REMOVAL OF PHARMACEUTICALLY ACTIVE COMPOUNDS FROM WATER SYSTEMS USING FREEZE CONCENTRATION

By

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Abstract

In the last few years, there has been a growing concern in the occurrence of pharmaceutically active compounds in the aquatic environment. Just in Europe, more than 3000 prescription and non-prescription drugs are used by human and animals and more than 80 drugs have been detected in municipal wastewater treatment plant effluent, surface water, groundwater, and in a few isolated cases, in drinking water, some at alarmingly high concentrations. Although no known human health effects have been associated with exposure to drinking water containing trace concentrations of drug residues, there is concrete evidence that these drug residues could cause numerous adverse health effects on aquatic life, even at very low concentrations.

Municipal wastewater treatment plant effluents have been identified as the major source of drug residues in surface waters. Conventional wastewater treatment systems cannot effectively remove pharmaceutically active compounds. The suitability of distinct wastewater treatment processes for the elimination of drug residues has not been studied. Freezing has been used successfully to treat various wastewaters. Application of freezing technology to purify water is based on the principle that when water freezes, ice crystals grow of pure water and impurities are rejected from the ice structure and become concentrated in the unfrozen liquid. Without addition of any chemicals, contaminants in a large volume of dilute wastewater can be effectively removed by freeze concentration. Objectives of this study are: 1) literature review of occurrence of pharmaceutically active compounds, treatment technologies and analytical methods; and 2) experimental study: evaluate the potential of the freeze concentration process for the removal of selected pharmaceutically active compounds, the effect of initial concentration of drug residues, freezing temperature and degree of freezing on the removal efficiency of pharmaceutically active compounds.

Five drugs aspirin, ibuprofen, gemfibrozil, metoprolol and sulfamethoxazole were selected to investigate the removal efficiency of freeze concentration. Gas chromatograph-mass spectrometry and total organic carbon were used to quantify the drug concentrations in water samples. After first freezing cycle, ibuprofen and gemfibrozil were concentrated 2-3 times compared to the feed water and the concentration factors reached 12 after second freezing cycle. The total removal efficiency of the selected pharmaceutically active compounds was about 80% after one stage freeze concentration. Results indicated that aspirin degraded during the treatment; therefore, the concentration of aspirin was difficult to measure. Analysis of sample total organic carbon concentration indicated that about 84% and 92% removal efficiency was achieved for ibuprofen and gemfibrozil one stage freeze concentration. Approximately 99% impurity removal efficiency and around 60% volume reduction was observed in refrozen ice obtained from the first freezing.

This research results indicated freeze concentration was effective and not sensitive to the natural of target pharmaceutically active compounds in the feed water. In addition, the analytical methodology for the determination of drug residues in complex environmental matrices is still evolving and it may takes years before the universally accepted methods are developed. The gas chromatograph-mass spectrometry method used by many researchers requires complex sample preparation, which could influence the accuracy of the analysis.

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Chapter 1 INTRODUCTION

In the past few years, there has been a growing concern in the occurrence of pharmaceutically active compounds (PhACs) in the aquatic environment (Daughton and Ternes 1999, Kümmerer 2001, Heberer et al. 2002 and Snyder et al. 2005). Various prescription and non-prescription human and veterinary drugs such as painkillers, antibiotics, antidepressants, lipid regulators have been detected in municipal wastewater treatment plant effluent, surface water, groundwater, and in a few isolated cases, in drinking water, some at alarmingly high concentrations (Khan and Ongerth 2004, EU-POSEIDON 2004 and Sedlak et al. 2005). Although no known human health effects have been associated with exposure to drinking water containing trace concentrations of drug residues, there is concrete evidence that these drug residues could cause numerous adverse health effects on aquatic life, even at very low concentrations (EU-POSEIDON 2004 and Nghiem et al. 2006). For example in the case of antibiotics, the possibility of inducing resistance in bacterial strains (Hectoen et al 1995, Migiore et al. 1996 and Gilliver et al. 1999), which could pass to humans via environmental exposure, is still under debate (Pascoe et al. 2003). In addition, the phenomena of pharmaceutical bioaccumulation have been found in fish of effluent-dominated stream by Brooks et al. (2005). Antidepressants such as the select serotonin reuptake inhibitors fluoxetine and setraline and their metabolites norfluoxetine and desmethylsetraline were detected at levels greater than 0.1 ng/g in all tissues examined from fish residing in a municipal effluent-dominated stream.

Human-use PhACs enter raw sewage via urine and feces and by improper disposal. Because of their specific properties including high chemical stability, low biodegradability, high water solubility, and low sorption coefficients (Zwiener 2007), municipal wastewater treatment effluents have been identified as the major source of drug residues in surface waters (Ternes et al. 2002, Miao 2003, Boyd et al. 2003 and Snyder et al. 2005). Additionally, although the concentrations of veterinary drug residues, found in aquatic environment, are generally in the range from a few nanograms to micrograms per liter (Kolpin et al 2002 and Calamari et al. 2003), it is really a beyond-neglect inflow into the environment.

In Europe alone, more than 3000 compounds are used as human and veterinary pharmaceuticals today (Zuccato et al. 2000) and a wide variety of new drugs with specific chemical and biological properties but often unknown (eco)toxicological effects are produced (Fent et al. 2006) and day by day released in unmatched and increasing quantities.

The presence of a number of PhACs in sewage treatment plant effluents has been confirmed in Germany (Ternes 1998 and Hirsch et al. 1999), the Netherlands (Belfroid et al. 1999), Switzerland (Golet et al. 2001), United Kingdom (Johnson et al. 2001), France, Greece, Sweden, Italy (Andreozzi et al. 2003), Spain (Calamari et al. 2004), the United States (Huggett et al. 2003 and Yang et al. 2004), Canada (Metcalfe et al. 2003), Brazil (Stumpf et al. 1999), and Australia (Braga et al. 2005). Obviously, the current wastewater treatment systems can not effectively remove PhACs residues. Also because of this situation, a sea of PhACs like antiphogistics, lipid regulators, neutral drugs and betablockers, antibiotics might be discharged into receiving waters and pollute source water for drinking water supplies. Therefore, more enhanced technologies are crucial for the future.

Research on the suitability of wastewater treatment methods for the elimination of drug residues has just started, however, numerous uncertain and knowledge gaps persist (EU-POSEIDON 2004, Sedlak et al. 2005 and Castiglioni et al. 2006). The potential of freeze concentration process for removal of drug residue in wastewater has not been investigated.

Objectives of this study are: 1) to review occurrence of PhACs in the aquatic environment; 2) to summarize analytical methods for detection of PhACs in aquatic environment; 3) to investigate removal efficiency of drug residues by currently treatment technologies; 4) to evaluate the potential of the freeze concentration process for the removal of selected PhACs; 5) to examine different factors influencing freeze concentration: a) freezing temperature, b) feed water drug concentration, and c) type of pharmaceuticals.

Baker (1966, 1967, 1969, 1970) and Halde (1979) reported that the effectiveness of the freeze concentration depends on a few of main factors as follow, freezing rate, impurity concentration, mixing intensity and ice washing. The freezing rate is important because it affects ice crystal growth and the subsequent rejection of impurities. Usually, a slow freezing rate is more effective than a fast freezing rate. Mixing is important because it sweeps away the accumulation of impurities ahead of the freezing front. For this very reason, a solution containing a high concentration of impurities is more difficult to purify than the same solution containing a lower concentration of impurities. Ice washing is necessary because it can obtain the concentrated liquid from growing ice front surface and achieve complete impurities recovery. In this research, all of experiments were run with mixing and with ice washing by distilled water of constant volume. Because we consider the differences in experimental conditions and the nature of detected impurities (drug residues) compared to that of literature, this study focuses examining the effects of freezing rate (freezing temperature) and impurities concentration (initial concentration of drug residues). In addition, we also investigate the effects of type of pharmaceuticals. Although the experimental results of Baker (1967) and Yang et al. (1990) indicated the non-selectivity of freeze concentration for organic impurities, characteristics of various pharmaceuticals are very different and literature (Gross, 1967) showed different behavior of impurities during freezing. Therefore, we selected different drugs to examine the effects of different types of pharmaceuticals.

Chapter 2 PHARMACEUTICALLY ACTIVE COMPOUNDS

Pharmaceutically active compounds (PhACs) are a group of compounds consisting of prescription drugs, over-the-counter medications, drugs used exclusively in hospital and veterinary drugs (Sedlak et al. 2005). PhACs in the environment are introduced primarily through excretion from humans or animals and the subsequent transport in sewage (Heberer et al. 2002). A large amount of pharmaceuticals are consumed by both humans and animals. For example, around 3000 different human medicines including analgesics and anti-inflammatory pharmaceuticals, contraceptives, antibiotics, beta-blockers, lipid regulators, neuroactive compounds and many others are used in Europe (Halling-Sørensen et al. 1998). Pharmaceuticals used in veterinary medicine include antibiotics, anti-inflammatory drugs and food additives.

2.1 A Variety of PhACs

Pharmaceuticals comprise a broad arrangement of chemical structures answering a wide array of medical needs. Because different groups have different preferences for the foundation of classifications, it is complex to classify pharmaceuticals. Generally, pharmaceuticals can be classified in different ways such as method of production, pharmaceutical criteria and material used. Pharmaceutical criteria can further be divided into three types: chemical group, e.g. alkaloids; pharmacologically, i.e. the way they work in the body; and according to their therapeutic uses.

