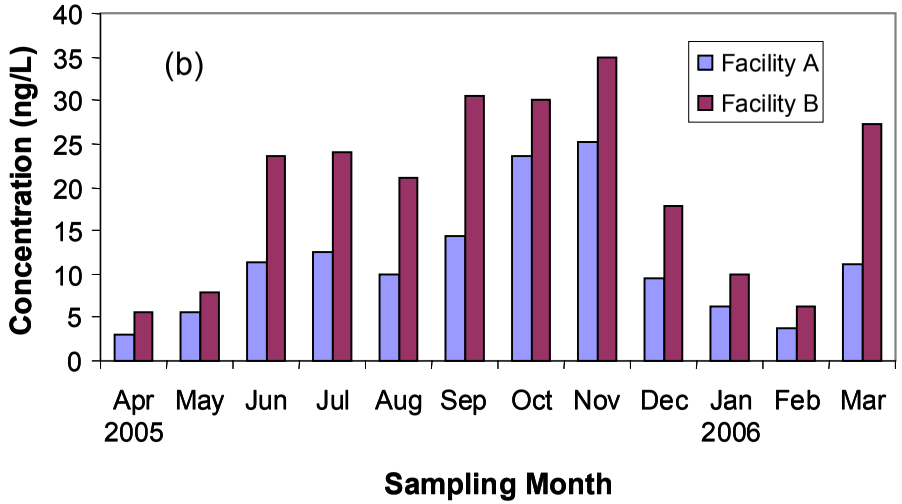

(b)
Concentration (ng/L)
1400
1200
1000
800
80
60
40
20
0
13C6-Sulfamethazine phenyl Calibration
External Calibration
Deuterated Surrogate Calibration
Apr 2006
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 2006
Feb
Mar
Sampling Month

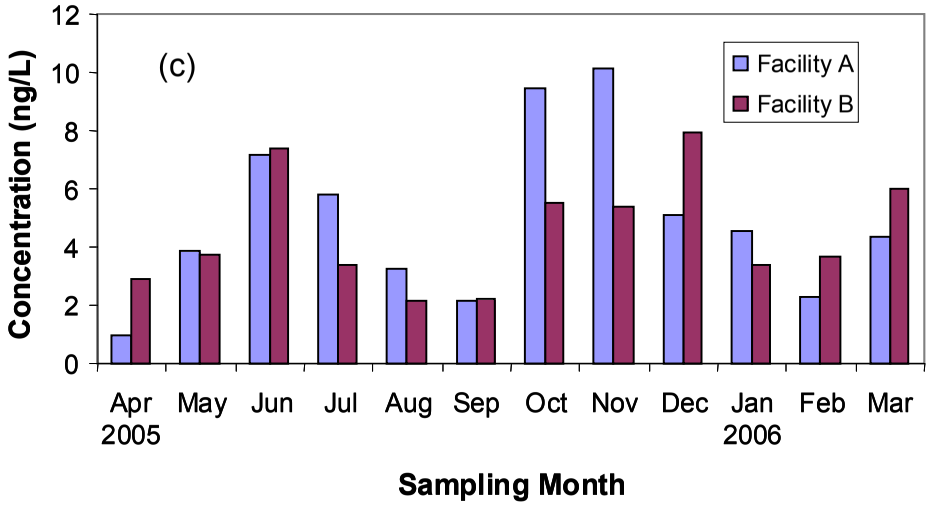
The three antibiotics (lincomycin HCl, sulfamethoxazole and trimethoprim) were detected in the raw water samples, but were not detected in the treated water samples. Figure 2.6 shows the mean concentrations of the three antibiotics in raw water samples collected from both facilities. Trimethoprim was detected at lower concentrations compared to the other two antibiotics, with the highest mean concentration of 10.1 ng/L (November 2005) in the samples collected from Facility A and 7.9 ng/L (December 2005) in samples collected from Facility B. In general, most of the concentrations detected were at or below the MDL of 10 ng/L for trimethoprim. Minimal seasonal trends were observed for trimethoprim with slightly higher concentrations detected during late spring/early summer, and then higher concentrations detected in the fall. Lincomycin HCl and sulfamethoxazole were detected at higher concentrations compared to trimethoprim, with higher mean concentrations detected in the raw water samples collected from Facility B for both compounds (Figure 2.6). The highest mean concentration for lincomycin HCl was 38.3 ng/L (April 2005) and the lowest mean concentration was 1.3 ng/L (September 2005) for samples collected at Facility A. For samples collected from Facility B, the range of mean concentrations of lincomycin HCl was 1.2 ng/L (July 2005) to 27.2 ng/L (December 2005). For sulfamethoxazole, the highest mean concentration was detected in November 2005 at 25.2 ng/L and the lowest mean concentration was 2.9 ng/L in April 2005 for samples collected from Facility A. The concentrations of sulfamethoxazole for raw water samples collected from Facility B ranged from 5.5 ng/L (April 2005) to 34.9 ng/L (November 2005).

Lincomycin HCl and sulfamethoxazole showed opposite seasonal trends with higher concentrations detected for lincomycin HCl in the spring and late fall/winter months, and higher concentrations of sulfamethoxazole detected in the summer and early fall months.

Figure 2.6: Mean concentrations of lincomycin HCl (a), sulfamethoxazole (b) and trimethoprim (c) in raw water samples collected from Facility A and Facility B from April 2005 to March 2006 (n=2).


(a)
Facility A
Facility B
Concentration (ng/L)
0
5
10
15
20
25
30
35
40
45
Apr 2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 2006
Feb
Mar
Sampling Month


(b)
Facility A
Facility B
Concentration (ng/L)
0
5
10
15
20
25
30
35
40
Apr 2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 2006
Feb
Mar
Sampling Month


(c)
Facility A
Facility B
Concentration (ng/L)
0
2
4
6
8
10
12
Apr 2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 2006
Feb
Mar
Sampling Month

Naproxen was detected at relatively high concentrations in the raw water samples collected at both facilities compared to other compounds investigated. In general, higher concentrations were detected in water samples collected from Facility A compared to Facility B (Figure 2.7). The highest mean concentration was 64.0 ng/L for naproxen (March 2006) in raw water samples collected from Facility B, and the highest mean concentration of 45.8 ng/L (September 2005) in raw water samples collected from Facility A. The seasonal variability for naproxen was not well defined with higher concentrations of naproxen present in water samples collected from Facility A in late spring/early summer and then again in the fall months. The seasonal variability of naproxen in the raw water samples collected from Facility B over the sampling period did not show the higher concentrations in the late spring/early summer months, but did show the higher concentrations in the fall months. In addition, there appeared to be a peak in concentration of naproxen in the raw water samples collected in March 2006 at Facility B.

Bezafibrate was one of the few compounds detected, which was found in both the raw water and treated water samples. In general, bezafibrate was not detected in the treated water samples collected at Facility A, but was found at detectable levels in the treated water samples collected from Facility B. Bezafibrate was found at higher concentrations in water samples collected from Facility B compared to Facility A. The concentrations were usually below 10 ng/L, with higher concentrations detected in the fall months (Figure 2.8). In most sampling months, the percent differences showed reduction of bezafibrate, but in some cases higher

concentrations were detected in the treated water samples collected from Facility B. The highest mean concentrations of bezafibrate in the raw and treated water samples collected at Facility B was 11.6 ng/L (November 2005) and 14.3 ng/L (November 2005), respectively.

Figure 2.7: Mean concentrations of naproxen in raw water samples collected from Facility A (a) and Facility B (b) from April 2005 to March 2006 (n=2).

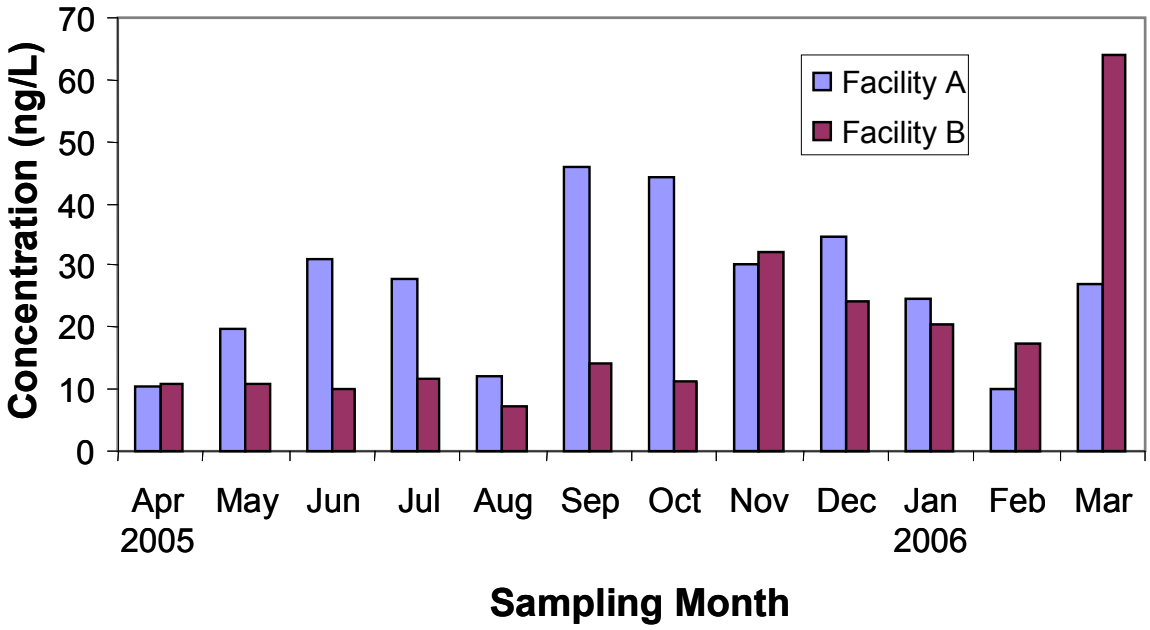
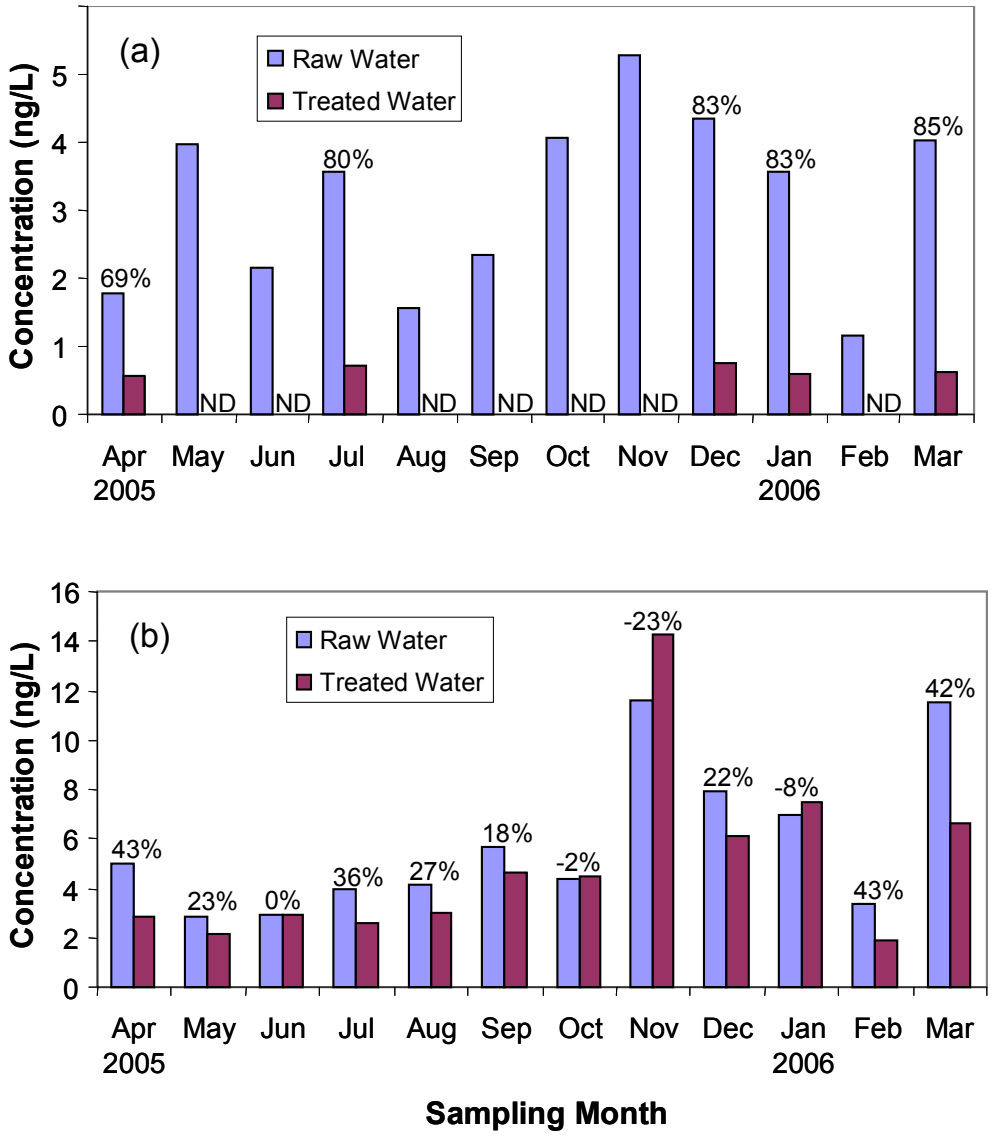

Concentration (ng/L)
70
60
50
40
30
20
10
0
Facility A
Facility B
Apr 2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 2006
Feb
Mar
Sampling Month

Figure 2.8: Mean concentrations and percent differences ([$Conc._{raw}$ − $Conc._{treated}$]/[$Conc._{raw}$] ×100) of bezafibrate in raw and treated water samples collected from Facility A (a) and Facility B (b) from April 2005 to March 2006 (n=2).


(a)
Raw Water
Treated Water
Concentration (ng/L)
0
1
2
3
4
5
69%
ND
ND
80%
ND
ND
ND
ND
ND
83%
83%
ND
85%
Apr 2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 2006
Feb
Mar
(b)
Raw Water
Treated Water
Concentration (ng/L)
0
2
4
6
8
10
12
14
16
43%
23%
0%
36%
27%
18%
-2%
-23%
22%
-8%
43%
42%
Apr 2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan 2006
Feb
Mar
Sampling Month

Carbamazepine, sulfamethoxazole and lincomycin HCl were the only compounds detected in the water samples collected from the groundwater wells. All three compounds were detected on each of the two sampling days at Well G, but only carbamazepine and lincomycin HCl were detected on both sampling days at Well H. The concentrations detected at the groundwater wells were lower then the concentrations detected at the two water treatment facilities during the same time period, with concentrations at or below their MDLs. Concentrations of lincomycin HCl, sulfamethoxazole and carbamazepine detected in the groundwater wells are summarized in Table A13.

2.5 Discussion

This study was one of the first to critically evaluate the application of liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) in determining the presence of pharmaceuticals in water samples, in which different quantification methods were applied. The purpose was to determine the extent matrix effects have on quantifying selected pharmaceuticals in raw water and treated water collected from two water treatment facilities in Southern Ontario, Canada.

The results from this study indicate the importance of addressing matrix effects when determining the presence of pharmaceuticals in environmental matrices. The use of an internal standard (preferably isotopically labeled surrogate) for each analyte has shown to be one of the best choices for addressing potential matrix effects (Richardson and Ternes, 2005; Hernando et al., 2006; Hummel et al., 2006; Vanderford and Snyder, 2006). These isotopically labeled surrogates have very similar physical and chemical properties to the analyte of interest, have similar retention times and undergo the same degree of ionization in a source interface (Hernando et al., 2006). The use of D_6-gemfibrozil, D_3-ibuprofen and D_{10}-

carbamazepine as isotopically labeled standards for the determination of gemfibrozil, ibuprofen and carbamazepine corrected for possible matrix effects as well as extraction efficiency. The comparisons made between the use of isotopically labeled surrogates, the use of $^{13}C_6$-sulfamethazine phenyl as an internal standard or the application of an external calibration approach showed how final concentrations can be overestimated or underestimated and inaccurate final concentrations reported (Figure 2.4). The low and variable recovery rates of $^{13}C_6$-sulfamethazine phenyl in the field samples (Figure 2.2), and the discrepancies between the recovery rates of $^{13}C_6$-sulfamethazine phenyl and the isotopically labeled surrogates in the field samples suggest that $^{13}C_6$-sulfamethazine phenyl was not an appropriate internal standard for representing how the analytes were acting in the matrix and what degree of ionization the analytes underwent in the interface. Figure 2.4 illustrates the variation in final concentrations of gemfibrozil and ibuprofen in raw water samples collected over a one year sampling period when $^{13}C_6$-sulfamethazine phenyl was used compared to when isotopically labeled surrogates were applied. The overestimation of the final concentrations of ibuprofen and gemfibrozil when using $^{13}C_6$-sulfamethazine phenyl as the internal standard is most likely the result of this internal standard being suppressed in the surface and treated water samples. The suppression of the signal for $^{13}C_6$-sulfamethazine phenyl resulted in the peak area ratio to be higher and therefore the calibration curve to be enhanced. The enhancement of the curve resulted in the unknown concentrations of the analytes to be higher.

Cahill et al. (2004) illustrated the importance of selecting an internal standard that is impacted to the same degree as the analyte in different matrices. The authors showed that ^{13}C-caffeine was not impacted by the matrix the same way as some of the analytes being

investigated. This resulted in another labeled internal standard being introduced which was not as impacted by the matrix and eluted from the separation column at a later time.

The same discrepancy was not observed when comparisons were made between the use of D_{10}-carbamazepine as an internal standard and $^{13}C_6$-sulfamethazine phenyl as an internal standard. In this case, $^{13}C_6$-sulfamethazine phenyl was a good choice for quantifying the concentrations of carbamazepine in this study because similar final concentrations were reported for the two different internal standard calibration approaches (Figure 2.5). One would assume that carbamazepine and $^{13}C_6$-sulfamethazine phenyl interacted with the matrix and underwent the same degree of ionization. This can also be explained by the higher and more constant recovery rates of $^{13}C_6$-sulfamethazine phenyl when analyzed in positive ionization mode. Similar results have shown that signal suppression is greater for analytes analyzed in negative ionization mode compared to positive ionization mode.

Gómez et al. (2006) showed lower signal suppression (45%) when analytes were analyzed in positive ionization mode. For analytes analyzed in negative ionization mode, signal suppression was greater at 60%. Carbamazepine was one of the few compounds investigated by Gómez et al. (2006), in which the degree of matrix suppression was minimal.

The results from Gómez et al. (2006) study also showed that the degree of signal suppression is sometimes dependent on where the analytes elute during separation in the chromatographic column, with some compounds eluting at the beginning or end of the chromatographic separation having greater signal suppression. Hernando et al. (2006) showed similar results, but with greater signal suppression for the analytes eluting at the beginning of the LC gradient. Vieno et al. (2006) reported higher signal suppression for analytes having a longer retention time. For the current study, ibuprofen, gemfibrozil and carbamazepine eluted

from the LC column during the middle of the run, with retention times of approximately 11.4 min, 14.3 min and 8.7 min, respectively.

Based on the recovery rates of $^{13}C_6$-sulfamethazine phenyl between positive and negative ionization modes, analytes analyzed in positive ionization mode were less impacted by matrix effects. Similar results were observed for statin drugs, in which positive ionization mode was more sensitive then negative ionization mode (Miao and Metcalfe, 2003). Hilton and Thomas (2003) found that the internal standard, ^{13}C-phenacetin, showed higher sensitivity in positive ionization, but poor ionization in negative mode.

An explanation for signal suppression being more apparent in negative ionization mode is that the formation of deprotonated analytes maybe more difficult to achieve in the presence of matrix components, and some mechanism could be preventing the deprotonated analytes from moving to the droplet surface and being effectively ionized. Until the mechanism of how ionization occurs in an ESI source is determined, it will be difficult to conclude why the polarity is influencing ionization efficiency of certain analytes. It would be beneficial to observe if other analytes analyzed in positive ionization mode are impacted to the same degree as carbamazepine, but this observation could be related to the persistence and resistant this compound exhibits in the natural environment and during treatment (Clara et al., 2004; Ternes et al., 2004; Hummel et al., 2006). It has been well documented that carbamazepine is persistent in different environmental matrices and a good marker for determining the presence of wastewater contamination (Clara et al., 2004). Environmental fate studies investigating the movement of carbamazepine in soil and groundwater aquifers have shown minimal removal and similar results have been observed in wastewater treatment

systems (Ternes, 1998; Stamatelatou et al., 2003; Clara et al., 2004; Strenn et al., 2004; Miao et al., 2005; Gómez et al., 2007).

The results from this study also support previous studies in which there is a relationship in recovery rates of analytes to the amount of matrix present in the sample. In general, recovery rates are usually lower in samples with more matrix present (e.g. soils, wastewater treatment effluents) compared to samples with less matrix present (e.g. drinking water, groundwater) (Hirsch et al., 1998; Miao and Metcalfe, 2003; Cahill et al., 2004; Hernando et al., 2006; Hummel et al., 2006). Cahill et al. (2004) investigated recovery rates of selected compounds in raw water samples to the recovery rates in organic-free reagent water. Some compounds showed similar recovery rates between the two matrices, like trimethoprim and ibuprofen, while others like gemfibrozil and sulfamethoxazole were suppressed in the surface water compared to the reagent water samples. Trimethoprim maintained a relatively constant concentration in serial dilutions of surface water with high dissolved organic matter compared to the other compounds tested. The impact that the matrix has on a compound is dependent on the analyte of interest as well as the composition of the matrix. The same matrix may have different degrees of ion suppression or enhancement when investigating different analytes (Miao and Metcalfe, 2003). Vanderford et al. (2003) investigated the presence of matrix effects using LC-ESI-MS/MS by spiking antibiotics and acidic drugs in surface water samples and the methanol samples. Trimethoprim was found to be least impacted by the surface water matrix with a percent change of 11% between the spiked solvent solution and the spiked surface water sample. For naproxen, which was analyzed in negative ionization mode, there was 85% change between the spiked solvent solution and the spikes surface water samples (Vanderford et al., 2003).

Ion suppression of carbamazepine ranged from just over 10% in river water to up to 60% for wastewater samples (Hummel et al., 2006). The ion suppression can be explained by the increase in matrix components present in the wastewater samples and therefore more competition with the analyte in the ionization source. This relationship was illustrated to some extent in this study, with lower recovery rates, as well as more variability, in the raw water samples compared to the treated water samples. It was well defined when a larger volume of sample was extracted. Figure 2.3 shows that increasing the volume extracted to 800 mL can dramatically impact the recovery of $^{13}C_6$-sulfamethazine phenyl. Ion suppression, and to a lesser extent ion enhancement, of the deuterated surrogates in the field samples was also observed based on the recovery rates of these compounds in 800 mL extracted samples but to a lesser extent then $^{13}C_6$-sulfamethazine phenyl. In most cases, recovery rates of D_3-ibuprofen and D_6-gemfibrozil in the 800 mL extracted samples were different compared to the recovery rates of $^{13}C_6$-sulfamethazine phenyl analyzed in negative ionization mode. The recovery rates of D_{10}-carbamazepine and $^{13}C_6$-sulfamethazine phenyl in 800 mL extracted samples analyzed in positive ionization mode were similar to each other.

Figure 2.4 and 2.5 also indicates to some extent that the choice of quantification method used does not have a dramatic impact on the seasonal trends observed. In general, similar seasonal trends determined by the deuterated surrogates were maintained when the external standard approach was applied, and to a lesser extent, when $^{13}C_6$-sulfamethazine phenyl was used. Seasonal trends were also found to be similar between the external calibration approach and $^{13}C_6$-sulfamethazine phenyl quantification approach for compounds with no isotopically labeled standards applied in the method (data not shown). This suggests that although, final concentrations cannot be determined accurately using $^{13}C_6$-sulfamethazine

phenyl or the external calibration approach, relative seasonal trends can be determined without the use of deuterated surrogates for the analytes investigated and the composition of the matrices used in this study.

Based on the discussion about the importance of addressing matrix effects, the final concentrations reported in the water samples from the drinking water treatment systems in Southern Ontario should be taken with caution when isotopically labeled standards were not applied. The final concentrations for most analytes were quantified by an external calibration approach or by the use of $^{13}C_6$-sulfamethazine phenyl. In either case, matrix effects were not addressed for these analytes. Figures 2.6 to 2.8 show the final concentrations quantified by the use of an external calibration approach. Despite the limitations of this analytical method of not addressing matrix effects, similar methods have been used in the literature (Hirsch et al., 1998; Castiglioni et al., 2004; Bendz et al., 2005; Castiglioni et al., 2005; Hao et al., 2006; Lissemore et al., 2006).

A limited number of compounds were detected in the water samples collected from Facility A and Facility B. A total of five analytes (excluding carbamazepine, gemfibrozil and ibuprofen) were detected in the raw water samples, and only one analyte was detected in the treated water samples. In most cases, the final concentrations reported were below their MDLs, but were still reported for qualitative purposes and to show possible seasonal variation.

The presence of the three antibiotics, bezafibrate and naproxen have been detected in surface waters in North America (Kolpin et al., 2002; Boyd et al., 2004a; Metcalfe et al., 2003b; Kolpin et al., 2004; Stackelberg et al., 2004; Hao et al., 2006; Hua et al., 2006b; Lissemore et al., 2006), and globally (Ternes, 1998; Stumpf et al., 1999; Heberer, 2002a;

Heberer et al., 2002; Thomas and Hilton, 2004; Bendz et al., 2005; Kim et al., 2006). The absence of most of these compounds in finished drinking water correlates with previous studies (Boyd et al., 2003; Stackelberg et al. 2004; Hua et al., 2006a; Hummel et al., 2006; Kim et al., 2006).

The presence of naproxen in environmental samples has been dominant with higher concentrations detected in wastewater treatment effluent samples and surface waters compared to other pharmaceuticals (Öllers et al., 2001; Boyd et al., 2003; Boyd et al., 2004a; Brun et al., 2006; Hua et al., 2006b; Lishman et al., 2006; Zhang et al., 2007). The current study showed a similar pattern with high concentrations of naproxen detected in the raw water samples compared to bezafibrate and the antibiotics. In Canada, naproxen is used predominantly as a human anti-inflammatory agent and is a commonly prescribed non-steroidal anti-inflammatory drug (NSAID) (Brun et al., 2006). There are a total of 46 products containing naproxen commercially available in the Canadian market and registered on Health Canada's Drug Product Database (Health Canada, 2006). The higher concentrations of naproxen detected in the raw water samples collected from Facility A and Facility B during the fall months could be explained by the population increasing their consumption of naproxen during flu and cold season (Tixier et al., 2003). A similar pattern was observed for ibuprofen with higher concentrations detected in the water samples collected during the fall and winter months (i.e. flu and cold season).

Limited work has investigated the removal of naproxen during drinking water treatment processes, but Boyd et al. (2003) reported naproxen to be below the detection limit after the addition of chlorine and filtration. The absence of naproxen in treated water samples is to be expected due to high reductions (greater than 80%) in concentrations during

conventional wastewater treatment processes (Lishman et al., 2006) and its reactivity to chlorine (Boyd et al., 2004b; Pinkston et al., 2004). Boyd et al. (2004b) investigated the removal of naproxen in synthetic waters with the addition of free chlorine and found that free chlorine reacted rapidly with naproxen at pH values 5 to 9. Similar results were observed by Pinkston et al. (2004), in which free chlorine attacked the aromatic ring of naproxen and resulted in complete transformation of this compound. The application of powdered activated carbon (PAC) was also found to have an impact on the reduction of naproxen during stimulated drinking water treatment processes, with reductions greater than 50% for all four water matrices used during the study (Westerhoff et al., 2005). Naproxen was also found to be oxidized by ozone with an average percentage oxidized of 91% (Westerhoff et al., 2005). The reduction of naproxen by PAC and ozone is consistent with similar treatment processes used at Facility B and Facility A, respectively.

Studies have also shown that photodegradation may be a natural elimination process for the removal of naproxen in surface waters (Boreen et al., 2003). The photodegradation of naproxen may help explain the overall lower concentrations of naproxen in the raw water samples collected from Facility B because the source raw water entering Facility B has a longer retention time in a small canal compared to Facility A which draws water from the main river system. The higher concentrations in the fall and winter months for samples collected at Facility B maybe the result of ice and snow covering the water surface and therefore naproxen not being photodegraded to the same extent as during the spring and summer months. Öllers et al. (2001) and Tixier et al. (2003) reported lower concentrations and higher removal of naproxen in the epilimnion (upper portion of the lake) compared to

hypolimnion, which could be a result of photodegradation occurring in the upper part of the lake water column and warmer temperatures helping the reduction of naproxen.

The seasonal variability of naproxen in the raw water samples collected from Facility A may be explained by changes in water flows. Some studies have shown that increases in water flow results in higher concentrations of pharmaceuticals (Tixier et al., 2003). The presence of naproxen has been found at higher concentrations during high flow conditions by Tixier et al. (2003), however the increases in water flows during the spring and winter months does not completely follow the observed concentration peaks in the current study, therefore suggesting other processes are taking place.

The most established seasonal trends for the compounds detected were observed for lincomycin HCl and sulfamethoxazole. Higher concentrations of lincomycin HCl were detected in the spring and fall months compared to the summer months (Figure 2.6). This can be explained by its predominant used as a veterinary antibiotic, it presence as a medicating agent in livestock feed and how this compound enters surface waters (Health Canada, 2006; Canadian Food Inspection Agency, 2004). This antibiotic is excreted by livestock animals as the parent compound or as a metabolite, and then the livestock manure is applied to agricultural land during the spring and fall months as a soil amendment to fields. The higher concentrations of lincomycin HCl in the raw water correspond to when livestock manure would be applied to agricultural fields and the greatest potential of surface runoff after land application.

For sulfamethoxazole, a predominantly used human antibiotic, higher concentrations were detected in the summer and the early fall months (Figure 2.6). The seasonal trends observed for this compound were opposite to the trends observed with lincomycin HCl. One

possible explanation for the higher concentrations of sulfamethoxazole in the summer and early fall months could be due to the decrease in river flow and levels, and as a result an increase in the percentage of wastewater effluents that comprised the volume of the river. Similar seasonal trends have been observed for other pharmaceuticals, in which higher concentrations of the pharmaceuticals are present during low flow conditions (Metcalfe et al., 2003b; Tixier et al., 2003; Kolpin et al., 2004; Lissemore et al., 2006; Loraine and Pettigrove, 2006). Kolpin et al. (2004) showed that lincomycin, sulfamethoxazole and trimethoprim were detected during low flow conditions, but were not detected during normal or high-flow conditions of streams in Iowa.