According to a recent report from WHO (2006), human pharmaceutical substances can be classified to fourteen main groups (Table 2-1) based on the Anatomical Therapeutic Chemical (ATC) classification of pharmaceuticals. In this literature review, we will focus on four groups of them covering various drugs such as pain relievers, antiphlogistics, analgesics, anti-inflammatories, non-steroidal drugs, beta blockers, lipid-regulator drugs, antibiotics and antiepileptics.

Table 2-1 Main groups of human pharmaceuticals (WHO, 2006)

NO.	ATC group
A	Alimentary tract and metabolism
B	Blood and blood forming organs
C	Cardiovascular system
D	Dermatologicals
G	Genito urinary system and sex hormones
H	Systematic hormonal preparations
J	Anti-infectives for systematic use
L	Antineoplastic and immunomodulating agents
M	Musculo-skeletal system
N	Nervous system
P	Antiparasitic agents, insecticides, repellents
R	Respiratory system
S	Sensory organs
V	Various

2.1.1 Group Pain Relievers, Analgesics, Antiphlogistics, Anti-inflammatories, and Non-steroidal Drugs

2.1.1.1 Analgesics

An analgesic (colloquially known as a painkiller) is any member of the diverse group of drugs used to relieve pain and to achieve analgesia. Analgesic drugs include paracetamol (acetaminophen), the nonsteroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, narcotic drugs such as morphine, synthetic drugs with narcotic properties such as tramadol, and various others. Some other classes of drugs not normally considered analgesics are used to treat neuropathic pain syndromes; these include tricyclic antidepressants and anticonvulsants (Internet-1 2007).

2.1.1.2 Antiphlogistic Pharmaceuticals

Antiphlogistic drugs and pain killers are agents, which are applied in medical therapy for relieving pains, fevers and against inflammatory caused by various diseases. They are substances used in many non-prescription drugs in the primary health sector (Internet-2 2007).

2.1.1.3 Non-steroidal Anti-inflammatory Drugs

Non-steroidal anti-inflammatory drugs, usually abbreviated as NSAIDs, are drugs with analgesic, antipyretic and anti-inflammatory effects - they reduce pain, fever and inflammation. The term "nonsteroidal" is used to distinguish these drugs from steroids, which (amongst a broad range of other effects) have a similar eicosanoid-depressing, anti-inflammatory action. NSAIDs are sometimes also referred to as non-steroidal anti-inflammatory agents/analgesics (NSAIAs). The most prominent members of this group of drugs are aspirin and ibuprofen. Paracetamol (acetaminophen) has negligible anti-inflammatory activity, and is strictly speaking not an NSAID. Beginning in 1829, with the isolation of salicylic acid from the folk remedy willow bark, NSAIDs have become an important part of the pharmaceutical treatment of pain (at low doses) and inflammation (at higher doses). Part of the popularity of NSAIDs is that, unlike opioids, they do not produce sedation, respiratory depression, or addiction. NSAIDs, however, are not without their own problems. Certain NSAIDs, including ibuprofen and aspirin, have become accepted as relatively safe and are available over-the-counter without prescription (Internet-3 2007).

2.1.2 Group C Cardiovascular System

2.1.2.1 Beta Blockers

Beta blockers (β-blockers) are a class of drugs used in the highest quantities within ATC group C used for various indications, but particularly for the management of cardiac arrhythmias and cardioprotection after myocardial infarction. Beta blockers are pharmaceuticals designed to block the β_1–receptor from stimulating the higher heart rate and the cardiac output in humans with mainly cardiovascular diseases, like hypertension and angina pectoris, but also some other diseases like migraine, thyrotoxicoses and the control of tremors. Some beta blockers have a high first pass metabolism, while others are excreted unchanged in the urine (Internet-4 2007). Most of the generic names for beta blockers end with *"olol"*(e.g. timolol, esmolol, carteolol, nadolol, propranolol, propranolol, betaxolol, penbutolol, metoprolol, acebutolol, atenolol, metoprolol, pindolol, bisoprolol).

2.1.2.2 Lipid-lowering Drugs

Lipid-lowering drugs reduce serum cholesterol levels by inhibiting a key enzyme involved in the biosynthesis of cholesterol; examples: 1)Resins cholestyramine (Cholybar, Questran) colestipol (Colestid); 2) HMG CoA Reductase Inhibitors lovastatin (Mevacor) pravastatin (Pravochol) simvastatin (Zocor); 3) Fibric Acid Derivatives gemfibrozil

(Lobid) clofibrate (Atromid-S); 4) Miscellaneous nicotinic acid (Niacin) probucol (Lorelco).

2.1.3 Group J Antibiotics

An antibiotic is a chemical compound that inhibits or abolishes the growth of microorganisms, such as bacteria, fungi, or protozoans. The original meaning of antibiotic includes any agent with biological activity against living organisms; however, the term is commonly used to refer to substances with anti-bacterial, anti-fungal, or anti-parasitical activity. The first antibiotic compounds used in modern medicine were produced and isolated from living organisms, for example, the penicillin class produced by fungi in the genus *Penicillium*, or streptomycin from bacteria of the genus *Streptomyces*. With the advent of organic chemistry many antibiotics are now also obtained by chemical synthesis, such as the sulfa drugs. Many antibiotics are relatively small molecules with a molecular weight less than 2000 Da (Internet-5, 2007).

2.1.4Group N Nervous System

Sedatives, tranquilizers, depressants, anxiolytics, soporifics, sleeping pills, downers, or sedativehypnotics these are different names for substances, which depress the central nervous system (CNS), resulting in calmness, relaxation, reduction of anxiety, sleepiness, slowed breathing, slurred speech, staggering gait, poor judgment, and slow, uncertain reflexes. At high doses or when they are abused, many of these drugs can cause unconsciousness and death (Internet-6, 2007). Carbamazepine is a representive of group N. It is an anticonvulsant and mood stabilizing drug, used primarily in the treatment of epilepsy and bipolar disorder. It is also used to treat schizophrenia and trigeminal neuralgia (Internet-7, 2007).

2.2 Properties of Pharmaceuticals

Pharmaceuticals are compounds characterised by complex chemical structures. Most pharmaceuticals are charged and hydrophilic. Many pharmaceuticals have multiple ionisable functional groups. The hydrophobic reactions dominate partitioning neutral organic compounds to sediments and suspended solids (limited sorption properties) are relatively unimportant for most of the pharmaceuticals. Many pharmaceutical are chiral and often administered as racemic mixtures (Williams 2005). Properties of a few pharmaceutical groups relevant for their behaviour in environment are given below.

2.2.1 Pain Relievers, Antiphlogistics, Analgesics, Anti-inflammatories, and Non-steroidal Drugs

Zwiener and Frimmel (2000) found a high degree of degradation for ibuprofen in the oxic biofilm reactor, which was attributed to adaptation of the biofilm to the residue. Two metabolites could be identified based on their mass spectra and comparison with literature data, viz. hydroxyibuprofen and carboxyibuprofen. Moreover, salicilic acid is found to be easily biodegradable.

2.2.2 Group β-blockers, Lipid - lowering Agents

High solubility (metoprolol >1000ppm) or moderate solubility (atenolol, propranolol 10-1000ppm) and low $\log K_{ow}$ (<3) correspond to a high affinity of β-blockers to water. The presence of beta blockers in the gaseous compartment is neglectable due to its low vapour pressure.

2.2.3 Antibiotics

Al-Ahmad et al. (1999) investigated biodegradability of some clinically important antibiotics, Cefotiam, Ciprofloxacin, Meropenem, Penicillin G, and Sulfamethoxazole, in the closed bottle test (CBT). These drugs possessed different chemical structures and mode of action (= antibiotic spectra). None of the investigated antibiotics were readily biodegradable (**Error! Reference source not found.**).

Table 2-2 Results of the closed bottle test (Al-Ahmad et al., 1999)

Test Compound	Supplied By	Test Concentration (μg/mL)	Biodegradation After 28 Days (%)	Biodegradation After 40 Days (%)
Cefotiam dihydrochloride	Takeda Pharma GmbH	4.8	7	10
Ciprofloxacin	Bayer MG, Lever Kusen	3.5	0	0
Meropenem	Zeneca-Grünenthal, GmbH Stolberg	2.5	7	7
Penicillin G	Zeneca-Grünenthal, GmbH Stolberg	3.0	27	36
Sulfamethoxazole	Sigma Aldrich Chemie GmbH, Steinbeim	3.8	0	0

Low biodegradation rates were also reported in soils. Adaptation of microorganisms was not concluded. Penicillin seems to be easier biodegradable than the rest of tested antibiotic compounds. These finding were in agreement with reported poor biodegradability in soils. On the other hand this could have been caused by adsorption resulting in poor bioavailability. Authors concluded that biodegradation of antibiotics in sewage treatment plants (STPs) might not be a reliable expectation for the removal of antibiotic substances. The CBT was a screening test using low bacterial density. In tests with higher bacterial density (biodegradation tests) or a higher degree of simulating an

STP, higher biodegradability and nonbiotic elimination processes like adsorption, hydrolysis, or partial degradation of active moieties may take place in a higher extension, but not necessarily.

2.2.4 Antidepressants

In a study by Black (2004), three selective serotonin reuptake inhibitors (SSRI's): paroxetine, sertraline and fluvoxamine, were tested for biodegradability using activated sludge inoculum from a wastewater treatment plant. No degradation was observed during a test period of 28 days. It was concluded that none of the compounds could be labelled readily biodegradable in wastewater treatment plants. Cunningham et al. 2004 found in a preliminary biodegradation study with sewage biomass a rapid depletion of paroxetine from solution over the first day of the studies followed by no further depletion despite culture acclimations and enrichments. In extensive aerobic biodegradability studies no depletion of paroxetine was observed in the period after sorption had reached equilibrium.