The absence of the three antibiotics in treated water samples can be explained by their potential to react with chlorine and ozone. Both sulfamethoxazole and lincomycin have reactive sites for ozone, with aromatic rings present in sulfonamides and tertiary amines present in most macrolides (Huber et al., 2003). Sulfamethoxazole showed high reactivity to 0.1 mg/L of ozone in bank infiltrated samples resulting in 70% transformation, and reactivity to an ozone dose of 0.5 mg/L in surface water samples resulting in over 95% transformation (Huber et al., 2003). Chlorinated drinking water samples taken from a drinking water treatment facility in the United States showed that trimethoprim and sulfamethoxazole had intermediate reactions with free chlorine residuals at 1.2 mg/L after 24 hours and complete reactivity with free chlorine after 10 days (Gibs et al., 2007). Lincomycin showed complete reaction with free chlorine within 24 hours (Gibs et al., 2007). The absence of these antibiotics in treated water samples correspond with data showing the reduction of these compounds during different treatment processes. Bezafibrate was the only compound (excluding carbamazepine, gemfibrozil and ibuprofen) that was found in treated water

samples during the sampling period, and the first to be reported in North America. To our knowledge, bezafibrate has only been detected in treated water samples once before with a concentration of 27 ng/L in German tap water samples (Jones et al., 2005). Although, the reported concentrations of bezafibrate in the water samples should be taken with caution based on the lack of an isotopically labeled surrogate for bezafibrate to address matrix effects and the concentrations at or below the MDL of 5 ng/L, the presence of this compound shows differences in the ability of the two facilities to reduce the concentrations of bezafibrate.

There has been minimal work investigating the possible elimination processes for this compound, but appears to follow the same pattern observed with carbamazepine during water treatment processes. In contrast to carbamazepine, bezafibrate was removed or transformed during wastewater treatment processes (Ternes, 1998; Stumpf et al., 1999; Strenn et al., 2004; Castiglioni et al., 2006), but minimal work has investigated the removal of bezafibrate during drinking water treatment processes (Ternes et al., 2002; Huber et al., 2003). Ternes et al. (2002) concluded that bezafibrate was ineffectively removed by flocculation with iron chloride, had a high sorption affinity to activated carbon during the adsorption isotherm tests and responded well to GAC filtration. Concentrations of bezafibrate have been reduced by 50% with the application of 1.5 mg/L of ozone in a lab-scale ozonation experiment, and during full-scale water treatment processes, concentrations of bezafibrate were reduced after GAC filtration at one facility without any oxidation processes. In addition, bezafibrate was not present after bank infiltration or after slow sand filtration processes (Ternes et al., 2002). Huber et al. (2003) reported that bezafibrate is oxidized to a greater extent by ozone then advanced oxidation processes, but has a lower rate constant with ozone than other compounds. For the bank infiltrated water, an ozone dose of greater than 0.5 mg/L was

required for 80% reactivity (transformation) of bezafibrate. A dose of greater than 1 mg/L of ozone was required for 70% reactivity of bezafibrate in the surface water samples. Bezafibrate was found to be effectively oxidized in water samples with low DOC and high alkalinity, which increases the ozone stability and results in bezafibrate effectively being oxidized directly by ozone. It was suggested that the limited reactivity with ozone is due to a functional group on one of the aromatic rings. This substitute cannot be deprotonated and therefore at certain pH values the rate constant with ozone is low (Huber et al., 2003).

Although, bezafibrate may be resistant to conventional treatment processes and show similar persistence in the natural environment and during treatment as carbamazepine, this compound has the potential to be removed or transformed by advanced treatment processes. The application of ozone and GAC filters, which were present at Facility A, were capable of reducing the concentrations of bezafibrate in treated water samples at Facility A.

The results from this study also show that bezafibrate is detected at higher concentrations than other fibrate drugs, which contradicts other reported concentrations with gemfibrozil found at higher concentrations. The presence of bezafibrate at higher concentrations is unexpected because of its limited use in Canada (active ingredient in only two drug products commercially available) (Health Canada, 2006). The high prevalence of bezafibrate and gemfibrozil was questioned by Metcalfe et al. (2003b), which found similar results with bezafibrate and gemfibrozil found at higher concentrations in surface waters compared to the highly prescribed statin drugs used by the Canadian population.

A limited number of pharmaceuticals were detected in two groundwater wells (Well G and Well H). Although sulfamethoxazole and lincomycin HCl were detected at concentrations below their MDLs, the results suggests that these two compounds are capable

of moving through soils into groundwater aquifers. The presence of sulfamethoxazole in Well G (urban located well) can be explained by higher loads of this human antibiotic being discharged into surface waters in areas with greater population density, and as a result an increase potential for the wells located in a population dense area to be exposed to this antibiotic. Sulfamethoxazole has been detected in groundwater wells as part of monitoring program in an area in Germany, with eleven samples containing this compound and a maximum concentration of 410 ng/L (Sacher et al., 2001). This compound was also detected in three groundwater samples collected in the Netherlands with concentrations below 25 ng/L (Stolker et al., 2004), and groundwater samples collected in the United States (Lindsey et al., 2001). Recently, sulfamethoxazole has been detected in wells where agricultural land was irrigated with treated wastewater (Ternes et al., 2007).

The presence of lincomycin HCl at the rural located well (Well H) can be explained by the application of livestock manure to agricultural fields in the fall months and runoff into the surface waters, however the presence of this compound at the urban located well (Well G) is more difficult to explain. One possible reason is the lack of degradation of this compound in surface water due to lower water temperatures, less penetration of sunlight for photodegradation in the water, or changes in water level and water flows (rainfall events), which all can result in higher concentrations in surface waters and a greater potential of the compound to infiltrate into susceptible wells (Lissemore et al., 2006). To our knowledge, this is the first study to report lincomycin HCl present in groundwater wells.

Bezafibrate and naproxen were not detected in the raw water samples collected from the selected groundwater wells, however studies have detected the presence of naproxen in groundwater wells. One study detected the presence of naproxen in groundwater wells

supplying drinking water to communities in Southern France, with concentrations 0.1 to 0.2 ng/L (Rabiet et al., 2006). The detection limits established for the GC/MS analytical method used in the study were lower than the detection limit used in the current study, which may explain the inability to detect naproxen in these groundwater wells. Naproxen was also detected in one groundwater monitoring well at 20 ng/L, in which wells are used to monitor the effectiveness of a soil-aquifer treatment when treated effluent is used for groundwater recharge (Drewes et al., 2002).

2.6 Conclusions

The application of LC-ESI-MS/MS is common for determining the presence of trace organics in the environment, and is widely used for investigating the presence of pharmaceuticals in different environmental matrices. Although, the use of LC-ESI-MS/MS is a sensitive tool, there are limitations that need to be addressed during method development. The major disadvantage is that the ESI source is prone to matrix effects, which can impact the accuracy and reliable of determining concentrations of pharmaceuticals in complex matrices.

Matrix effects can be addressed by a number of different approaches, with one being the use of an internal standard (structural analogues or isotopically labeled standards) to compensate for analyte suppression or enhancement in different matrices. Internal standard calibration approaches were applied in this study and showed that an inappropriate selection of an internal standard, $^{13}C_6$-sulfamethazine phenyl, can impact the final concentrations reported. The use of isotopically labeled standards was found to be the best choice in addressing matrix effects.

The use of $^{13}C_6$-sulfamethazine phenyl as an internal standard was found to overestimate the concentrations of the analytes analyzed in negative mode, but was an

appropriate internal standard for carbamazepine, which was analyzed in positive mode. Seasonal variability was retained for the analytes when using different calibration approaches, but the concentrations were enhanced compared to what was estimated using isotopically labeled standards. The average overestimation of the estimated final concentrations using $^{13}C_6$-sulfamethazine phenyl as the internal standard was two to three times higher compared to the final concentrations determined when the isotopically labeled standards were used. The degree of overestimation when using $^{13}C_6$-sulfamethazine phenyl was found to be compound dependent and sometimes dependent on the season.

The lack of commercially available isotopically labeled standards during development of the analytical method restricted the ability of most of the analyte concentrations being corrected for matrix effects. The final concentrations for analytes not corrected using an isotopically labeled standard are reported but must be considered with caution, even though similar methods have been used in recent reports in the scientific literature.

Three antibiotics (lincomycin HCl, sulfamethoxazole and trimethoprim), bezafibrate and naproxen were detected frequently during the sampling period. Bezafibrate was the only compound detected in the treated water samples, with higher percent reduction during treatment at Facility A compared to Facility B. Bezafibrate was found to follow carbamazepine, in which advanced treatment processes were required for reducing this compound to non-detectable levels.

The absence of most analytes in the treated water samples collected from both treatment facilities, suggest that current treatment technologies are capable of reducing these compounds to non-detectable levels or creating transformation by-products, which cannot be detected using the current analytical method. The one year sampling study provided

information on the capabilities of detecting low levels of pharmaceuticals in raw and treated water samples, and provided insight into the ability of two full scale water treatment facilities to reduce the concentrations of these compounds. The information will provide direction into future research initiatives assessing the impacts of these compounds in drinking water treatment systems in Ontario.

CHAPTER 3

Presence and Seasonal Variability of Carbamazepine, Gemfibrozil and Ibuprofen in Southern Ontario Drinking Water Supplies

3.1 Abstract

The presence and seasonal variability of selected human pharmaceuticals in two Southern Ontario drinking water treatment systems and eight groundwater wells was investigated. Surface water (raw water) and treated water samples were collected at both treatment facilities each month for a period of one year, and raw water samples were collected from the wells on two sample dates. Water samples were extracted by solid phase extraction techniques and analyzed using liquid chromatography with electrospray tandem mass spectrometry (LC-ESI-MS/MS). Carbamazepine, gemfibrozil and ibuprofen were detected in raw and treated water samples usually below 100 ng/L, with higher concentrations detected in the raw water samples. Carbamazepine was detected at higher concentrations during the summer and early fall months, while ibuprofen was detected during the fall and winter months. In addition, carbamazepine was detected in groundwater wells within the same watershed, indicating the movement of this compound from surface waters to groundwater supplies. A comparison of the treatment methods employed at the two drinking water facilities suggests differing treatment processes (i.e. ozonation) may differentially reduce the concentrations of detected compounds in drinking water. The results from this study illustrate that human pharmaceuticals are present in full scale drinking water treatment facilities, and that some current treatment technologies are capable of reducing these compounds to non-detectable levels.

3.2 Introduction

It has been well documented that pharmaceuticals are considered environmental contaminants (Halling-Sørensen et al., 1998; Daughton and Ternes, 1999; Jones et al., 2001; Heberer, 2002b) detected in a number of environmental matrices, including wastewater

effluent samples (Ternes, 1998; Metcalfe et al., 2003a; Stamatelatou et al., 2003; Castiglioni et al., 2005; Castiglioni et al., 2006). Research has found that these compounds are not completely removed by conventional wastewater treatment technologies, which results in a major pathway for these compounds entering aquatic ecosystems (Ternes, 1998; Stumpf et al., 1999; Miao et al., 2005; Kim et al., 2006; Lishman et al., 2006; Gómez et al., 2007). The presence of these compounds in treated wastewater effluents results in a continual replenishment of these compounds in surface waters, many of which are used downstream for drinking water purposes.

Recent advancements in environmental chemistry has allowed researchers to determine the presence of pharmaceuticals at low concentrations in the environment, with only a few studies having investigated the presence of these compounds in drinking water supplies (Heberer et al., 1998; Zuccato et al., 2000; Stackelberg et al., 2004; Hua et al., 2006a; Kim et al., 2006). There is limited data available in North America about the concentrations of these pharmaceuticals in drinking water, how current treatment technologies are reducing their concentrations in treated water and the seasonal trends observed. Currently, there is some monitoring data at various locations within Canada and the United States, but no extensive investigation has looked at the presence of these compounds in drinking water systems over an extended period (Boyd et al., 2003; Stackelberg et al., 2004; Hua et al., 2006a).

In the current study, three human pharmaceuticals (carbamazepine, gemfibrozil and ibuprofen) were selected based on their presence in environmental matrices, limited environmental fate information and a high consumption rate in the North American population. In Canada, carbamazepine, ibuprofen and gemfibrozil are active ingredients in a

number of drug products in the Canadian market. Carbamazepine and gemfibrozil require a prescription, while ibuprofen is prescribed or can be purchased without a prescription.

The aim of this study was to apply an analytical method using solid phase extraction techniques and liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS) to determine the presence of carbamazepine, gemfibrozil and ibuprofen in two full scale drinking water treatment facilities in a Southern Ontario watershed. Further, this study investigates if concentrations of these pharmaceuticals vary seasonally and are reduced following water treatment.

3.3 Materials and Methods

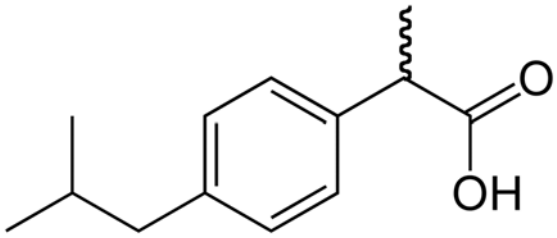
3.3.1 Chemicals and Standards

Carbamazepine, gemfibrozil and ibuprofen were purchased from Sigma-Aldrich (St. Louis, MO, USA), along with ammonium hydroxide (ACS reagent grade), heptafluorobutyric acid (HFBA), and ethylenediaminetetraacetic acid (EDTA) disodium salt dehydrate (ACS reagent grade). A summary of the physical and chemical properties of the three selected human pharmaceuticals can be found in Table 3.1, with the chemical structures in Figure 3.1. Ammonium acetate (above 99% purity) was purchased from Fluka Chemika (Mississauga, ON, Canada). Sulfamethazine-phenyl-$^{13}C_6$ (^{13}C-90%) was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Carbamazepine-D_{10} (98.2 atom %D), gemfibrozil-D_6 (2,2-dimethyl-D_6, 99.7 atom %D) and (+)-ibuprofen-D_3 (α-methyl-D_3, 99.4 atom %D) were purchased from CDN Isotopes (Pointe-Claire, PQ, Canada). These deuterated compounds were used to monitor extraction efficiencies, and to correct for potential matrix effects commonly observed with the use of LC-ESI-MS/MS instrumentation. Methanol (distilled in glass grade), acetronitrile (HPLC grade), water (HPLC grade) and sulphuric acid were

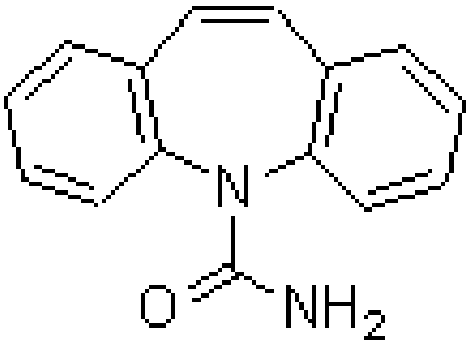
Table 3.1: Physical and chemical properties of three human pharmaceuticals evaluated over a 12 month interval in a Southern Ontario watershed.

Pharmaceutical	CAS No.	Molecular Weight (g/mol)	Chemical Formula	Application
Carbamazepine	298-46-4	236.3	$C_{15}H_{12}N_2O$	Anti-epileptic and anti-depressant drug
Gemfibrozil	25812-30-0	250.3	$C_{15}H_{22}O_3$	Lipid regulating drug
Ibuprofen	15867-27-1	206.3	$C_{13}H_{18}O_2$	Analgesic and anti-inflammatory drug

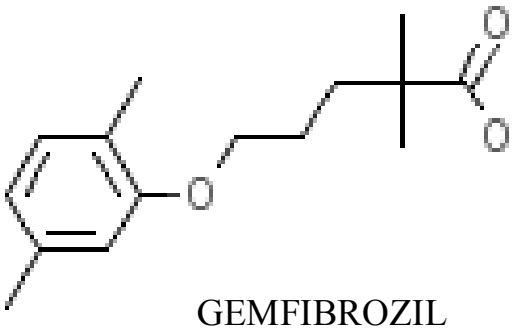
Figure 3.1: Chemical structures of the selected pharmaceuticals, carbamazepine, gemfibrozil and ibuprofen.



IBUPROFEN



CARBAMAZEPINE



GEMFIBROZIL

purchased from Caledon Laboratories Ltd. (Georgetown, ON, Canada), and sodium hydroxide pellets (ACS reagent grade) was purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA) for preparation of a 5% sodium hydroxide solution (w/v) in nanopure water. A Barnstead NANOpure Diamond^{TM} water purification system (set at 18Ω) was used to provide nanopure water for sample preparation and extraction.

3.3.2 Preparation of Solutions

Stock solutions of carbamazepine, gemfibrozil, ibuprofen, and the three deuterated labeled compounds (D_{10}-carbamazepine, D_6-gemfibrozil and D_3-ibuprofen) were prepared by weighing out approximately 10 mg of the powdered neat standard and dissolving the contents in methanol. The final stock solution concentrations were approximately 1 mg/mL, and these solutions were used to prepare calibration standards and spiking solutions (including the mixed surrogate standard solution with the three deuterated compounds). All standard solutions were stored at -20 °C.

3.3.3 Study Sites

Description of Drinking Water Treatment Facilities

A detailed description of the two water treatment facilities, Facility A and Facility B, the sampling locations at the facilities and the watershed can be found in Section 2.3.3. In brief, both facilities obtain their source water from the main river system with Facility B located further downstream of Facility A. The locations of the drinking water treatment facilities relative to each other and to the wastewater treatment facilities within the watershed are illustrated in Figure 2.1.

Both facilities are considered full conventional water treatment facilities with coagulation, flocculation, sedimentation and filtration processes present. The addition of chlorine gas and anhydrous ammonia are used for disinfection at both facilities. The major difference between the two facilities is the presence of ozonation, granular activated carbon (GAC) filters and ultra-violet (UV) irradiation at Facility A. Details of the operational parameters and treatment processes at each facility can be found in Table 3.2.

Duplicate raw and treated water samples were collected at each facility on the first Tuesday of each month from April 2005 to March 2006. Details on the sample collection procedures can be found in Section 2.3.3. The collection of the raw and treated water samples did not account for retention time during treatment. The samples collected were analyzed for the presence of pharmaceuticals and general water chemistry parameters.

Description of the GUDI Well Locations

In addition to water samples being collected from the two drinking water treatment facilities, water samples were also collected from groundwater wells. A total of eight groundwater wells were sampled, including two deep groundwater reference wells (Well C and Well D) and six susceptible groundwater wells under the direct influence of surface water (referred to as GUDI) (Well A, Well B, Well E to H). A description of each well and the sample collection procedure can be found in Section 2.3.3. In brief, all eight wells are located in the same watershed as the two drinking water treatment facilities. Most of the susceptible GUDI wells were located in rural areas, except Well G. Duplicate grab raw water samples were collected from each well on Tuesday September 27th 2005 and Tuesday October 11th 2005 for pharmaceutical and water chemistry analyses.

Table 3.2: Summary of the treatment processes employed at two full scale drinking water treatment facilities, Facility A and Facility B, during the evaluation of selected human pharmaceuticals in Ontario drinking water supplies.

Facility	Population Served	Coagulation	Flocculation	Sedimentation	Disinfection	Filtration	Other Treatment Processes
Facility A	200,000*	Yes	Yes	Yes	chlorine gas, anhydrous ammonia, ultra-violet irradiation	granular activated carbon (GAC) and sand filters	ozonation (1.5 to 3 mg/L)
Facility B	98,000	Yes	Yes	Yes	chlorine gas, anhydrous ammonia	anthracite coal and sand filters	powder activated carbon (PAC), activated silica, sodium silicofluoride

*population is supplied with drinking water from Facility A and groundwater wells located near the facility

3.3.4 Sample Analysis – Water Chemistry Parameters

A number of different analytical methods were used to analyze for general water chemistry parameters. A summary of the methods used can be found in Section 2.3.4. All sample analysis was conducted at the Ontario Ministry of the Environment Laboratory in Etobicoke, Ontario.

3.3.5 Sample Analysis – Pharmaceuticals

A detailed description of the preparation and extraction of the water samples for pharmaceutical analysis is outlined in Section 2.3.5. In brief, the water samples collected were prepared along with one blank sample and two method spike samples for a total of eleven samples being extracted at one time. The samples were prepared and extracted within 24 to 36 hours after being collected. A total volume of 400 mL was extracted after the samples were prepared, and appropriate spiking solutions and the mixed surrogate standard solution were added.

The water samples were extracted using preconditioned Waters Oasis® HLB cartridges (6cc, 200mg) solid phase extraction cartridges, and the analytes were eluted using 5 mL of methanol. The final extract was mixed and 1 mL was transferred to a clean 2 mL clear glass vial.

The 1 mL extracts were evaporated using a gentle stream of nitrogen gas, and were reconstituted by adding an aqueous solution to each vial after evaporation. The aqueous solution consisted of a known volume of $^{13}C_6$-sulfamethazine phenyl, which corrected for volume injection problems into the LC-ESI-MS/MS and to calculate absolute recovery rates.

The vials were placed into the freezer until analysis. The samples were usually analyzed within one week of being extracted.

3.3.6 LC-ESI-MS/MS Instrumentation

A detailed description of how the samples were analyzed can be found in Section 2.3.6. In summary, all samples were analyzed using liquid chromatography with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). The analytes were separated by an Agilent Hewlett Packer 1100 Series liquid chromatograph system (Mississauga, ON, Canada), which consisted of a vacuum degasser, binary pump, column compartment and an autosampler. The sample injection volume was 20 µL, except for samples collected in April 2005 when the injection volume was 15 µL.

The analytes were detected using an Applied Biosystems/MDS Sciex 4000 Q Trap™ mass spectrometer with an electrospray ionization source interface (TurboIonSpray™) and multiple reaction monitoring (MRM) was selected as the scan mode to quantify the compounds present in the water samples.

The three human pharmaceuticals were analyzed according to analytical method used, with carbamazepine analyzed in positive ESI mode, and gemfibrozil and ibuprofen analyzed in negative ESI mode. For both analytical methods, the same LC column was used (ThermHyperSil Gold RP-C_{18}, 2.0 mm × 100 mm, particle size of 3 µm). For positive ionization mode, the flow rate was 0.2 mL/min and 0.03% heptafluorobutryic acid (HFBA) (mobile phase A) and 100% HPLC grade acetronitrile (mobile phase B) was used for the binary gradient elution. The mobile phase gradient elution was as follows: at 0 min A/B was 85:15, at 13 min and 15 min A/B was 0:100, at 17 min A/B was 85:15 and remained at this gradient until the end of the 28 min run. For negative ionization mode, the flow rate was 0.18

mL/min and 10 mM ammonium acetate (mobile phase A) and 100% HPLC grade acetronitrile (mobile phase B) was used for the binary gradient elution. The mobile phase gradient elution was as follows: at 0 min A/B was 90:10, at 15 min A/B was 20:80, at 18 min A/B was 90:10 and remained at this gradient until the end of the 28 min run. The column was kept at room temperature during LC analysis.

Details on the MRM ion transitions, dwell time and collision energy for the three analytes and deuterated compounds are located in Table 3.3. Details of the different source parameters required for the ESI interface and the triple quadrupole mass spectrometer are summarized in Table 2.4.

3.3.7 Data Analysis

The Analyst® software (version 1.4.1) was used to control the instrument, create acquisition methods, inject/submit the samples and perform data analysis.

Quantification

A multi-point internal standard calibration curve was used to quantify the concentrations of the three human pharmaceuticals in the water samples. The calibration standards were prepared with different known concentrations of the three analytes and a known concentration of each deuterated surrogate. The known concentrations of analytes and deuterated compounds were plotted against the signal of the instrument (i.e. peak area of the analyte and surrogate) in each calibration standard. A linear through zero calibration curve was plotted for each of the three human pharmaceuticals, with a ratio of analyte peak area to corresponding peak area of deuterated compound (peak $area_{analyte}$/peak $area_{surrogate}$) on the y-axis and a ratio of known analyte concentration to known concentration of surrogate

Table 3.3: Multiple reaction monitoring (MRM) ion transitions, dwell time, and collision energies for carbamazepine, gemfibrozil, ibuprofen, and corresponding deuterated surrogates.

Pharmaceutical	**Precursor Ion (m/z)**	**Product Ion (m/z)**	**Dwell Time (msec)**	**Collision Energy (eV)**
Carbamazepine	237	194	10	20
D_{10}-Carbamazepine	247	204	10	25
Gemfibrozil	249	121	35	-15
D_6-Gemfibrozil	255	121	15	-15
Ibuprofen	205	161	40	-10
D_3-Ibuprofen	208	164	20	-10

(concentration$_{analyte}$/concentration$_{surrogate}$) on the x-axis. The unknown concentrations in the field samples were determined from the linear through zero regression line, with a correlation coefficient (r^2 value) usually greater than 0.98.

In contrast to other analytical methods, in which the calibration standards are prepared in an environmental matrix, calibration standards for this method were prepared in a solvent, evaporated and then reconstituted with an aqueous solution ($^{13}C_6$-sulfamethazine phenyl in nanopure water).

It has been well documented that LC-ESI-MS/MS analysis is susceptible to matrix effects in which components of the matrix co-elute with analytes of interest during the formation of gas-phase ions in the electrospray ionization source interface. For this analytical method, deuterated compounds (isotopically labeled compounds) were added into the blank sample, spiked nanopure water samples and field samples before extraction to determine the extraction efficiency of the deuterated surrogates within the different matrices and correct for potential matrix effects. Without the addition of the labeled surrogates for each analyte of interest, it is very difficult to correct for potential matrix effects in the field samples and have confidence in the response of the instrument knowing that the signal could be suppressed or enhanced by the matrix.

Recovery Rates

Recovery rates were determined for the three analytes and deuterated surrogates in the spiked nanopure samples, and for the deuterated surrogates in the field samples. The recovery rates were determined by comparing the peak area of the analyte or deuterated surrogate to the average peak area of the control standards (known volume of spiking solutions).

The range of recovery rates calculated for the deuterated surrogates in the raw and treated water samples is summarized in Table 2.5. The recovery rates for the three deuterated compounds in the field samples were very different from the recovery rates of these compounds in the nanopure water samples. The difference in recovery rates between the nanopure water samples, the raw water samples and the treated water samples, confirms that the matrix has a significant impact on the response of the analyte during LC-ESI-MS/MS analysis. The recovery rates of the deuterated surrogates in the field samples also shows that certain compounds are impacted to a greater extent by the matrix than other compounds. In general, more suppression was observed with gemfibrozil and ibuprofen, which were both analyzed in negative ion mode. Carbamazepine was impacted by the matrix, but to a lesser degree compared to the ibuprofen and gemfibrozil.

MDLs

The method detection limit (MDL) was based on ten 400 mL spiked nanopure water replicates, and the lowest value found in the linear range of the calibration curve. The MDLs for carbamazepine, gemfibrozil and ibuprofen were 1 ng/L, 1 ng/L and 5 ng/L, respectively. The signal to noise ratio for determining the MDLs was 3:1.

3.4 Results

3.4.1 Drinking Water Treatment Facility Study

Presence of Selected Human Pharmaceuticals

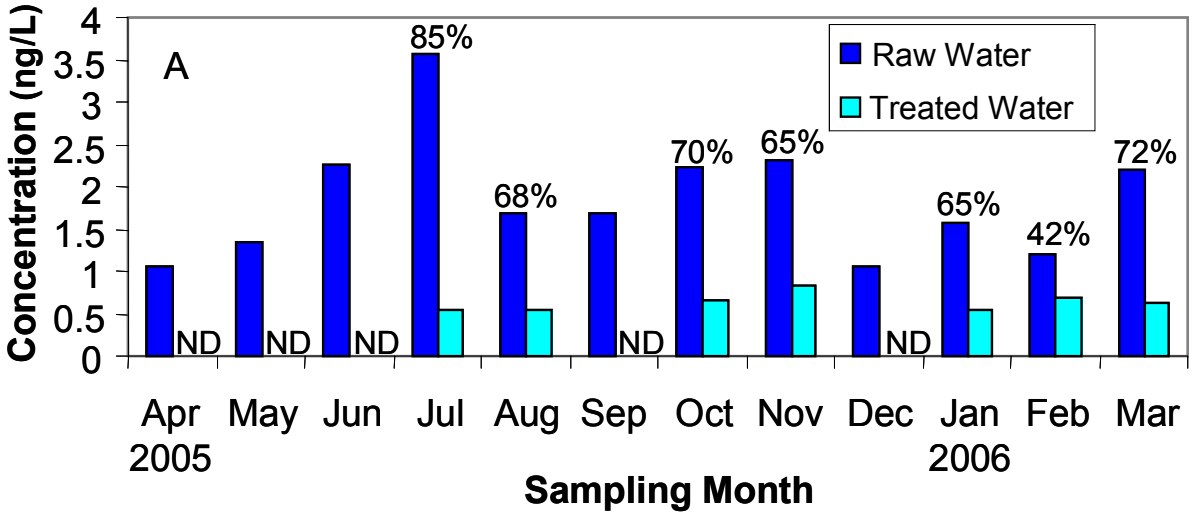
Carbamazepine, gemfibrozil and ibuprofen were routinely detected in most duplicate raw and treated water samples collected at Facility A and Facility B over the sampling period. In general, higher concentrations were present in the raw water samples collected from Facility B.

Gemfibrozil, a lipid regulating agent, was present in all raw water samples collected from both facilities during the sample collection period (Figure 3.2). Although there was a detection frequency of 100% in the raw water samples, the mean concentrations in the raw water samples collected at each facility were very low, usually below 5 ng/L. The only exception was for the raw water samples collected in March 2006 from Facility B, when the mean concentration approached 6 ng/L. The range of mean concentrations in raw water samples collected from Facility A was from 1.1 ng/L in December 2005 to 3.6 ng/L in July 2005. Higher mean concentrations of gemfibrozil were present in the raw water samples collected from Facility B, with a range in concentrations of 1.5 ng/L in April 2005 to 5.9 ng/L in March 2006.

The mean concentrations of gemfibrozil detected in the treated water samples were lower at both water treatment facilities. In some months, gemfibrozil was not detected in the treated water samples collected at either facility. This occurred in the spring and early summer months, as well as December 2005. Concentrations of gemfibrozil present in treated water samples collected at Facility A were below the MDL of 1 ng/L. For the treated water samples collected at Facility B, the mean concentrations were above the MDL for samples collected in the summer and fall months, but were below the MDL during the winter months. The highest mean concentration of gemfibrozil was 2.9 ng/L in the treated water samples collected from Facility B in October 2005.