The antidepressants are not volatile from water or they have a very slow rate of volatilisation as Henry constants are low, $K_H < 1.3 \cdot 10^{-7}$ atm·m^3 ·mole^{-1}. They have rather little mobility or are immobile in soils and will most likely sorb to soils and sludge. It is expected that the compounds tend to bioaccumulate as $\log K_{OW}$ values are around 3 and higher. In a study performed by Brooks et al., 2003, the fluoxetine, sertraline and the metabolites norfluoxetine and desmethylsertraline were detected at levels greater than 0.1 ng/g in all tissues examined from fish residing in a municipal effluent-dominated stream.

2.3 PhACs Consumption by Human

Table 2-3 summarizes the annual consumption of various common-use PhACs in different countries. Up to one hundred tons of individual pharmaceuticals were sold in Germany, England, Switzerland and Japan and other countries. The consumption pattern of pharmaceuticals for the different countries is not identical and some pharmaceuticals may be forbidden or replaced by related pharmaceuticals. However, a few pharmaceuticals are regularly documented within the most frequently applied range: the class of NSAIDs including acetylsalicylic acid, paracetamol, ibuprofen, naproxen and diclofenac, the oral antidiabetic metformin and the antiepileptic carbamazepine. The quantity of each pharmaceutical consumed ranged from several tons to about one thousand tons in each country.

Additionally, North Americans have become one of the world's greatest producers and consumers of pharmaceuticals. In 2005, North America accounted for 47% of global pharmaceutical sales (Robinson 2006). The US top 200 prescriptions were prescribed more than two billion times within the United States in 2003 (Jjemba 2006). In Canada, approximately 9,100 products and 1,870 active ingredients are available on the market. Canadians spent more than $18 billion in 2005 alone on pharmaceutical products (IMS Health 2005).

It is worthy to say that the number of pharmaceuticals and their availability to the general population will continue to increase dramatically with modernization and scientific advancement, especially, since many drugs can be purchased without prescription. For instance, according to a recent IMS report (IMS Health 2007), in the 12 month period from 2005 through to December 2006, a 5% growth in drug sales had

Table 2-3 Annual consumption (tons) of different classes of prescribed drugs for different countries

Coumpounds	Germany 1995[a]	Germany 1999[b]	Germany 2000[b]	Germany 2001[b]	Austria 1997[c]	Denmark 1997[d]	Australia 1998[e]	England 2000[f]	Italy 2001[g]	Switzerland July 2001- June 2002[h]	Japan 2002[i]	Switzerland 2004[j]
Analgesics, abtuoyretics and anti-inflammatory												
Acetylsalicylic acid		902.27(1)	862.60(1)	836.26(1)	78.45(1)	0.21(7)	20.4(9)				403(1)	43.80(3)
Salicylic acid		89.70(12)	76.98(17)	71.67(17)	9.57(11)							5.30(6)
Paracetamol		654.42(2)	641.86(2)	621.65(2)	35.08(2)	0.24(6)	295.9(1)	390.9(1)				95.20(1)
Naproxen					4.63(16)		22.8(7)	35.07(12)			22-33(6)	1.70(12)
Ibuprofen	105(1)	259.85(5)	300.09(5)	344.89(5)	6.7(13)	0.03(19)	14.2(13)	162.2(3)	1.9(15)	17.982(1)	99(4)	25.00(4)
Diclofenac	75(3)	81.79(16)	82.20(14)	85.80(14)	6.14(15)			26.12(16)		3.883(3)		4.50(7)
β-Blocker												
Atenolol								28.98(13)	22.07(4)			3.20(9)
Metorolol	50(4)	67.66(18)	79.15(16)	92.97(11)	2.44(20)							3.20(9)
Antilipidemic												
Gemfibrozil	6(8)						20(10)					0.399(18)
Bezafibrate	30(5)				4.47(17)				7.60(8)			0.757(15)
Neuroactive												
Carbamazepine	80(2)	86.92(13)	87.71(13)	87.60(12)	6.33(14)		9.97(18)	40.35(8)			107-162(2)	4.40(8)
Diazepam						0.21(8)						0.051(21)
Antiacidic												
Ranitidine		85.41(15)	89.29(12)	85.81(13)			33.7(5)	36.32(10)	26.67(3)			1.60(13)
Cimetidine								35.65(11)				0.063(20)
Diuretics												
Furosemide						3.74(1)			6.40(9)			1.00(14)
Sympatominetika												
Terbutalin						0.46(3)						0.0099(23)
Salbutamol						0.17(9)						0.035(22)
Various												
Metformin		368.01(4)	433.46(4)	516.91(3)	26.38(3)		90.9(2)	205.8(2)				51.40(2)
Iopromide		64.93(19)	63.26(19)	64.06(19)								6.90(5)

For every country a top 20 sold-list is taken into account. Data in bracket represent the position in the ranking list within a country.

[a] Ternes (1998)
[b] Huschek et al. (2004).
[c] Sattelberger (1999).
[d] Stuer-Lauridsen et al. (2000).
[e] Khan and Ongerth (2004).
[f] Jones et al. (2002).
[g] Calamari et al. (2003).
[h] Tauxe-Wuersch et al. (2005)
[i] Nakada et al. (2006)
[j] ©IMS Health Incorporated or its affiliates.

occurred through retail pharmacies in the 13 key markets closing at US$388.28 billion, in which North America posted a 7% sales growth at $211.52 billion in sales in the 12 months to December, 2006; that growth was slightly higher than that of in Europe, which has a 3% constant exchange growth (IMS Health 2007).

2.3.1 Non-steroidal Anti-inflammatory Drugs (NSAIDs)

NSAIDs such as acetylsalicylic acid (aspirin) and ibuprofen are an important group in pharmaceuticals. Their widespread application in high doses makes up the largest tonnage of one group of pharmaceuticals (Daughton and Ternes 1999, Sattelberger 1999, Huschek et al. 2004, Tauxe-Wuersch et al. 2005 and Nakada et al., 2006). For example, the sale amount of aspirin constantly lies on top of the sold-list in France as well as in Germany between 1999 and 2001, with annual sales of up to 800 tons (Buser et al. 1999 and Huschek et al. 2004). Furthermore, from 1998 to 2004, paracetamol came forth frequently predominance among all sold parmaceuticals in Australia (Ternes 1998), England (Jones et al. 2002) and Switzerland (IMS Health 2004), of whose consumption are almost beyond hundreds of tons. NSAIDs are even available without prescription.

2.3.2 Betablockers and Lipid Regulators

β-blockers including nadolol, bisoprolol atenolol, metoprolol and propranolol are important therapies for the treatment of angina, glaucoma, heart failure, high blood pressure and other related conditions, and have been widely employed in cosmopolitan sites. Different countries seem to have different predilection for use of β-blockers, for instance, metorolol was employed comprehensively in Germany (Huschek et al. 2004), while it seems atenolol was preferred in England (Jones et al. 2002) and Italy (Calamari et al. 2003).

Lipid regulators were also consumed largely at tens of tons yearly deals in different countries. Gemfibrozil is a representive of a lipid regulator and was consumed 20 tons in 1998 by Australian (Khan and Ongerth 2004).

2.3.3 Antibiotics

Sulfonamides and (fluoro)quinolones, among the most powerful antibiotics, are nowadays used extensively to treat a wide variety of diseases (Daughton and Ternes 1999 and Sacher et al. 2001). The original antibacterial sulfonamides (sometimes called simply sulfa drugs) are synthetic antimicrobial agents that contain the sulfonamide group (Figure 2-1). The quinolones are a family of broad-spectrum (an antibiotic with activity against a wide range of disease-causing bacteria) antibiotics. The parent of the group is nalidixic acid (Figure 2-1). The majority of quinolones in clinical use belong to the subset of fluoroquinolones, which have a fluoro group (Figure 2-1) attached the central ring system, typically at the 6-position.

Bacteriostatic sulphonamide (sulpha) antibiotics such as sulphamethoxazole, sulphathiazole and sulphadiazine etc., are used in the treatment of infections in livestock and to a lesser extend in the treatment of human infection such as bronchitis and urinary tract infection. On the other hand, antibiotics belonging to the quinolones, including fluoroquinolones, pipemidic acid, ofloxacin, norfloxacin, ciprofloxacin, lomefloxacin, enrofloxacin, difloxacin, sarafloxacin, and tosufloxacin, are applied to treat various bacterial infections such as bronchitis, gonorrhea, skin infections, and urinary tract infections etc. in many different parts of the body. Over 50 million pounds of antibiotics are produced annually in the US, with approximately 60% for human use and 40% for animal agriculture (Levy 1998).

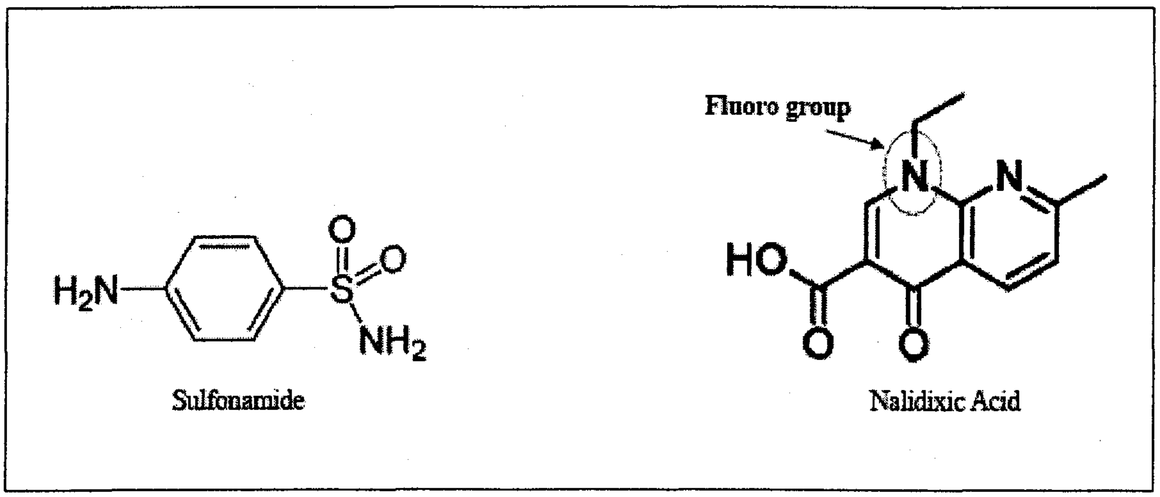


Figure 2-1 Schematic structure of sulfonamides and quinolones

2.3.4 Antiepileptics

For the other low production pharmaceuticals, they are usually prescribed as prescription and their consumption depend on the different applied conditions (e.g., different countries). For example, the antiepileptic carbamazepine is an established drug for the control of grand mal and psychomotor epilepsy and is also effective in the treatment of trigeminal neuralgia. Furthermore, it is used in bipolar depression (Clara et al. 2004). In 1997, the consumption of carbamazepine in Austria amounted to 6 tons (Sattelberger 1999). However, in Canada, approximately 28 tons of carbamazepine was sold as prescriptions in 2001 (IMS Health in Canada 2001).

2.4 Veterinary Use of PhACs and Food Additives

A variety of pharmaceuticals, including antimicrobial agents, anthelminthics, insecticides, vitamins, digestive medicines, and vaccines, have been utilized in animal husbandry. Most frequently used are antimicrobial agents, followed by antiparasitic drugs consisting of anthelmintics and insecticides.

Table 2-4 Annual consumption (kg) of different classes of veterinary drugs in Denmark and the U.S.

Coumpounds	Denmark 1995[a]	U.S.[b]	
		Min.	Max.
Growth promoters (livestock production pigs)			
Single substances			
Carbadox	1,181	215	538
Olaquindox	16,213		
Avilamaycin	1,665		
Avoparcin	5,690		
Bacitracin	7,910	1,399	2,799
Flavomycin	48		
Monesin	5,007		
Salinomycin	850		
Tylosin	52,275	1,076	
Virginiamycin	2,590	108	215
Therapeutics applied in livestock production			
Antibiotics	49,687		
Therapeutics applied in poultry industry			
Coccidiostatics	16,165		
Antibiotics feed additive	2,491		
Fish farms, feed additive			
Single substances			
Branzil (Trimetoprim Sulfamethizol)	3,356		
Oxolinic acid and Inoxyl	208		
Oxytetracycline	82		
Sulfa contaning substances (therapeutics)	182		

[a] Halling-Sørensen et al., 1997
[b] Sedlak et al., 2005

According to the exiguous literature about trade circumstances of these veterinary pharmaceuticals, it is not hard to image a mass of pharmaceuticals being employed in animal husbandry in worldwide medication market. As shown in Table 2-4, every year, pharmaceuticals of a few kilogram to tens of tons were used in animals as growth promoters, therapeutics and feed additive in Denmark (Halling-Sørensen et al. 1997) and the U.S. (Sedlak et al. 2005).

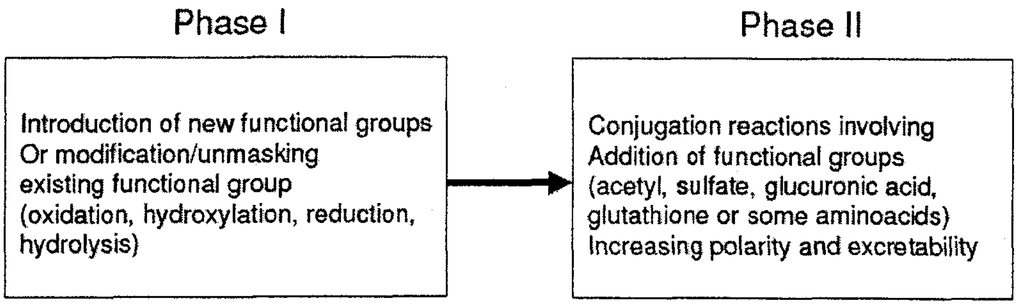
Chapter 3 FATE OF PHARMACEUTICALLY ACTIVE COMPOUNDS IN THE ENVIRONMENT

3.1 Exposure Routes

There are two stages of drugs enterint the environment. First is the reaction in the received and another one is environmental fate of PhACs. These stages are introduced as followed sections.

3.1.1 Fate before Entering the Environment

After applied, all pharmaceuticals will pass through a series of reactions in the receiving body before entering the environment. Figure 3-1 and Figure 3-2 show an overview of the metabolisation phases of a parent compound. Before being retrieved from the body with the urine and exposed to the environment, most pharmaceutical compounds such as ibuprofen are metabolized to two phases of metabolites through specialized enzymatic systems. Phase I metabolism usually precedes Phase II, though not necessarily. During these reactions, polar bodies are either introduced or unmasked, which results in (more) polar metabolites of the original chemicals. Phase I reactions usually consist of oxidation, reduction or hydrolysis, and the products are often more reactive and sometimes more toxic than the parent drug (Halling-Sørsensen et al. 1998).



Introduction of new functional groups Or modification/unmasking existing functional group (oxidation, hydroxylation, reduction, hydrolysis)

Conjugation reactions involving Addition of functional groups (acetyl, sulfate, glucuronic acid, glutathione or some aminoacids) Increasing polarity and excretability

Figure 3-1 Two phase of drug metabolism; all drugs under go both phases; conjugation reactions can be reversed (Kujawa-Roeleveld et al., 2006)

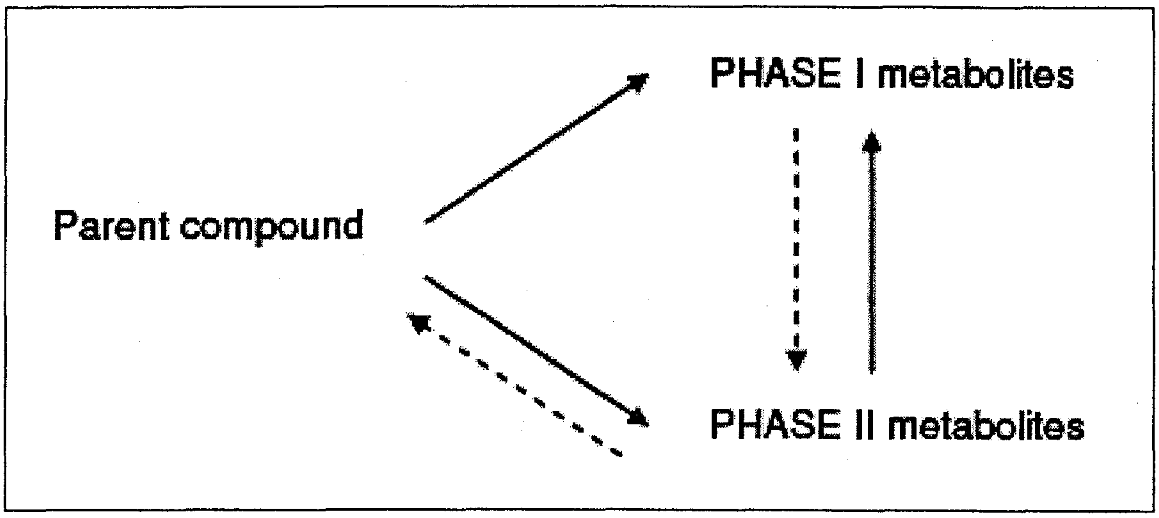


Figure 3-2 Metabolism of pharmaceutical compounds; Solid lines transformation into a more water soluble compound; Dotted lines indicate a reactivation of the phase II metabolites into a less water soluble compound. (Halling-Sørsensen et al., 1998)

If the metabolites of phase I reactions are sufficiently polar, they may be readily excreted at this point. However, many phase I products are not eliminated rapidly and undergo a subsequent reaction in which an endogenous substrate combines with the newly incorporated functional group to form a highly polar conjugate (Kujawa-Roeleveld et al. 2006). Phase II reactions involve conjugation (e.g., with glucuronic acid, sulfonates (commonly known as sulfation), glutathione or amino acids), which normally results in inactive compounds and involve the interactions of the polar functional groups of phase I metabolites (Internet-8, 2007). Both phase I and phase II reactions change the physical chemical behavior of the substance because metabolisation always renders the metabolites more water soluble than the parent compounds (Halling-Sørsensen et al. 1997). For example, Berger et al. (1986) showed that chloramphenicol glucoronide and N-4-acetylated sulphadimidine, phase II metabolites, were converted to chloramphenicol and sulphadimidine respectively in samples of liquid manure, and thus reactivated into the parent compounds. Therefore, often it is not only the parent compound, which should be the subject of a risk assessment but also the main metabolites.