Ibuprofen, an analgesic and anti-inflammatory drug, was detected in raw water samples collected in July 2005 and from September 2005 to March 2006 (Figure 3.3). In contrast to gemfibrozil that was detected in all raw water samples, the frequency of

Figure 3.2: Mean concentrations and percent differences ([$Conc._{raw}$ − $Conc._{treated}$]/[$Conc._{raw}$] ×100) of gemfibrozil in raw and treated water samples collected from Facility A (A) and Facility B (B) from April 2005 to March 2006 (n=2).


A
Concentration (ng/L)
4
3.5
3
2.5
2
1.5
1
0.5
0
Raw Water
Treated Water
ND
ND
ND
85%
68%
ND
70%
65%
ND
65%
42%
72%
Apr
2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
2006
Feb
Mar
Sampling Month

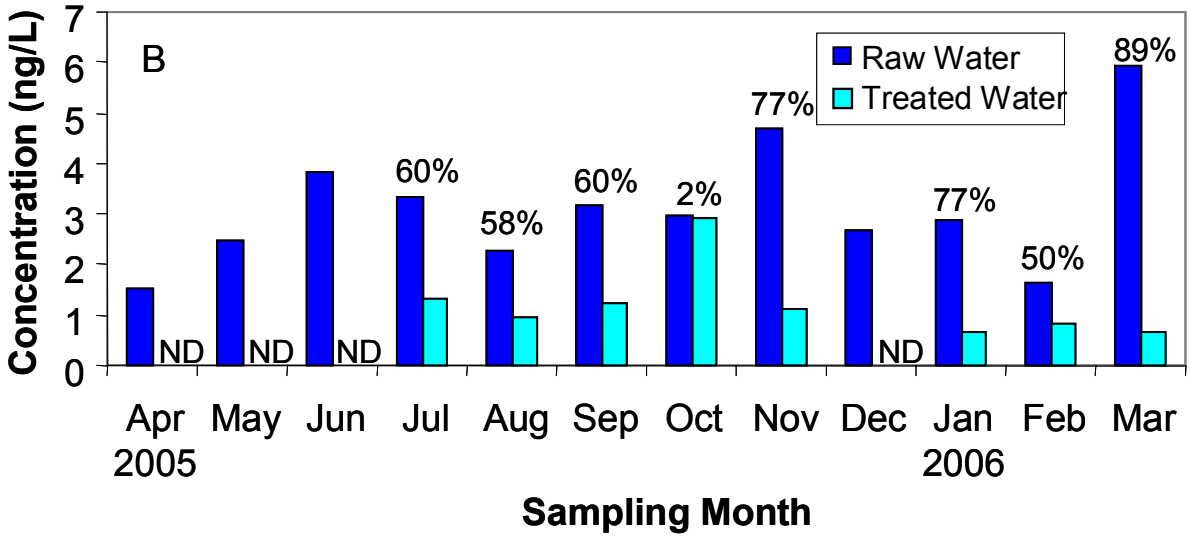
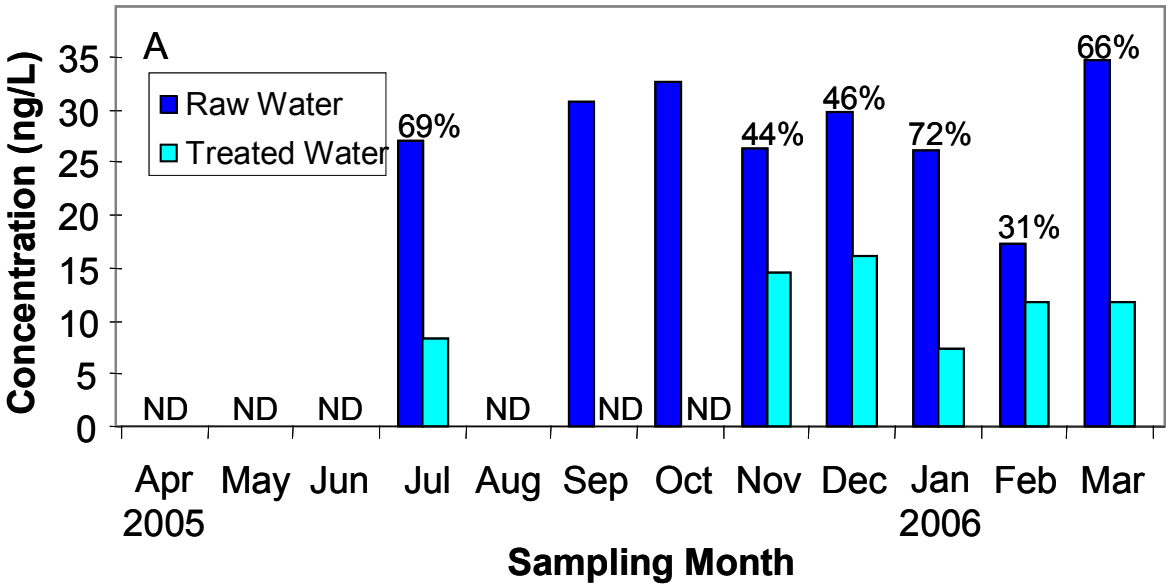

B
Concentration (ng/L)
7
6
5
4
3
2
1
0
Raw Water
Treated Water
ND
ND
ND
60%
58%
60%
2%
77%
ND
77%
50%
89%
Apr
2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
2006
Feb
Mar
Sampling Month

Figure 3.3: Mean concentrations and percent differences ([$Conc._{raw}$ − $Conc._{treated}$]/[$Conc._{raw}$] ×100) of ibuprofen in raw and treated water samples collected from Facility A (A) and Facility B (B) from April 2005 to March 2006 (n=2).


A
Raw Water
Treated Water
Concentration (ng/L)
0
5
10
15
20
25
30
35
ND
ND
ND
69%
ND
ND
ND
44%
46%
72%
31%
66%
Apr
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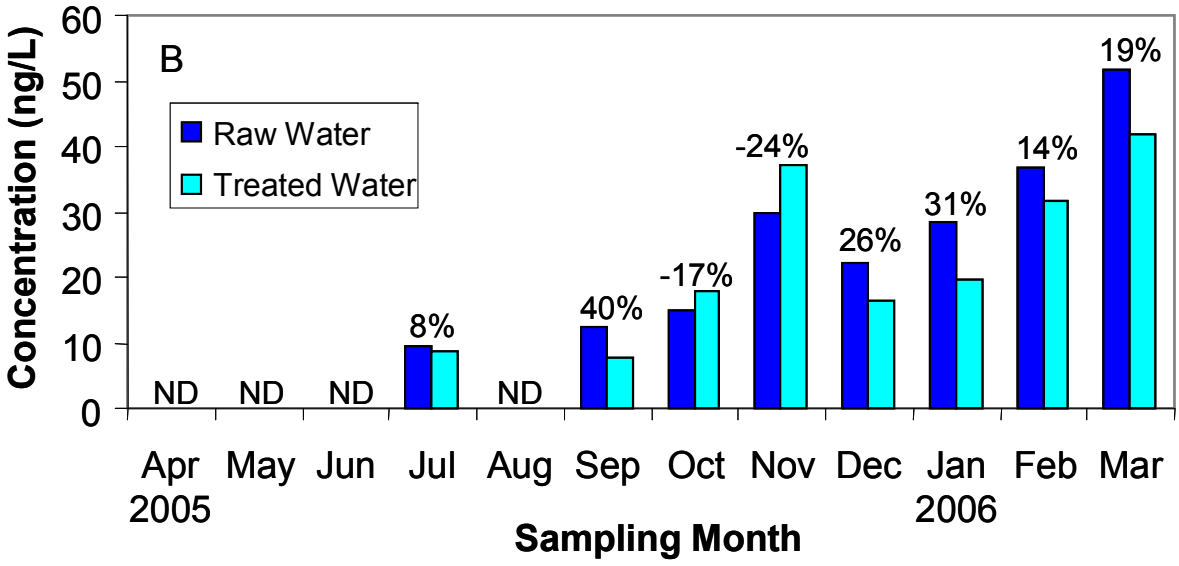
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
2006
Feb
Mar
Sampling Month


B
Raw Water
Treated Water
Concentration (ng/L)
0
10
20
30
40
50
60
ND
ND
ND
8%
ND
40%
-17%
-24%
26%
31%
14%
19%
Apr
2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
2006
Feb
Mar
Sampling Month

ibuprofen detected in the raw water samples was 67%. Higher mean concentrations of ibuprofen were detected in the raw water samples, except for samples collected in October 2005 and November 2005 when slightly higher concentrations were present in the treated water samples collected from Facility B. During the winter months (January, February and March) higher mean concentrations were detected in the raw water samples collected from Facility B compared to Facility A. The opposite trend was observed for the other sampling months, in which higher mean concentrations of ibuprofen were detected in the raw water samples collected from Facility A. The range of mean concentrations detected in the raw water samples from Facility A was 16.4 ng/L (February 2006) to 33.0 ng/L (March 2006). In the raw water samples collected at Facility B, there was more variability in the concentrations detected. The lowest mean concentration detected at this facility was 9.4 ng/L (July 2005) and highest mean concentration was 51.6 ng/L (March 2006).

Ibuprofen was detected in only half of the treated water samples collected during the sampling period at Facility A. Detectable concentrations of ibuprofen were found in July 2005 and November 2005 to March 2006. The highest mean concentration was found in December 2005 at 15.3 ng/L, and the lowest mean concentration was found in the treated water samples collected in January 2006 at 7.1 ng/L at Facility A. The mean concentrations of ibuprofen in the treated water samples collected from Facility B were more variable, with concentrations ranging from 7.5 ng/L to 41.7 ng/L. Detectable concentrations of ibuprofen in treated water samples were found in more sampling months at Facility B compared to Facility A, in which ibuprofen was found in samples collected in September and October 2005.

Carbamazepine is a prescribed pharmaceutical mainly used in the treatment of epilepsy, but can also be used to treat some types of depression. Carbamazepine was detected in all raw water samples collected at both facilities, with higher concentrations detected in the samples collected at Facility B (Figure 3.4). The mean concentrations detected in the samples collected at Facility B were 2 to 3 times higher than the concentrations detected at Facility A in the raw water samples. The only exception was August 2005, when the mean concentration of carbamazepine in the raw water samples collected at Facility B was close to 1µg/L.

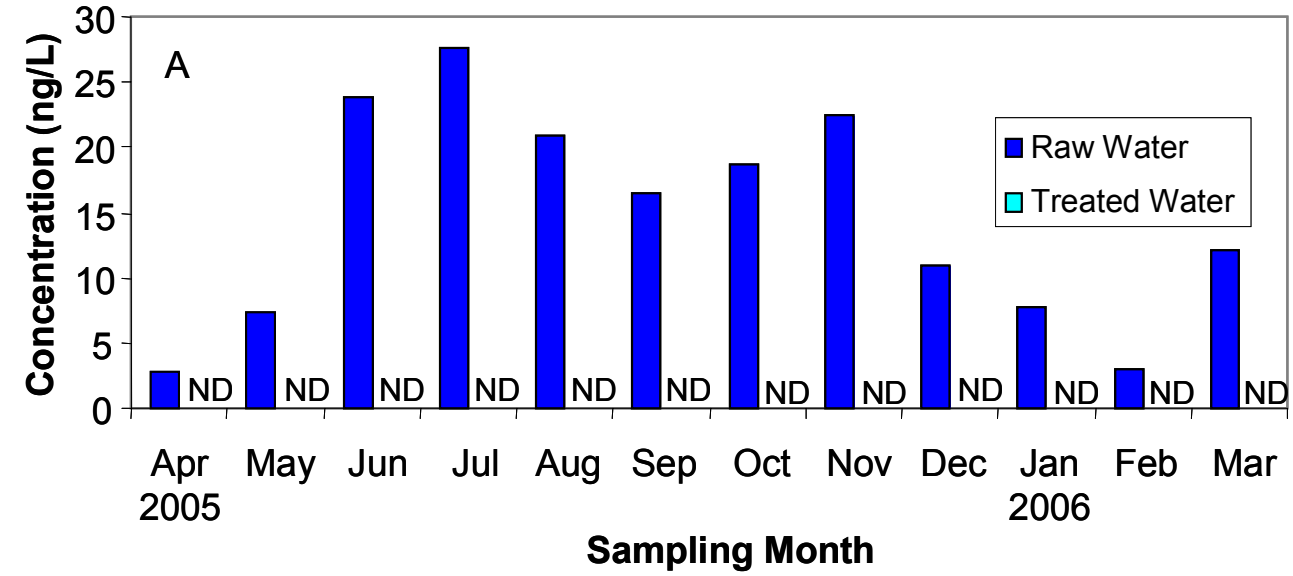
The concentrations of carbamazepine detected at Facility A ranged from 2.8 ng/L in April 2005 to 27.6 ng/L in July 2005 in the raw water samples. The concentrations detected in the raw water samples collected from Facility B covered a wider range, with the lowest mean concentration of 7.8 ng/L and the highest mean concentration of 988.3 ng/L (Figure 3.4).

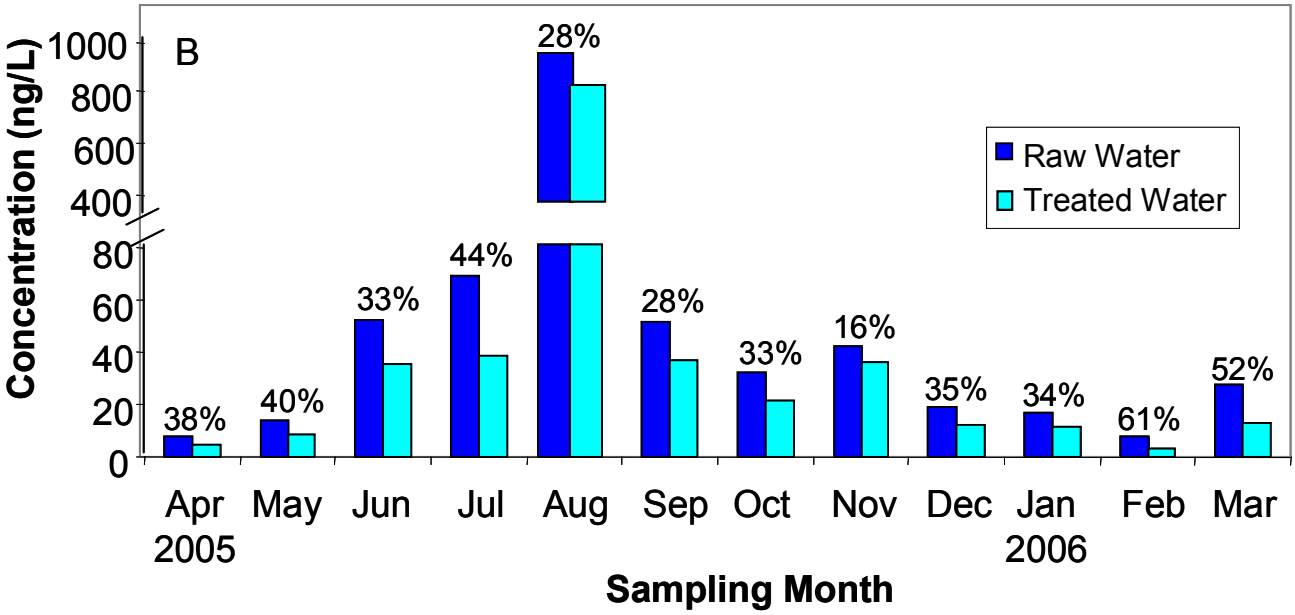
In contrast to ibuprofen and gemfibrozil, carbamazepine was not detected in any of the treated water samples collected at Facility A during the sampling period. However, carbamazepine was detected each month in all treated water samples collected from Facility B. The maximum mean concentration of carbamazepine in the treated water samples was 713.6 ng/L in August 2005.