3.1.2 Environmental Fate of PhACs

Due to the increasing consumption of pharmaceuticals in daily life, more parent and metabolized drugs will enter the environment via the municipal sewage systems at the end of their application cycles. Although PhACs can enter the environment by many routes, municipal wastewater treatment plant effluents have been identified as a main source because conventional wastewater treatment system cannot effectively remove residual PhACs (McQuillan et al. 2002, Alder et al. 2003, Clara et al. 2004 and Castiglioni et al. 2006). Chen et al. (2006) detected a widespread of residual pharmaceuticals in wastewater treatment effluents and in the water supply systems in Canada.

There are differences between the fates of veterinary and human drugs after urinal or fecal excretion. As shown in Figure 3-3, in general, excreted human pharmaceuticals can be partly removed since passing through a sewage treatment plant prior to entering rivers or streams (surface water). However, the route of veterinary pharmaceutical products to the environment is predominantly via manure deployed to soils, which ultimately results in ground water contamination (Stumpf et al. 1999).

Additionally, after rainfall incidents, abundant human or veterinary pharmaceutials can enter surface waters by run-off from fields treated with digested sludge or livestock slurries respectively, which can lead to surface water pollution. Richardson et al. (1985) suggested industrial wastewater may be another possible source for the contamination of surface waters, but are surely not responsible for their ubiquitous occurrence.

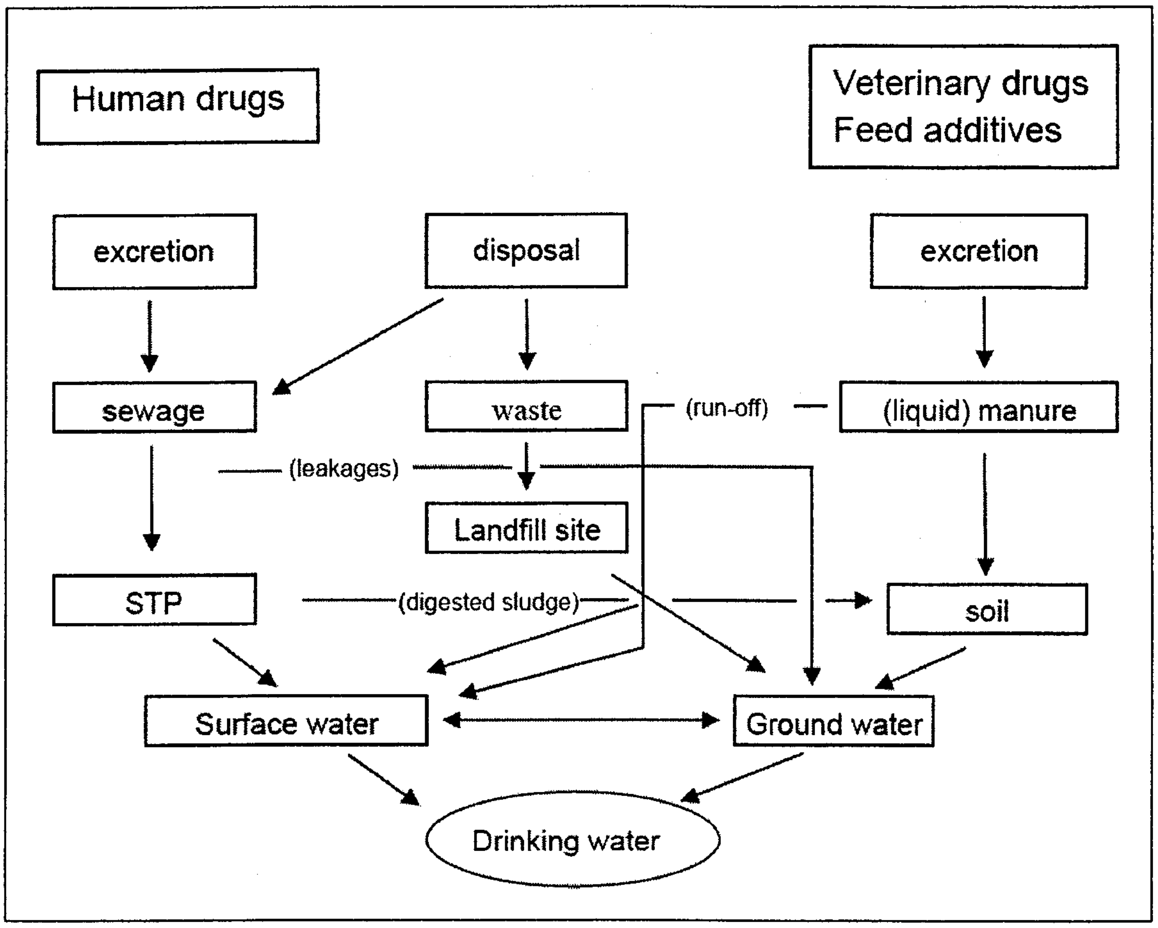


Figure 3-3 Scheme for the main fates of pharmaceuticals in the environment after application (STP: sewage treatment plant) (Ternes, 1998)

The application of digested sludge from municipal sewage treatment plants on agricultural areas possibly causes the contamination of soils and ground water. Transport to groundwater is of relevance for all compounds with low sorption efficiency and high water solubility. Furthermore, groundwater can be affected by bankfiltration or artificial recharge of loaded surface waters, by leaks from sewer systems in urban areas, and, occasionally, by residues from production (Hall and Medmenham 1992). Holm et al. (1995) also found that drugs disposed together with domestic waste can reach landfill sites which could lead to ground water contamination by leaching.

Medicinal residues derived from human application have a unique pathway for the contamination of soils and ground water that may be the disposal of raw sewage or STP effluents by spray and board irrigation in agricultural areas. Veterinary pharmaceuticals used in animal feeding operations may be released to the environment with animal wastes through overflow or leakage from storage structures or land application (Kolpin et al. 2002).

3.2 Occurrence of PhACs in Wastewater Treatment Plant Effluents

As discussed in section 3.1, the majority of pharmaceutical compounds after ingestion and subsequent excretion, in the form of the original compound or as metabolites with STP effluents, enter aquatic systems. Moreover, many of the conjugates of PhACs can be converted back into active parent compounds by enzymes present in wastewater (Rodríguez et al. 2003 and Sedlak et al. 2005). The high application and the high levels of excretion of intact drugs as well as their persistence against biodegradation are the major reasons for PhACs' occurrence in the environment (Van der Heide and Hueck-Van der Plas 1984, Richardson and Bowron 1985).

Table 3-1 gives an overview of published literatures describing occurrences of residue PhACs in the aquatic environment. About 30 initial commom-use pharmaceuticals have been alphabetically listed in the table. These PhACs include NSAIDs, β-blockers, hormones and antibiotics, and all those commonly employed in human therapy and animal husbandry. The first column gives the name of PhACs or residues and the corresponding therapeutic types are shown in the second column. The concentrations of observed PhACs in the aquatic environment and sampling sites are illustrated in columns 3 and 4, respectively. The final column supplies the matching authors and issued date to each pharmaceutical. According to the table, most PhACs were detected in μg/L-level in sewage, for example, the concentrations of ibuprofen was found up to 20 μg/L and 4 μg/L, respectively, in STP influents and effluents in Finland. In contrast to wastewater or treated wastewater, the concentrations of residue PhACs ranged at the ng/L-level in surface water and ground water. The literature on aspect of the occurrence of veterinary drug residues is limited, only two data have been found, therefore, veterinary drugs will not be further the subject of this study.

Table 3-1 Occurrence of PhACs in the aquatic environment

PhACs	Therapeutic use	Concentration detected in the aquatic environment	Source or receiving body	Reference
Human Therapy				
Aspirin	Analgesic (Pain killer)	~1μg/L	STP effluents	Richardson and Bowron, 1985
		<150-1510ng/L	Effluent from sedimentation tank	Stumpf et al., 1996
		500-3000ng/L	STP influents in Tokyo	Nakada et al., 2006
		50-200 ng/L	Secondary effluents	
Atenolol	β-Blocker	190 ng/L	STP effluents in Italy	Castiglioni et al., 2005
		30ng/L	STP influents in Sweden	Bendz et al., 2005
		160ng/L	STP effluents in Sweden	
		160-1100 ng/L	STP effluents in Canada	Nikolai et al., 2006
Bleomycin	Antinoplastic agent	11-19 ng/L	STP effluent (radioimmuno assay)	Aherne et al., 1990
		<5-17 ng/L	River water	
		5-13 ng/L	Potable water	
Caffeine	Psychomotor stimulants	~ 1 μg/L	STP effluents	Richardson and Bowron, 1985
		> 1 μg/L	Potable water	
		16-292 μg/L	STP effluents	Rogers et al., 1986
		1.7-8.1 μg/L	STP effluents in Western Canada	Verenitch et al., 2006
		29-53 ng/L	River water	
Carbamazepine	Antiepileptic	0.25μg/L	River and stream water in German	Ternes, 1998
		2.1 μg/L	STP effluents in German	Ternes, 1998
		< 900ng/L	Groundwater in German	Sacher et al., 2001
		1.7 μg/L	STP effluents in Calgary, Canada	Metcalfe et al., 2002
		0.7 μg/L	STP effluents in Canada	Metcalfe et al., 2003
Ciprofloxacin	Antibiotic	118 ng/L	STP effluents in Canada	Chen et al., 2006
		850-2000ng/L	Hospital effluents in New Mexico	Brown et al., 2006
		200-1000ng/L	STP effluents in New Mexico	
Clofibrate	Liquid lowering agent	~ 40 ng/L	River water	Richardson and Bowron, 1985
Clofibric acid		< 0.5-1750 ng/L	River water Berlin	Heberer, 1995
		< 0.5-220 ng/L	River water Europe	
		< 5-180 ng/L	River water	Stumpf et al., 1996
		<50-1560 ng/L	Effluent from sedimentation tank	
		< 20 ng/L	River water	Stumpf et al., 1999
		<25 ng/L	STP effluents in U.S.	Drewes et al., 2002
		50 ng/L	Surface water of lake Wannsee	Heberer et al., 2002

(continued on next page)

Table 3.1 (Continued) Occurrence of PhACs in the aquatic environment

PhACs	Therapeutic use	Concentration In the aquatic environment	Source or receiving body	Reference
Cyclophosphamide	Antinoplastic agent (chemotherapy)	146ng/L (Estimated 1-10μg/L)	Treated hospital effluent from STP	Steger-Hartmann et al., 1996
Dextropropoxyphene	Analgesic agent	~1 μg/L	River water	Richardson and Bowron, 1985
Diazepam	Anxiolytic agent	<1 μg/L	STP effluents	Waggott, 1981
		~10 ng/L	River water	
		~10 ng/L	Potable water	
Diclorfenac	Analgesic agent	Up to 2 μg/L	Effluent from sedimentation tank	Stumpf et al., 1996
		15-304 ng/L	River Rhine	
		38-489 ng/L	Different rivers	
		<590ng/L	Groundwater in German	Sacher et al., 2001
		15-80 ng/L	STP effluents in U.S.	Drewes et al., 2002
		50 ng/L	Surface water of lake Wannsee	Heberer et al., 2002
		<20-568 ng/L	STP downstream river water in U. K.	Ashton et al., 2004
		0.68-5.45μg/L	STP effluents in Sweden	Bendz et al., 2005
		<10-200 ng/L	STP effluents in U.S.	Sedlak et al., 2005
		32-448 ng/L	STP effluents in Western Canada	Verenitch et al., 2006
Erythromycin	Antibiotic	~1 μg/L	River water	Watts et al., 1983
		<50-366 ng/L	STP downstream rivers water in U. K.	Ashton et al., 2004
		4.5-15.9 ng/L	River water	Zuccato et al., 2005
Estrogen	Hormone	Measurable concentration	Treated sewage water for irrigation	Shore et al., 1992
		0.2 -0.5 nmol/L	Raw sewage water, Tei Aviv, Israel	Shore et al., 1993
Estrogen/estradiol and estrone	Hormones	10 μmol/day	Daily excreate with urine from pregnant women	Fostis, 1987
		10-500 ng/L	STP influents in Tokyo	Nakada et al., 2006
		<1-100 ng/L	STP secondary effluents	
Ethinylestradiol	Hormone	< 0.2 ng/L	Surface water	Kalbfus, 1995
		0.3-0.6 ng/L	Effluent from sedimentation tank	
		2-15 ng/L	River water	Aherne and Briggs, 1989
		1-3 ng/L	Reservoir	
		< 5 ng/L	Drinking water	
Gemfibrozil	Lipid regulator	<10-1235 ng/L	STP effluents in U.S.	Drewes et al., 2002
		<10- 4600 ng/L	STP effluents in U.S.	Sedlak et al., 2005
		0.84-4.76μg/L	STP effluents in Sweden	Bendz et al., 2005

(continued on next page)

Table 3.1 (Continued) Occurrence of PhACs in the aquatic environment

PhACs	Therapeutic use	Concentration In the aquatic environment	Source or receiving body	Reference
		80-478 ng/L	STP effluents in Canada	Verenitch et al., 2006
		13.7 ng/L	Surface water in Canada	Lissemore et al., 2006
		23ng/L	Surface water in Canada	Chen et al., 2006
Ibuprofen	Analgesic agent	Up to 12μg/L	Effluent sedimentation tank	Stumpf et al., 1996
		< 5-41 ng/L	River Rhine	
		17-139 ng/L	Different river water samples	
		1.5-7.8 ng/L	River or lake water	Buser et al., 1999
		1-5 μg/L	STP effluents in Switzerland	
		5-3380 ng/L	STP effluents in U.S.	Drewes et al., 2002
		<10-2300 ng/L	STP effluents in U.S.	Sedlak et al., 2005
		<0.02-5 μg/L	STP downstream rivers water in U. K.	Ashton et al., 2004
		9.7-19.7 μg/L	STP influents in Finland	Lindqvist et al., 2005
		<3.9μg/L	STP effluents in Finland	
		0.05-7.11 μg/L	STP effluents in Sweden	Bendz et al., 2005
		1-4.6 μg/L	STP effluents in Switzerland	Tauxe-Wuersch et al., 2005
		2.2-6.7μg/L	STP effluents in Western Canada	Verenitch et al., 2006
		< 24.6μg/L	STP effluents in Canada	Metcalfe et al., 2003
Ifosfamide	Antinoplastic agent (chemotherapy)	24 ng/L (estimated 1~10 ng/L)	Treated hospital effluent from SPT	Steger-Hartmann et al., 1996
Indometacin	Analgetic agent	<5-26 ng/L	River Rhine	Stumpf et al., 1996
		17-121 ng/L	Different rivers	
Methaqualone	Hypnotics	~ 1μg/L	STP effluents	Richardson and Bowron, 1985
Methotrexate	Antinoplastic agent (chemotherapy)	~ 1μg/L	STP effluents	Aherne and English, 1985
		<6.25 ng/L	River water	
		<6.25 ng/L	Potable water	
Metoprolol	Beta-blocker	730 ng/L	STP effluents in Germany	Ternes, 1998
		<10-160 ng/L	STP effluents in U.S.	Sedlak et al., 2005
		160ng/L	STP influents in Sweden	Bendz et al., 2005
		190 ng/L	STP effluents in Sweden	
		170-520 ng/L	STP effluents in Canada	Nikolai et al., 2006
Morphinan structure	Narcotic analgesic	< 1 μg/L	River water	Richardson and Bowron, 1985
Naproxen	Analgesic, anti-inflammitory	16-292μg/L	STP effluents	Rogers et al., 1986
		70 ng/L	River and stream water in German	Ternes, 1998

(continued on next page)

Table 3.1 (Continued) Occurrence of PhACs in the aquatic environment

PhACs	Therapeutic use	Concentration In the aquatic environment	Source or receiving body	Reference
		< 33.9 µg/L	STP effluents in Canada	Metcalfe et al., 2003
		~100 ng/L	STP effluent in German	Ternes et al., 2003
		100-3200 ng/L	STP effluents in U.S.	Sedlak et al., 2005
		<6.8µg/L	STP effluents in Canada	Lishman et al., 2006
Norethisterone	Hormone	8-20 ng/L	Effluent from sedimentation tank	Aherne and Briggs, 1989
		< 17 ng/L	River water	
		< 10 ng/L	Reservoir	
		< 10 ng/L	Drinking water	
Norfloxacin	Antibiotic	50ng/L	STP effluents in Canada	Chen et al., 2006
Oral contraceptive	Hormone	< 0.2 µg/L	River water	Aherne and English, 1985
		< 0.1 µg/L	STP effluents	
Ofloxacin	Antibiotic	330-510ng/L	STP effluents in France	Andreozzi et al., 2003
		290-580ng/L	STP effluents in Italy	
		460 ng/L	STP effluents in Greece	
		94ng/L	STP effluents in Canada	Chen et al., 2006
		4900-35,500ng/L	Hospital effluents in New Mexico	Brown et al., 2006
		110-1000ng/L	STP effluents in New Mexico	
Penicilloyl groups	Antibiotic	> 25 ng/L	River water	Richardson and Bowron, 1985
		> 10 ng/L	Potable water	
Propranolo	Beta-blocker	170 ng/L	STP effluents in Germany	Ternes, 1998
		30 ng/L	STP effluents in U.K.	Paxeus et al., 2004
		50ng/L	STP influents in Sweden	Bendz et al., 2005
		30ng/L	STP effluents in Sweden	
		60ng/L	STP effluents in U.S.	Fono and Sedlak, 2005
		20-92 ng/L	STP effluents in Canada	Nikolai et al., 2006
Sulphamethoxazole	Antibiotic	~ 1µg/L	River water	Watts et al., 1983
		300-1500	STP effluents in Germany	Hartig et al., 1999
		<410ng/L	Groundwater in German	Sacher et al., 2001
		<50 ng/L	STP downstream river water in U. K.	Ashton et al., 2004
		<30-2000 ng/L	STP effluents in U.S.	Sedlak et al., 2005
		50-90 ng/L	STP effluents in Sweden	Bendz et al., 2005
		243ng/L	STP effluents in Canada	Chen et al., 2006
		400-2100ng/L	Hospital effluents in New Mexico	Brown et al., 2006

(continued on next page)

Table 3.1 (Continued) Occurrence of PhACs in the aquatic environment

PhACs	Therapeutic use	Concentration In the aquatic environment	Source or receiving body	Reference
		310-1000ng/L	STP effluents in New Mexico	
Tetracycline	Antibiotic	~ 1μg/L	River water	Watts et al., 1983
Theophylline	Psycomotor stimulant	~ 1μg/L	River water	Watts et al., 1983
Testosterone	Hormone	0.8-1.1 nmol/L	Raw sewage water, Tel Aviv, Israel	Shore et al., 1983
Animal Husbandry				
Ivermectin	Antiparacitic agent	> 60%	Excreated with feces the 3 first days after injection (dose 0.3 mg/kg of cattle body weight)	Chiu et al., 1990
		2%	Concentration in urine (see above)	
Testrosterone and estrogen	Hormones growth promoters	1 μmol/g manure	Chicken manure	Shore et al., 1988

3.2.1 Occurrence of Antiphlogistics in STP Effluents

Researches in the water industry have had concerns about the occurence of PhACs residues in STP effluents since the early 1970's. Hignite and Azarnoff (1977) reported loads of antiphlogistics such as salicylic acid up to 28.7 kg/d in the effluents of the municipal sewage treatment plant of Kansas City (USA). Later, Waggott (1981), Watts et al. (1983) and Richardson and Bowron (1985) noticed that the same drug was present in the aquatic environment at concentrations up to approximately 1μg/L in the U.K.