Percent Differences in Concentrations

The percent differences of the three human pharmaceuticals were calculated based on the mean concentrations found in the raw and treated water samples during the sampling period. The percent differences should be interpreted carefully because sample collection did not take into account the retention times within the water treatment facilities. The percent

Figure 3.4: Mean concentrations and percent differences ([$Conc._{raw}$ − $Conc._{treated}$]/[$Conc._{raw}$] ×100) of carbamazepine in raw and treated water samples collected from Facility A (A) and Facility B (B) from April 2005 to March 2006 (n=2).


A
Concentration (ng/L)
30
25
20
15
10
5
0
ND
Raw Water
Treated Water
Apr
2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
2006
Feb
Mar
Sampling Month


B
Concentration (ng/L)
1000
800
600
400
80
60
40
20
0
38%
40%
33%
44%
28%
28%
33%
16%
35%
34%
61%
52%
Raw Water
Treated Water
Apr
2005
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
2006
Feb
Mar
Sampling Month

difference of gemfibrozil was found to be variable over the sample collection period, especially at Facility B. Higher percent differences were observed at Facility A, with percent differences ranging from 32% to 85% (Figure 3.2). In general, higher percent differences were observed in samples collected in the summer and early fall months. The percent difference of gemfibrozil at Facility B was more variable, ranging from 2% to 89%. Higher percent differences were observed in the winter months at Facility B, which was the opposite trend observed at Facility A. The 2% difference of gemfibrozil at Facility B in October 2005 was an exception compared to the percent differences observed during the other sampling months (Figure 3.2).

The percent difference of ibuprofen in the water samples collected at both facilities shows that Facility A is more effective at reducing ibuprofen to non-detectable levels. The percent difference of ibuprofen during treatment was between 31% and 72% at Facility A, in comparison to a maximum percent difference of 40% at Facility B. In October and November 2005, ibuprofen was found at higher concentrations in the treated water samples collected at Facility B compared to the raw water samples.

The percent difference of carbamazepine in the water samples collected from Facility A was the most significant with 100% difference for each sampling month. These results show that the treatment processes at Facility A were able to reduce the concentrations of carbamazepine to non-detectable levels in the treated water samples for all sampling months. The treatment processes at Facility B were not as efficient in reducing carbamazepine in the treated water, with percent differences ranging from16% to 61%. The lowest percent

difference was for samples collected in November and the highest percent difference was for samples collected in February.

Seasonal Variability

There were no distinct seasonal trends observed with the concentrations of gemfibrozil in the water samples collected over the sampling period. The low concentrations detected made it difficult to observe any distinct seasonal trends, especially when most of the concentrations in the treated water samples are close to the MDL. Distinct seasonal variability was observed for ibuprofen, with high concentrations (i.e. greater than 15 ng/L) detected in the late fall and winter months. The most significant seasonal variability was observed for carbamazepine, where the highest mean concentrations were observed in the water samples collected in the summer and early fall months. This seasonal trend was observed in water samples collected from both facilities. In addition, the seasonal profile of carbamazepine also showed a large peak in the concentration of carbamazepine in August 2005.

3.4.2 GUDI Study

Presence of Selected Human Pharmaceuticals

A preliminary study investigated the presence of the three human pharmaceuticals in raw water samples collected from six susceptible GUDI wells and two deep groundwater wells in the fall of 2005. The results showed that carbamazepine was the only target analyte detected in the raw water samples, with detectable concentrations found in Well G and Well H on both sample collection days.

Lower concentrations of carbamazepine were detected in the GUDI wells compared to the concentrations detected in the raw water samples collected from the drinking water

treatment facility study during the fall season. In addition, lower mean concentrations were present in the raw water collected from Well H compared to Well G. Carbamazepine was detected at concentrations of 10 ng/L in samples collected from Well G and approximately 4 ng/L in samples collected from Well H (Table 3.4).

3.5 Discussion

This study was one of the first research initiatives to investigate the presence and seasonal variability of carbamazepine, gemfibrozil and ibuprofen in Canadian drinking water supply systems. The results from this study demonstrate that ibuprofen and carbamazepine, and to a lesser extent gemfibrozil, are routinely detected in water samples collected from two full scale drinking water treatment facilities in Southern Ontario, Canada. The study also provides evidence that carbamazepine is found in groundwater supplies in Southern Ontario, Canada. The concentrations detected were in the low ng/L range, which is comparable to the limited studies that have reported these compounds in drinking water systems (Stackelberg et al., 2004; Vieno et al., 2005; Hua et al., 2006a; Hummel et al., 2006; Kim et al., 2006).

The presence of these human pharmaceuticals in the raw water samples collected from the two facilities was expected based on the numerous wastewater treatment facilities located within the watershed. It has been well documented that treated wastewater effluents are one of the major pathways for pharmaceuticals entering surface waters and the aquatic environment (Heberer, 2002b). Studies have shown that samples collected further downstream from wastewater treatment effluent discharges have lower concentrations detected, indicating that dilution and higher water flows have an impact on the concentrations of these contaminants (Stumpf et al., 1999; Metcalfe et al., 2003b; Wiegel et al., 2004; Bendz et al., 2005; Hua et al., 2006b).

Table 3.4: Concentrations (ng/L) of carbamazepine, gemfibrozil and ibuprofen in duplicate raw water samples collected from two groundwater wells (Well G and Well H) in fall 2005.

Pharmaceutical	Sampling Date	Well G	Well H
Carbamazepine	September 27th 2005	11.5	4.2
	September 27th 2005	12.2	4.1
	October 11th 2005	9.8	3.2
	October 11th 2005	11.0	4.1
Gemfibrozil	September 27th 2005	ND	ND
	September 27th 2005	ND	ND
	October 11th 2005	ND	ND
	October 11th 2005	ND	ND
Ibuprofen	September 27th 2005	ND	ND
	September 27th 2005	ND	ND
	October 11th 2005	ND	ND
	October 11th 2005	ND	ND

ND equals not detected

Relatively high concentrations of carbamazepine and ibuprofen were detected in the raw water samples collected at both facilities, despite the nearest wastewater treatment facility being located approximately 15 km upstream of Facility A, and relatively high water flows. Environmental persistence and inefficient dilution of the treated wastewater effluents provides an explanation of the concentrations detected in the raw water samples at both facilities. In addition, a large number of wastewater treatment facilities discharge into the tributaries and main water system of this particular watershed. The higher concentrations in the raw water samples could also be attributed to the inefficiency of the wastewater treatment technologies to reduce the concentrations of these contaminants or the effectiveness of natural biodegradation and sorption processes in the surface waters to lower the concentrations of these contaminants (Andreozzi et al., 2003; Bendz et al., 2005; Vieno et al., 2005).

When comparing the concentrations of the pharmaceuticals found in the raw water samples collected from each facility, higher mean concentrations were detected in the raw water samples collected from Facility B. This particular facility is located further downstream of Facility A, and therefore has a greater potential for source water being exposed to more point sources of contamination (i.e. application of biosolids to agricultural land). There are a number of wastewater treatment facilities that discharge into receiving waters upstream of the raw water intake for Facility B. In addition, one of the tributaries entering the main river system just above the intake pipe of Facility B comes from an intensively agricultural area, which could provide another source of contamination. Although carbamazepine has been detected at higher concentrations in urbanized areas, Hao et al. (2006) and Lissemore et al. (2006) have detected the presence of carbamazepine in surface waters near agricultural inputs in this watershed.

For the most part, lower concentrations were present in the treated water samples compared to the raw water samples, which suggests that the current treatment technologies employed are capable of reducing the parent compound to some extent. The only exception was for the water samples collected in October and November 2005 at Facility B, in which ibuprofen was detected at higher concentrations in the treated water samples compared to the raw water samples. One possible explanation could be due to the presence of organic matter in the samples collected during these months and as a result extraction efficiency of the treated water samples being compromised. This could also explain the low percent difference (about 2% difference) of gemfibrozil in the raw water and treated water samples collected in October 2005 from Facility B. Another explanation for the higher concentrations of ibuprofen in the treated water samples could be due to the conjugated forms of ibuprofen entering the treatment facility being degraded during treatment and being released as the parent form. Since the conjugated forms of these human pharmaceuticals were not investigated during this study, it is difficult to say if these ibuprofen conjugates entered the facility, were degraded during treatment, and then persisted in the treated water samples.

Recent studies have suggested the cleavage of the conjugated pharmaceuticals during treatment processes may result in higher concentrations in the treated wastewater and water samples. Ternes (1998) reported that 14% of ibuprofen is excreted in a conjugated form (i.e. via glucuronidation). This would explain the increase in ibuprofen detected in the treated water samples by the treatment processes cleaving the glucuronide from the rest of the molecule. However, one would have to assume that the wastewater treatment facilities did not degrade the conjugated form and this form persisted in the natural surface waters.

There were differences in the ability of the two facilities to reduce the concentrations of these compounds, with Facility A being more effective compared to Facility B. As the sample collection times did not take into account the retention times with the treatment facilities, actual removal rates could not be accurately calculated, but percent differences could be reported. The percent differences compared the mean concentration in the raw water samples to the mean concentration in the treated water samples for each month and for each compound. The percent differences for gemfibrozil were higher at both facilities compared to the other two compounds of interest. This would suggest that the concentrations of gemfibrozil were reduced to greater extent compared to ibuprofen and carbamazepine. As the concentrations of gemfibrozil were quite low, it was challenging to make conclusions when the concentrations detected were around the method detection limit for this compound.

The percent differences for ibuprofen were quite variable, ranging from -24% to close to 100%. Higher percent differences were observed for samples collected at Facility A, indicating that this facility is more effective in reducing ibuprofen from raw water. However, relatively low percent differences were observed at both facilities compared to the removal rates reported for ibuprofen in previous studies, which showed greater than 80% removal during wastewater and water treatment (Ternes, 1998; Strenn et al., 2004; Westerhoff et al., 2005; Kim et al., 2006). A possible explanation is the differences in treatment technologies available at both facilities compared to other studies. Huber et al. (2003) and Zwiener and Frimmel (2000) both found that ibuprofen was not effectively removed with the addition of ozone, but was more effectively removed with advanced oxidation processes (i.e. ozone and hydrogen peroxide). The lack of advanced oxidation processes may contribute to the lower removal rates of ibuprofen observed at both facilities. In addition, Castiglioni et al. (2006)

found that biodegradation of ibuprofen appears to be a major elimination process in natural waters; although other studies have suggested that log K_{ow} value of 3.5 or higher may allow ibuprofen to sorb to particulate matter and be removed by sorption processes (Aston et al., 2004). Ternes et al. (2004) determined solid-water distribution coefficient values for a number of pharmaceuticals in sewage sludge, and found that sorption did not have a major role in the removal of ibuprofen during wastewater treatment. In either case, concentrations of ibuprofen can be reduced by different elimination processes and appears to be site-specific, and in some cases dependent on the season (Tixier et al., 2003; Castiglioni et al., 2006).

The most interesting data reported from this study was the percent differences for carbamazepine between the two treatment facilities. The percent differences between the raw and treated water samples was close to 100% for all samples collected from Facility A, while percent differences at Facility B ranged from 16% to 61%. This suggests that the treatment processes used at each facility have an impact on the ability to reduce the concentrations of carbamazepine. Facility A was very effective at reducing this pharmaceutical, which may be explained by the use of ozonation at this facility. There is a reasonable amount of information in the literature that would suggest that ozone was likely responsible for the non-detectable levels of carbamazepine observed in the treated water samples collected from Facility A. Studies have shown that oxidation processes, like ozonation, are effective at reducing carbamazepine by greater than 90% during treatment (Ternes et al., 2002; Ternes et al., 2003; McDowell et al., 2005; Hua et al., 2006a). Although ozonation may not be completely responsible for the reduction of carbamazepine, it is a reasonable explanation for the differences observed between facilities. Another possible explanation for the high removal of carbamazepine at Facility A is that the surface water taken from the river is stored in a large

reservoir for a selected amount of time, which may allow carbamazepine to be photodegraded. Limited work has investigated the degradation of carbamazepine by photolysis, but carbamazepine does not seem to be impacted by sunlight, especially in the presence of organic matter (i.e. humic acids) (Boreen et al., 2003; Vogna et al., 2004; Chiron et al., 2006). Chiron et al. (2006) reported that chloride may enhance photodegradation of carbamazepine by the chloride interacting with Fe(III) colloids under irradiation to create chloride radicals. A recent study by Pereira et al. (2007) investigated the reduction of carbamazepine and other pharmaceuticals by UV photolysis and UV/H_2O_2 photolysis. The results showed direct UV photolysis was not effective at reducing carbamazepine, but the application of UV and hydrogen peroxide was effective at reducing this compound in water samples.

The results from this part of the study show that low doses of ozone (1.5 to 3 mg/L) appears to be able to reduce the concentrations of carbamazepine to non-detectable levels and that seasonal changes in water quality and quantity do not necessarily impact the ability of this facility to reduce the concentrations of carbamazepine.

The absence of ozonation in Facility B may explain the detectable levels of carbamazepine in the treated water samples. Studies have demonstrated that conventional drinking water treatment processes are not very effective at reducing pharmaceuticals, especially persistent compounds, like carbamazepine (Ternes et al., 2002). In some cases, advanced oxidation processes are the only treatments that are capable of reducing selected pharmaceuticals, in which ozone and hydrogen peroxide or UV and hydrogen peroxide are applied to increase the oxidants available for transforming the compounds of interest (Huber et al., 2003; Vogna et al., 2004; Miao et al., 2005). In addition, recent studies have reported that the concentrations of some pharmaceuticals are reduced to various extents by the

application of new membrane technologies and innovative filtration devices (Nghiem et al., 2005; Radjenovic et al., 2007).

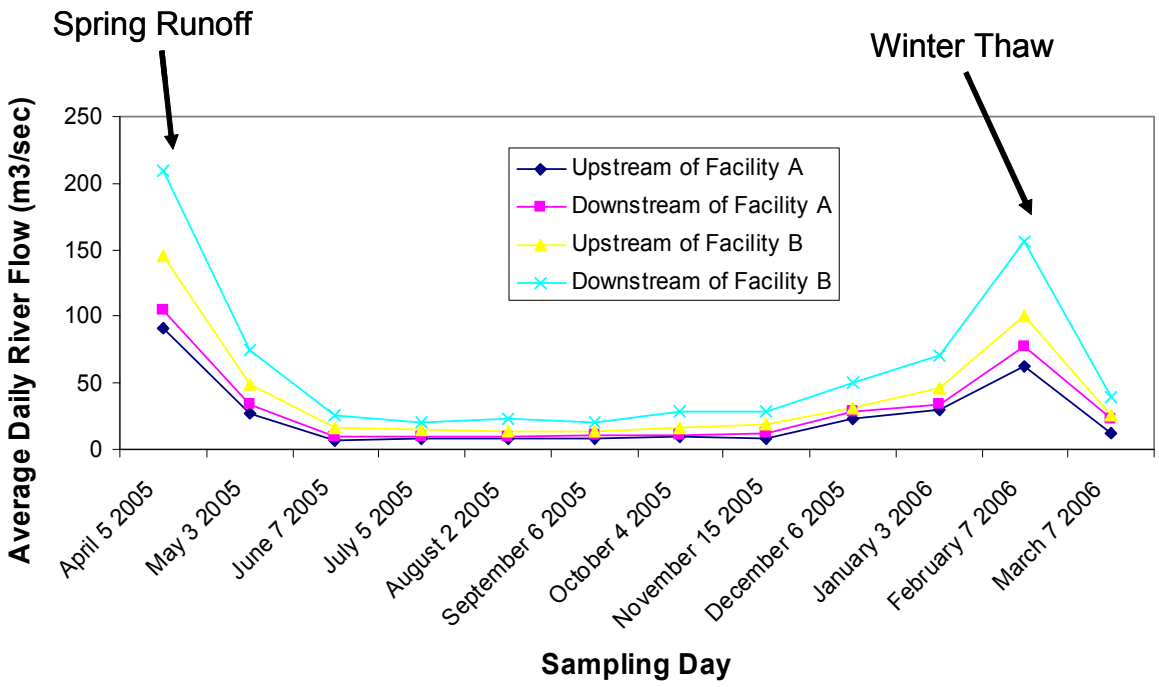
The concentrations of carbamazepine and ibuprofen detected showed seasonal variability. Ibuprofen was detected at higher concentrations during the fall and winter months, while carbamazepine was detected at higher concentrations during the summer and early fall months. The presence of ibuprofen can be explained by the increase in consumption of this analgesic and anti-inflammatory drug during cold and flu season (Tixier et al., 2003; Vieno et al., 2005). The increase in consumption of this compound increases the amount being excreted and entering wastewater treatment facilities. These facilities can be overwhelmed with the amount of ibuprofen entering the facility (i.e. increase in water flow rates), and therefore more ibuprofen is released into surface waters through treated wastewater effluents. The increase in ibuprofen concentrations, and other pharmaceuticals, detected in surface waters may also be the result of increases in rainfall events, with treatment facilities having a lower efficiency during precipitation events (Tixier et al., 2003; Boyd et al., 2004a).

Another explanation is that during the fall and winter months, the water temperature tends to be cooler. The low temperatures may hinder the microorganisms in the wastewater treatment facilities and as a result, ibuprofen is still present after treatment. It has been suggested that ibuprofen is most likely removed by biodegradation processes both in the natural surface waters and the treatment facilities (Andreozzi et al., 2003; Tixier et al. 2003; Páxeus, 2004). The effectiveness of biodegradation is correlated to temperature, biodegradation decreases with decreases in water temperatures (Vieno et al., 2005). During the fall and winter months, the lower temperatures would result in less biodegradation in the wastewater treatment facilities, which would result in the concentrations of ibuprofen not

being reduced as much by biodegradation processes in either the wastewater treatment facilities or surface waters (Andreozzi et al., 2003; Vieno et al., 2005). The process of photodegradation may also result in a possible elimination pathway for ibuprofen and may explain the higher concentrations found in the fall and winter months. Research to date has shown that photodegradation is not a major elimination pathway for ibuprofen based on its chemical structure and resistance to absorb sunlight (Boreen et al., 2003; Tixier et al., 2003). Studies have shown that sorption to sediment may be a possible elimination pathway for ibuprofen. An elimination rate constant for ibuprofen through sedimentation was determined at 0.005 to 0.01/day in a study by Tixier et al. (2003) in the epilimnion of lake water.

Loraine and Pettigrove (2006) confirmed the presence of more compounds and higher concentrations detected in the dry and summer months (August to November in Southern California). The presence of carbamazepine in the summer and early fall months can be contributed to the low water levels and water flows during the hot and dry months (Hua et al., 2006b; Loraine and Pettigrove, 2006). Figure 3.5 illustrates the water flow levels at monitoring sites located upstream and downstream of the two facilities over the sampling period. This figure shows that lower water flows occurred during the summer and early fall months, and higher flows were observed during the spring and winter months. The lower water flows would result in less dilution of the treated wastewater effluents and therefore a higher proportion of the surface waters would be comprised of treated effluent, which could then be used for drinking water production. The seasonal trends observed for carbamazepine cannot be explained by consumption patterns because carbamazepine is mainly used as an anti-epileptic drug, which is prescribed and taken by patients throughout each season.

Figure 3.5: Average daily flow rates (m^3/sec) at sampling sites located upstream and downstream of Facility A and Facility B from April 2005 to March 2006.


Spring Runoff
Winter Thaw
Average Daily River Flow (m3/sec)
250
200
150
100
50
0
Upstream of Facility A
Downstream of Facility A
Upstream of Facility B
Downstream of Facility B
April 5 2005
May 3 2005
June 7 2005
July 5 2005
August 2 2005
September 6 2005
October 4 2005
November 15 2005
December 6 2005
January 3 2006
February 7 2006
March 7 2006
Sampling Day

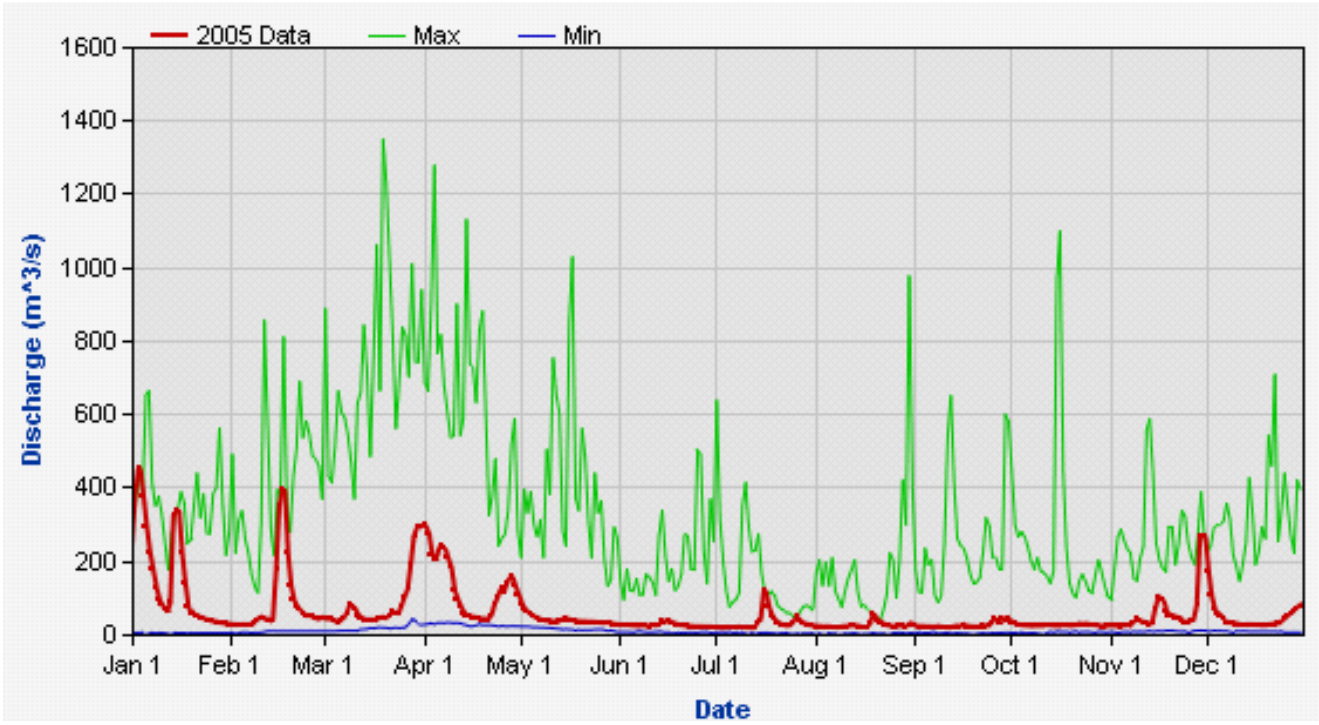
In addition to carbamazepine being detected in the samples collected from the drinking water treatment facilities, this compound was also found at low concentrations in raw water samples collected from two groundwater well locations, Well G and Well H. Both of these wells are located in the flood plain of the river, and therefore under a greater threat of being influenced by surface waters. Higher concentrations were present in the Well G (urban well) compared to the rural well (Well H). The presence of carbamazepine found at Well G can be explained by higher loads of this human pharmaceutical being discharged into surface waters in areas with greater population density, and as a result, an increased exposure for the wells located in a population dense area. A study by Lissemore et al. (2006) showed similar results in which carbamazepine was detected at low concentrations in surface waters from agricultural areas and higher concentrations in surface waters collected in urbanized areas. The authors concluded that one reason for the presence of carbamazepine in rural or agricultural areas is the application of biosolids to agricultural land as a soil amendment and possibility of surface runoff. The results from this study confirm the persistence of carbamazepine in different aquatic environments, and its ability to resist elimination processes (Clara et al., 2004). Field studies have shown that this compound is not attenuated during bank filtration and has been detected in shallow wells, water supply wells and groundwater samples (Heberer, 2002b; Drewes et al., 2002; Heberer et al., 2002; Clara et al., 2004; Stolker et al., 2004; Rabiet et al., 2006; Ternes et al., 2007). Gemfibrozil and ibuprofen were not detected in any of the selected groundwater wells investigated in the current study, but have been detected in groundwater wells located near contaminated surface waters in Germany at concentrations above 200 ng/L (Heberer, 2002a).

The large peak of carbamazepine in the water samples collected from Facility B in August 2005 appears to be a rare event, but can be expected based on previous data collected by Lissemore et al. (2006) which has shown similar peaks in concentrations of pharmaceuticals, including carbamazepine, in the summer months. Data collected by the Water Survey of Canada showed that the water flow rates at a monitoring site close to Facility B were low, which may explain the lack of dilution of the wastewater effluents during this time period (Figure 3.6) (Water Survey of Canada, 2006). However, the flow rates observed during the rest of the summer of 2005 were comparable. Other possible explanations for the large peak in concentration of carbamazepine could be related to wastewater treatment facilities not operating at optimal levels, changes in surface water hydrology preventing elimination processes from reducing carbamazepine, the saturation of certain processes capable of reducing carbamazepine during this time period or the release of the parent compound from the conjugated form (Stamatelatou et al., 2003; Bendz et al., 2005; Lissemore et al., 2006).

3.6 Conclusions

The study shows that carbamazepine, gemfibrozil and ibuprofen are detected in raw water and treated water samples collected from two full scale drinking water treatment facilities in a Southern Ontario watershed. The concentrations detected were in the low ng/L range, which is comparable to concentrations detected throughout North America and Europe. In addition, this study clearly illustrates that current advanced treatment technologies (i.e. oxidation) applied in some drinking water systems are capable of reducing these compounds from raw water, compounds which are known to be quite persistent in environmental

Figure 3.6: Daily flow rates (m^3/sec) from January 2005 to December 2005 at a monitoring site located near Facility B (data obtained from the Water Survey of Canada).


2005 Data
Max
Min
1600
1400
1200
1000
800
600
400
200
0
Discharge (m^3/s)
Jan 1
Feb 1
Mar 1
Apr 1
May 1
Jun 1
Jul 1
Aug 1
Sep 1
Oct 1
Nov 1
Dec 1
Date

matrices. The consequences and possible impacts of these compounds in drinking water supplies are largely unknown, especially during chronic, sub-therapeutic exposure of a mixture of these compounds to populations who maybe immune compromised. This study is one of the first to look at the presence and variability of pharmaceuticals in operational drinking water treatment supply systems over a long-term period (over each season). The information gathered from this study will provide exposure data for selected pharmaceuticals in Ontario drinking water systems and serve as a starting point for attempting to assess the impacts of these compounds in drinking water supplies.

CHAPTER 4

General Discussion

This thesis investigated the occurrence and seasonal variability of selected human and veterinary pharmaceuticals in drinking water supply systems in a Southern Ontario watershed. The studies conducted provide evidence that selected pharmaceuticals, representing different therapeutic classes, are present in raw and treated water samples collected from two full-scale municipal drinking water treatment facilities, and selected groundwater wells. The most frequently detected compounds were carbamazepine, gemfibrozil, ibuprofen, bezafibrate, naproxen, trimethoprim, sulfamethoxazole and lincomycin HCl, all of which have recently been identified as dominant pharmaceuticals found in the environment (Nikolaou et al., 2007). Three compounds, carbamazepine, lincomycin HCl and sulfamethoxazole, were also detected in raw water samples collected from groundwater wells within the same watershed. To our knowledge, this was one of the first studies that detected bezafibrate in drinking water samples collected in North America, and the presence of lincomycin in groundwater supplies.

The presence of selected pharmaceuticals in surface water (raw water) samples was expected due to the large number of wastewater treatment facilities, which discharge effluent into the same receiving environment as the source water used for drinking water production. Numerous studies have concluded that wastewater treatment facilities are not effectively eliminating these compounds during treatment and therefore pharmaceuticals are entering the aquatic environment through the discharging of wastewater effluents into surface waters (Ternes, 1998; Stumpf et al., 1999; Ashton et al., 2004; Carballa et al., 2004; Strenn et al., 2004; Thomas and Foster, 2005; Castigiloni et al., 2006). Although the concentrations detected were below 100 ng/L for the most part, these concentrations will likely increase as

more wastewater treatment capacity is required to compensate for urbanization and population growth, and water reuse becomes more common to meet the demands of the growing population (Jones et al., 2005).

This study was one of the first research initiatives to determine the occurrence of a wide variety of pharmaceuticals over an extended period of time in operational drinking water treatment facilities. Carbamazepine and sulfamethoxazole showed similar seasonal variability with higher concentrations detected in the summer and early fall months compared to the rest of the year. One possible explanation for this seasonal variability was the low flow conditions in the hot and dry summer months, and as a result less dilution of the wastewater effluent discharges during this time of year. Researchers have found similar trends with higher concentrations of pharmaceuticals detected during low flow conditions compared to normal or high flow conditions (Kolpin et al., 2004; Loraine and Pettigrove et al., 2006). Ibuprofen was detected most of the time during the fall and winter months, which could be explained by its increase use during flu and cold season, and treatment facilities not able to effectively lower high concentrations of ibuprofen entering the treatment facility at one time. Another explanation was the wastewater treatment facilities were not operating at optimal levels during the winter months because the cooler water temperature decreased biodegradation processes, which resulted in less ibuprofen being biodegraded before being released into the receiving environment.

Lincomycin HCl was detected at higher concentrations in the spring and fall months compared to the summer months. One explanation for the seasonal variability was how this compound enters the aquatic environment. Lincomycin HCl is predominantly used as a veterinary drug in Canada, in which this compound would be excreted by the livestock

animals directly onto pasture or enter the aquatic environment by surface runoff after the land application of stored manure. In Ontario, manure is applied to agricultural land as a soil amendment during the spring (before planting of crops) and during the fall (after harvesting the crops and before the ground is frozen). The land application of manure corresponds to peaks in concentrations of lincomycin HCl.

The presence of carbamazepine, sulfamethoxazole and lincomycin in selected groundwater wells implies that these compounds are capable of moving through soils to groundwater aquifers. The locations of the wells are in close proximately to surface waters and therefore the contamination was likely the result of surface waters infiltrating groundwater supplies. The concentrations were lower compared to the concentrations detected in the raw water samples at the treatment facilities during the same time of year, which implies that the concentrations of these compounds are reduced to some extent by soil infiltration processes.