Rogers et al. (1986) identified the two antiphlogistics ibuprofen and naproxen in untreated sewage on Iona Island, British Columbia, Canada. Up to now, antiphlogistics such as ibuprofen, naproxen, mefenamic and diclofenac, and the rest frequently utilized acidic pharmaceuticals are constantly protagonists in allL statements relative to PhACs in wastewater (Drewes et al. 2002, Lee et al. 2005, Lindqvist et al. 2005, Tauxe-Wuersch et al. 2005, Chen et al. 2006). The concentrations of these anti-inflammatory drugs in wastewater fluctuate between ng/L and μg/L, of which naproxen was found to be the most abundant compound. In Canada, concentrations of naproxen and ibuprofen were found up to 33.9 μg/L and 24.6 μg/L in STP effluents (Metcalfe et al., 2003).

3.2.2 Occurrence of Betablockers and Lipid-Lowing Agents in STP Effluents

Mean concentrations of β-blockers were in the μg/L-range in many wastewater treatment plant effluents (Huggett et al. 2003, Paxeus 2004, Fono and Sedlak 2005, Castiglioni et al. 2006, Roberts and Thomas 2006, Nikolai et al. 2006). For example, metoprolol had concentrations below 2μg/L in different countries like Germany, the U.S.

and Canada have been reported by several researchers (Ternes 1998, Huggett et al. 2003, Nikolai et al. 2006). In Europe, atenolol concentrations varied in a wide ng/L range (Paxeus 2004, Roberts and Thomas 2006, Nikolai et al. 2006), with median concentrations of 190 ng/L in Italy (Castiglioni et al. 2006). Concentrations of propranolol have been kept with the lower median concentrations (20-92 ng/L) in Canada (Nikolai et al. 2006) compared to the other two β-blockers (atenolol and metoprolol) observed in the U.S. and the U.K. STP effluents (*ca.* 30ng/L) (Fono and Sedlak 2005 and Paxeus 2004) and 170 ng/L in German STP effluents (Ternes 1998).

Lipid regulator gemfibrozil and clofibric acid (a metabolite of the lipid regulator clofibrate) occurred in high concentration in wastewater treatment plant effluents (Drewes et al. 2002, Lee et al. 2003, Chen et al. 2006). Gemfibrozil was found at concentrations around 5μg/L was detected in a STP effluent in Sweden (Bendz et al. 2005). In addition, Hignite and Azarnoff (1977) found clofibric acid of up to 3 kg/d in the STP effluents in U.S.

3.2.3 Occurrence of Antibiotics in STP Effluents

Among a variety of pharmaceuticals, antibiotics are of particular environmental concern. The occurrences of antibiotics have been revealed in several European countries, such as Switzerland (Golet et al. 2002), France, Italy, Sweden, Greece (Andreozzi et al. 2003) as well as in Canada (Chen et al. 2006).

Several studies have reported the occurrence of antibiotics with the quinolone group in hospital effluents (Guiliani et al. 1996, Guardabassi et al. 1998, Hartmann et al. 1998, Alder et al., 2003). Brown et al. (2005) found ciprofloxacin and ofloxacin with concentrations over hundreds of part per billion (ppb) in hospital effluents and wastewater treatment plants in New Mexico. High concentrations of ofloxacin have been found in the effluents from sewage treatment plants in different countries, such as France (330–510ng/L), Italy (290–580ng/L), Greece (460ng/L) (Andreozzi et al., 2003), and Canada (max. 506ng/L) (Chen et al. 2006).

Widespread use of bacteriostatic sulphonamide (sulpha) antibiotics in factory farming without proper withdraw periods led to the accumulation of sulphaonamides in wastewater treatment plant influents (Brown et al. 2006, Chen et al. 2006) and futher more washed out with wastewater treatment plant effluents due to the incomplete elimination during wastewater treatment processes. For instance, sulfamethoxazole was detected in the effluents of wastewater treatment plants in Germay (Hartig et al. 1999) and Switzerland (Golet et al. 2002) as well as several European countries (Andreozzi et al. 2003) and Canada (Chen et al. 2006).

3.2.4 Occurrence of Carbamazepine in STP Effluents

Many researchers described the resistance of antiepileptic carbamazepine to treatments in the STPs (Metcalfe et al. 2003, Joss et al. 2005, Carballa et al. 2005) thus resulted in its frequent occurrence in wastewater treatment plant effluents (Ternes 1998, Öllers et al. 2001; Heberer 2002, Metcalfe et al., 2003). Also, its concentrations are usually up to the μg/L-level in sewage treatment plant effluents.

3.3 Occurrence of PhACs in Surface Water

All concentrations of PhACs found in sewage treatment plant effluents indicated a potential contamination of surface water. Over 80 PhACs have been detected in surface waters by investigations carried out in Austria (Hohenblum 2002), Brazil (Stumpf et al.

1999), Denmark (ENDS 2000), the U.K. (Richardson and Bowron 1985), Germany (Ternes 1998), Italy (Zuccato et al. 2005), the Netherlands (Mons et al. 2000), Canada, Croatia, Greece, Spain, Switzerland and the U.S. (Heberer 2002).

3.3.1 Trace of Pain Killers in Rivers or Lakes

Because of the sufficiently large applications and the appropriate physicochemical properties (i.e. nondegradability, polarity), analgesics have reached detectable concentrations in the environment. If this type of compound is mobile in the aquatic environment, they may then become detectable in rivers and lakes in very much the same way as other environmental contaminants. An analgesic agent such as ibuprofen, diclofenac and naproxen etc. were found in concentrations in excess of 0.1μg/L distinguished in various lakes or rivers in Switzerland (Buser et al. 1999) and in 18 Brazilian rivers and streams (Stumpf et al. 1999) as well as in surface waters in the U.S. (Boyd et al. 2003). Additionally, Ashton et al. (2004) and Bound and Voulvoulis (2006) consistently found ibuprofen at the highest concentrations (up to 3μg/L) in U.K. rivers that were downstream to the sewage treatment plant.

3.3.2 Trail of Cardiovascular Drugs in Surface Water

Several studies showed that β-blockers could be located in surface water and indicate incomplete degradability in sewage treatment plants (Stolker et al. 2004, Wiegel et al. 2004, Thomas and Hilton 2004, Ashton et al. 2004, Bendz et al. 2005, Fono and Sedlak 2005, Castiglioni et al. 2006, Roberts and Thoma 2006). Bendz et al. (2005) reported a high degree of persistence of propranolol and metoprolol to biological degradation in the aquatic environment.

The lipid lowering agent clofibric acid and gemfibrozil were detected in rivers, lakes, and in the open sea (Buser et al. 1998 and Rabiet et al. 2006). Clofibric acid was perceived to have a similar concentration of a hundred-level nanogram per liter in North American (Boyd et al. 2003) and in European surface waters (Stan et al. 1994, Stumpf et al. 1996, Ternes 1998, Daughton and Ternes 1999).

3.3.3 Occurrence of Antibiotics and Antiepieptics in Surface Water

Wastewater treatment processes achieve variable and often incomplete removal of antibiotics, resulting in the discharge of antibiotics into surface waters. Watts et al. (1983) described the presence of several antibiotics (erythromycin, sulphamethoxazole, tetracyclines and theophlline) with mg/L-level concentration in river water samples near a fish farm. From 1999 to 2001, tetracyclines and sulfonamides were detected in samples from nine sites in the U.S. in concentrations ranging from 70 ng/L to >15µg/L (Lindsey et al. 2001).

Antiepieptic carbamazepine displayed its high-ng/L (>100ppb) concentrations in surface waters in Germany (Zuehlke et al., 2004) and in northern Italy (Loos et al. 2007).

3.4 Occurrence of PhACs in Groundwater

As discussed in section 3.1.2, groundwater can be contaminated by digested sludge from municipal sewage treatment plants and by leaks from sewer systems in urban areas and occasionally, residues from production. Owing to their polar structure, several PhACs are not significantly adsorbed in the subsoils (Heberer 1995; Heberer and Stan, 1997, Heberer et al. 2001); thus they may reach the groundwaters. Sacher et al. (2001) analysed samples from 105 groundwater wells in Baden-Württemberg, Germany and located traces of PhACs including analgesics (phenazone, propyphenazone), β-blockers (metoprolol, bisoprolol, sotalol), the antiepileptic carbamazeppine and some antibiotics like the sulfonamides sulfamethoxazol in more than one-third of the samples.

3.4.1 Anti-inflammatories and Lipid-lowering Drugs

Analgesics, like phenazone-type pharmaceuticals, as well as their metabolites (Reddersen et al. 2002) and paracetamol (Rabiet et al. 2006) were discovered at trace-level concentrations in groundwater samples from Germany and France, respectively.