The ability of the two treatment facilities to reduce the concentrations of these compounds during drinking water production was also investigated. In general, higher concentrations were detected in the treated water samples collected from Facility B compared to Facility A. These differences provide evidence that water treatment technologies have an influence on the ability to reduce the concentrations of these contaminants during drinking water production. Both facilities were able to reduce the concentrations of the three antibiotics and naproxen to non-detectable levels in the treated water samples, which suggests that the different treatment technologies applied at each facility were effective in decreasing the concentrations to non-detectable levels. For carbamazepine and bezafibrate, there were clear differences in the capability of the two facilities to reduce the concentrations of these

two compounds during drinking water production. Facility B was not very effective in reducing the concentrations of these compounds in drinking water, which provides evidence that the conventional treatment technologies used at this facility provide minimal reduction of these compounds from drinking water. Facility A showed the opposite effect, in which the concentrations of carbamazepine and bezafibrate were reduced to non-detectable levels in treated water samples collected during most sampling months. The application of ozonation and GAC filtration at Facility A may be responsible for carbamazepine and bezafibrate not being detected in treated water samples collected from this facility. Both compounds have shown the ability to react with oxidants and respond effectively to GAC filtration processes. Another explanation for the reduction of these two compounds at Facility A was the storage of raw water in large reservoirs before the water entered the treatment facility. The storage of the water in the reservoir may allow time for elimination processes to reduce the concentrations of these compounds before the water enters the facility.

Ibuprofen and gemfibrozil were detected in treated water samples collected from both facilities, but higher percent differences were found at Facility A compared to Facility B. In some cases, ibuprofen was detected at higher concentrations in the treated water samples compared to the raw water samples collected at Facility B. This provided evidence that gemfibrozil and ibuprofen require advanced treatment to reduce the concentrations of these compounds to lower levels. Treatment processes at either facility were not effective in reducing these compounds to non-detectable levels. Previous research has suggested that advanced oxidation processes (e.g. ozone and hydrogen peroxide or UV and hydrogen peroxide) are required for the reduction of ibuprofen compared to selective oxidants, like ozonation (Zwiener and Frimmel, 2000; Ternes et al., 2003). Evidence provided from this

project suggests that there was variability in the reduction of these compounds during water treatment.

In addition to determining the occurrence, seasonal variability and potential reduction of these compounds during water treatment, limitations of the analytical method were also investigated. Although the use of LC-ESI-MS/MS instrumentation is a very sensitive and selective tool for determining the presence of polar organic contaminants in complex environmental matrices, the major disadvantage is matrix suppression or enhancement and the impact matrix effects have on the quantification of these contaminants in environmental matrices (Vanderford and Snyder, 2006; Vieno et al., 2006). Recently studies have shown that the use of isotopically labeled standards is the best choice for compensating for possible matrix effects (Vanderford and Snyder, 2006). These standards are able to compensate for matrix effects by eluting from the chromatographic column at the same time as the analytes of interest and undergoing the same degree of ionization in the ESI source. Carbamazepine, gemfibrozil and ibuprofen were the only compounds in which isotopically labeled standards were available at time of method development. Therefore, only the final concentrations of these three compounds were corrected for potential matrix effects (Chapter 3). For the remaining compounds detected, the final concentrations could not be corrected for potential matrix effects, so caution must be taken when using or reporting these concentrations. Chapter 2 provided comparisons between the different quantification methods and the discrepancies in concentrations when matrix effects were not addressed. The results showed that compounds analyzed in negative ionization mode were impacted to a greater degree by the matrix compared to compounds analyzed in positive ionization mode. The results from Chapter 2 also provided evidence that the matrix effects impacted the final concentrations

reported, but did not have an impact on the seasonal trends observed. Although, the concentrations of the three antibiotics, naproxen and bezafibrate cannot be accurately determined, these compounds were detected, percent differences were determined and seasonal variability was compared.

This thesis provided evidence that selected pharmaceuticals were detected in drinking water supplies in a susceptible Southern Ontario watershed, with a limited number present in treated water samples. The compounds detected provide evidence of seasonal variability which can be related to consumption and use patterns, changes in water quantity and quality, and changes operational parameters in wastewater treatment facilities during the seasons. The results also provide information on the capability of current water treatment technologies to reduce the concentrations of pharmaceuticals from drinking water supplies by comparing two facilities with different treatment processes. For the most part, conventional treatment technologies (e.g. coagulation, flocculation and sedimentation) were able to reduce the concentrations of some compounds, however more advanced treatments were required for persistent pharmaceuticals.

The data obtained from this thesis provided information which can be used for future research initiatives on investigating these contaminants in drinking water supplies. It provides direction for which compounds should be further evaluated, what seasons are more susceptible to contamination, and possible treatment technologies to reduce the concentrations of these compounds in drinking water. The information from this thesis provides exposure data in helping to support the assessment of potential implications of these contaminants in drinking water supplies and the impact they have on human health.

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APPENDIX A

Table A1: Final concentrations (ng/L) of bezafibrate in duplicate raw water samples collected from Facility A and Facility B over a 12 month interval from April 2005 to March 2006.

Facility	April 2005	May 2005	June 2005	July 2005	August 2005	September 2005	October 2005	November 2005	December 2005	January 2006	February 2006	March 2006
Facility A	1.5*	4.1*	2.6*	3.3*	1.6*	2.2*	4.5*	5.3	4.2*	3.8*	1.1*	4.2*
Facility A	2.0*	3.9*	1.7*	3.8*	1.5*	2.5*	3.7*	5.2	4.5*	3.3*	1.2*	3.9*
Facility B	4.7*	1.8*	2.6*	4.2*	3.7*	5.3	4.5*	11	8.2	6.9	3.4*	12
Facility B	5.2	4.0*	3.2*	3.8*	4.5*	6.0	4.3*	12	7.6	7.0	3.3*	12

*Below MDL of 5 ng/L

Table A2: Final concentrations (ng/L) of bezafibrate in duplicate treated water samples collected from Facility A and Facility B over a 12 month interval from April 2005 to March 2006.

Facility	April 2005	May 2005	June 2005	July 2005	August 2005	September 2005	October 2005	November 2005	December 2005	January 2006	February 2006	March 2006
Facility A	0.5*	ND	ND	0.8*	ND	ND	ND	ND	0.8*	0.7*	ND	0.7*
Facility A	0.6*	ND	ND	0.7*	ND	ND	ND	ND	0.7*	0.6*	ND	0.6*
Facility B	3.1*	2.0*	2.6*	2.4*	2.8*	5.1	4.3*	14	6.4	7.6	1.9*	6.5
Facility B	2.6*	2.3*	3.2*	2.7*	3.2*	4.2*	4.8*	14	5.9	7.5	1.9*	6.8

*Below MDL of 5 ng/L
ND equals not-detected

Table A3: Final concentrations (ng/L) of carbamazepine in duplicate raw water samples collected from Facility A and Facility B over a 12 month interval from April 2005 to March 2006.

Facility	April 2005	May 2005	June 2005	July 2005	August 2005	September 2005	October 2005	November 2005	December 2005	January 2006	February 2006	March 2006
Facility A	2.8	7.6	25	27	22	16	19	24	11	7.1	3.2	12
Facility A	2.7	7.1	22	28	20	17	18	22	11	8.5	2.8	12
Facility B	7.9	14	52	72	1015	51	33	42	18	19	8.2	27
Facility B	7.6	14	53	67	961	52	31	43	21	16	7.9	29

Table A4: Final concentrations (ng/L) of carbamazepine in duplicate treated water samples collected from Facility A and Facility B over a 12 month interval from April 2005 to March 2006.

Facility	April 2005	May 2005	June 2005	July 2005	August 2005	September 2005	October 2005	November 2005	December 2005	January 2006	February 2006	March 2006
Facility A	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Facility A	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Facility B	5.0	7.9	36	39	721	36	21	36	13	13	2.9	13
Facility B	4.7	9.0	34	38	706	38	22	36	12	10	3.3	13

ND equals not-detected

Table A5: Final concentrations (ng/L) of gemfibrozil in duplicate raw water samples collected from Facility A and Facility B over a 12 month interval from April 2005 to March 2006.

Facility	April 2005	May 2005	June 2005	July 2005	August 2005	September 2005	October 2005	November 2005	December 2005	January 2006	February 2006	March 2006
Facility A	1.0	1.4	2.8	3.5	1.9	1.8	2.1	2.7	1.4	1.5	1.2	2.2
Facility A	1.1	1.3	1.7	3.7	1.5	1.6	2.3	1.9	0.7*	1.7	1.2	2.2
Facility B	1.4	3.0	3.0	3.3	2.3	2.6	2.8	5.1	2.1	2.7	1.9	5.9
Facility B	1.7	2.0	4.7	3.4	2.2	3.7	3.2	4.3	3.2	3.1	1.4	6.0

*Below MDL of 1 ng/L

Table A6: Final concentrations (ng/L) of gemfibrozil in duplicate treated water samples collected from Facility A and Facility B over a 12 month interval from April 2005 to March 2006.

Facility	April 2005	May 2005	June 2005	July 2005	August 2005	September 2005	October 2005	November 2005	December 2005	January 2006	February 2006	March 2006
Facility A	ND	ND	ND	0.5*	0.4*	ND	0.7*	0.8*	ND	0.5*	0.7*	0.6*
Facility A	ND	ND	ND	0.6*	0.7*	ND	0.7*	0.8*	ND	0.6*	0.7*	0.7*
Facility B	ND	ND	ND	1.3	0.9*	1.1	2.9	1.0	ND	0.6*	0.7*	0.6*
Facility B	ND	ND	ND	1.3	1.0	1.4	3.0	1.2	ND	0.7*	0.9*	0.7*

*Below MDL of 1 ng/L
ND equals not-detected

Table A7: Final concentrations (ng/L) of ibuprofen in duplicate raw water samples collected from Facility A and Facility B over a 12 month interval from April 2005 to March 2006.

Facility	April 2005	May 2005	June 2005	July 2005	August 2005	September 2005	October 2005	November 2005	December 2005	January 2006	February 2006	March 2006
Facility A	ND	ND	ND	31	ND	32	30	23	30	27	16	32
Facility A	ND	ND	ND	21	ND	27	32	27	27	23	17	33
Facility B	ND	ND	ND	8.7	ND	13	16	34	20	29	35	54
Facility B	ND	ND	ND	10	ND	Volume injection problem	14	26	24	28	38	50

ND equals not-detected

Table A8: Final concentrations (ng/L) of ibuprofen in duplicate treated water samples collected from Facility A and Facility B over a 12 month interval from April 2005 to March 2006.

Facility	April 2005	May 2005	June 2005	July 2005	August 2005	September 2005	October 2005	November 2005	December 2005	January 2006	February 2006	March 2006
Facility A	ND	ND	ND	5.8	ND	ND	ND	14	16	6.8	12	12
Facility A	ND	ND	ND	10	ND	ND	ND	14	15	7.4	11	11
Facility B	ND	ND	ND	7.9	ND	7.8	17	38	14	20	32	44
Facility B	ND	ND	ND	9.3	ND	7.2	18	36	19	19	31	39

ND equals not-detected

Table A9: Final concentrations (ng/L) of lincomycin HCl in duplicate raw water samples collected from Facility A and Facility B over a 12 month interval from April 2005 to March 2006.

Facility	April 2005	May 2005	June 2005	July 2005	August 2005	September 2005	October 2005	November 2005	December 2005	January 2006	February 2006	March 2006
Facility A	37	7.8*	2.8*	2.1*	1.9*	1.1*	10	9.6*	20	13	15	18
Facility A	40	7.1*	2.6*	2.0*	1.7*	1.6*	9.9	11	18	12	17	19
Facility B	22	6.3*	1.6*	1.3*	1.5*	1.6*	19	14	27	4.1*	18	6.6*
Facility B	19	6.2*	2.4*	1.1*	1.4*	1.6*	15	14	28	17	16	6.5*

*Below MDL of 10 ng/L

Table A10: Final concentrations (ng/L) of naproxen in duplicate raw water samples collected from Facility A and Facility B over a 12 month interval from April 2005 to March 2006.

Facility	April 2005	May 2005	June 2005	July 2005	August 2005	September 2005	October 2005	November 2005	December 2005	January 2006	February 2006	March 2006
Facility A	6.3*	21	30	26	12	38	47	27	33	26	11	25
Facility A	15	19	31	29	13	54	42	34	36	23	9.7*	29
Facility B	16	10	8.7*	11	4.8*	14	16	34	20	8.6*	18	64
Facility B	5.1*	12	12	12	9.6*	14	6.3*	30	28	21	17	64

*Below MDL of 10 ng/L

Table A11: Final concentrations (ng/L) of sulfamethoxazole in duplicate raw water samples collected from Facility A and Facility B over a 12 month interval from April 2005 to March 2006.

Facility	April 2005	May 2005	June 2005	July 2005	August 2005	September 2005	October 2005	November 2005	December 2005	January 2006	February 2006	March 2006
Facility A	2.6*	5.7*	12*	13*	10*	14*	21	25	9.3*	6.8*	3.7*	11*
Facility A	3.2*	5.4*	11*	12*	9.7*	15*	26	26	9.5*	5.6*	3.6*	12*
Facility B	6.2*	7.4*	24	24	21	31	29	35	19*	4.7*	6.7*	27
Facility B	4.8*	8.5*	24	24	21	30	32	35	16*	10*	5.9*	28

*Below MDL of 20 ng/L

Table A12: Final concentrations (ng/L) of trimethoprim in duplicate raw water samples collected from Facility A and Facility B over a 12 month interval from April 2005 to March 2006.

Facility	April 2005	May 2005	June 2005	July 2005	August 2005	September 2005	October 2005	November 2005	December 2005	January 2006	February 2006	March 2006
Facility A	0.4*	4.1*	7.5*	5.7*	3.6*	1.7*	14	11	4.7*	4.1*	2.6*	4.0*
Facility A	1.6*	3.6*	6.8*	5.9*	2.9*	2.5*	5.2*	9.3*	5.6*	5.1*	2.0*	4.7*
Facility B	2.8*	3.6*	6.9*	3.8*	2.2*	2.2*	6.5*	6.2*	8.1*	1.4*	3.7*	5.8*
Facility B	3.0*	3.9*	7.8*	3.0*	2.1*	2.2*	4.5*	4.6*	7.7*	5.4*	3.5*	6.1*

*Below MDL of 10 ng/L

Table A13: Final concentrations (ng/L) of carbamazepine, lincomycin HCl and sulfamethoxazole in duplicate raw water samples collected from Well G and Well H on September 27th 2005 and October 11th 2005.

Pharmaceutical	Well Location	September 27th 2005	October 11th 2005
Carbamazepine	Well G	11	9.8
	Well G	12	11
	Well H	4.2	3.2
	Well H	4.1	4.1
Lincomycin HCl	Well G	4.0*	3.0*
	Well G	4.1*	4.8*
	Well H	1.7*	3.0*
	Well H	1.5*	3.2*
Sulfamethoxazole	Well G	8.3*	6.7*
	Well G	13*	8.9*
	Well H	ND	ND
	Well H	ND	ND

*Below MDL of 10 ng/L for lincomycin HCl and MDL of 20 ng/L for sulfamethoxazole
ND equals not-detected