Contamination of groundwater by clofibric acid (a lipid regulator) was investigated in Spain and Germany (Galceran et al. 1989, Stan and Linkerhäger 1994, Heberer and Stan 1996). These papers showed that samples, taken from different districts of Berlin, all contained clofibric acid with concentration levels ranging up to 165 ng/L.

3.4.2 Antibiotics

Investigations related to antibiotics in the past decade have resulted in publications documenting their presence in the groundwater. For example, sulfonamides have the potential to enter groundwater rapidly (Stuer-Laurdsen et al. 2000) and have been detected in groundwater in Germany (Sacher et al., 2001), because they are water soluble, demonstrate low chelating ability and have a low binding constant for soil (Thurman and Lindsey 2000). Studies in the U.S. have identified antibiotics (sulfonamides and trimethoprim) in groundwater down-gradient from a landfill containing hospital waste (Eckel et al. 1993).

In addition, tetracylines have been shown to be strong chelators (Blanchflower et al. 1997) and sorb to soils strongly (Thurman and Lindsey 2000), therefore, it was hypothesized by Lindsey et al. (2001) that although tetracylines might be detected in surface water, they may not occur in groundwater. However, Thurman and Hostetler (1999) as well as Meyer et al. (2000) tracked tetracylines in groundwater samples collected near waste and wastewater lagoons (>1 μg/L,) and liquid hog lagoon samples (5 to 700μg/L), respectively.

3.5 Occurrence of PhACs in Treated Drinking Water

Because of the difficulties associated with measuring trace concentrations of PhACs, it is extremely difficult to detect PhACs in most water supplies. Nevertheless, concentrations of PhACs in the range of several hundreds of ng/L have been reported in the drinking water of several large European cities. These cities include Berlin (Heberer and Stan 1997, Heberer et al. 1998, Heberer 2002 and Redderson et al. 2002), Prague (Möller et al. 2002), or Milano (Zuccato et al. 2000). Additionally, trace-concrntration PhACs were also found in treated drinking water of Southern California, U.S. (Loraine and Pettigrove 2006).

3.5.1 Antiphlogistics

Reddersen et al. (2002) detected NSAIDs such as phenazone (400ng/L) and propiphenazone (120ng/L) in drinking water. Although the concentrations of phenazone and propiphenazone in drinking water seem harmless to human health over a lifetime consumption of drinking water according to the recommended concentration level 3μg/L by the German Federal Environmental Protection Agency (Reddersen et al. 2002), other data are needed to prove the potential human health risk due to increasing PhACs residues in aquatic environment. Loraine and Pettigrove (2006) conducted an investigation about the occurrence of thirteen polar PhACs in finished drinking water in Southern California, and found ibuprofen and its metabolite at low-µg/L levels.

3.5.2 Others

Anitiotic sulfonamides have been detected in Berlin drinking water wells for which almost all of the groundwater was bank-filtered surface water (Hartig and Jekel 2001), and in water supply wells in a Nebraska bank filtration site (Heberer et al. 2001).

From 1992 to 1994, Heberer and Stan (1999) found clofibric acid in Berlin tap water at a concentration of 270 ng/L. Later, Heberer et al. (2002) reported discovery of carbamazepine and primidone at concentrations as high as 100ng/L in the water-supply wells in Berlin.

In 2004, antiepileptic carbamazepine at a high concentration of 258ng/L was found in finished water in the U.S. by Stackelberg et al. (2004) using high performance liquid chromatography-mass spectrometry (HPLC-MS).

Based on discussions in this chapter, it has been known that through two stages, reactions in received bodies and environmental fate, the occurrence of PhACs in water systems presented in high concentration ranges from hundreds of nanogram per liter to a few of microgram per liter. What effects on the environment have these drug residues of high concentrations? This topic will be introduced as the followed chapter.

Chapter 4 EFFECT OF RESIDUAL PHARMACEUTICALLY ACTIVE COMPOUNDS ON THE ENVIRONMENT

With the increasing concentration of residual pharmaceuticals in water, scientists are beginning to realize that the health of aquatic organisms might be adversely affected (Sonnenschein and Soto 1998, Stumpter 1998) if they were continually exposed to residual pharmaceuticals became most of pharmaceutical are biologically active.

4.1 Effects of NSAIDs on the Aquatic Environment

The anti-fungal activity of ibuprofen was found to be enhanced with lowered pH, especially at values below pH 7 by Sanyal et al. (1993) as well as Elvers and Wright (1995). They also pointed out *Staphylococcus aureus* (a spherical bacterium frequently living on the skin or in the nose of a healthy a living beings) was susceptible to ibuprofen when its concentrations were greater then 150 μg/mL at initial pH 7. When pH decreased to 6, *Staphylococcus aureus* was significantly prevented growth.

4.2 Effects of Betablockers for Aquatic Organisms

Ecotoxicological studies show that aquatic organisms are sensitive to β-blockers (Huggett et al. 2002, Villegas-Navarro et al. 2003, Ferrari et al. 2004, Fent et al. 2006). Escher et al. (2005, 2006) demonstrated that most β-blockers had a specific toxicity towards green algae (*Desmodesmus subspicatus*).

For most mammals including human beings, β-receptors are located in many peripheral regions including the heart and lungs. In the heart, β-receptor blockade causes a decrease in heart rate and contractile strength (Insel 1991).

4.3 Side Effect of Antibiotics on the Environment

Intensive use of antibiotics in human and veterinary medicine and in industrial farming (food additives) has led to a significant increase in antimicrobial resistance, with important consequences on public health (Hartmann et al. 1998, ASM 2002). Just due to such diversified influences as disruption of aquatic ecology, promotion of antibiotic resistance and potential genotoxicity, antibiotics have exceptional concerns from water reaseachers compared to other PhACs in the environment (Daughton and Ternes 1999).

4.3.1 Toxicity of Antibiotics

The immediate concern of antibiotics is the potential toxicity of these compounds to aquatic organisms and humans through drinking water (ASM 2002). Harrass et al. (1985) reported that growth of six blue-green algae species were prevented by streptomycin (the first antibiotic remedy for tuberculosis) at concentrations level of 0.09 to 0.86 mg/L. Additionally, a motile single celled green algae (*Chlamydomonas reinhardtii*) growth was prevented at streptomycin concentrations of 0.66 mg/L. Algae growth in sub-lethal concentrations of streptomycin was slowed or delayed, and the maximum density attained by several species was decreased.

Macrì et al. (1988) and Migliore et al. (1997) showed that the acute toxicity of several agricultural antibiotics to *daphnia magna* (the most popular live food for aquarium fishes) or *artemia salina* (a brine shrimp), for example, furazolidone, an antibiotic largely used in medicated fish feed, has significant toxicity on daphnia magna and the mosquito (*culex pipiens*) larvae. In this regard, recent work by Golet et al. (2002) used acute ecotoxicity data to develop a risk assessment for total fluoroquinolone concentrations in wastewater treatment plants for *Pseudomonas putida*, a common wastewater treatment plant bacterium, and in surface water with regard to fish, daphnia, and algae. The predicted no-effect concentration (PNEC) of total fluoroquinolones for wastewater treatment plant organisms was found to be 8 μg/L while the PNEC for surface water species was 3 μg/L.

4.3.2 Promotion of Antibiotic Resistance

There is growing concern that releases of antibiotics to the environment contribute to the emergence of strains of disease-causing bacteria that are resistant to even higher doses of these drugs and makes treatment of infections more difficult (ASM 2002). Leff et al. (1993) detected resistance to kanamycin (an aminoglycoside antibiotic used to treat a wide variety of infections) and neomycin (an aminoglycoside antibiotic used topically in the treatment of skin and mucous membrane infections, wounds, and burns) in the bacterial assemblage of a coastal plain stream of South Carolina in U.S. by growth of colonies on media containing antibiotics. Attrassi et al. (1993) reported that resistance of bacterial flora to some antibiotics from water and mussels sampled at three marine sites located in Morocco, especially, resistance to penicillin and ampicillin, two β-lactam antibiotics used in the treatment of bacterial infections. Guardabassi et al. (1998) found that sewers downstream from a hospital displayed an increased prevalence of bacteria resistant to oxytetracycline (a tetracycline used to treat many infections common and rare), while sewers downstream from a pharmaceutical plant showed an increased prevalence of bacteria resistant to multiple drugs, including sulfamethoxazole. These results lend credence to the concern that antibiotic-resistant bacteria might develop from long-term environmental exposure to relatively low concentrations of antibiotics (Brown et al. 2005).

4.3.3 Genotoxic Effects of Antibiotics

Genotoxic substances are often mutagenic and carcinogenic, and are therefore, potentially suspect in the development of antibiotic-resistant organisms. Giuliana et al. (1994 and 1996) showed genotoxic activity in 13% of the 800 analyzed unconcentrated wastewater samples from a hospital. Fluoroquinolone antibiotics exhibited genotoxic effects for the genetically modified bacterial strain, *Salmonella typhimurium*, at concentrations as low as 5μg/L for norfloxacin and 25μg/L for ciprofloxacin (Hartmann et al. 1998).

It should be pointed out that although the concentration of these drug residues mentioned above in the aquatic environment is too low to pose an acute risk in an individual manner, it is unknown whether the combination of drugs that share a common mechanism of action can exhibit synergistic effects (Tyler et al. 1998). Due to that possibility, the last two decades have witnessed extensive efforts to identify the occurrence, fate and effects of pharmaceuticals in water analysis as discussed in Chapter3.

As discussions above, although no know human health effects have been associated with exposure to drinking water containing trace concentrations of drug residues; there is concrete evidence that these residual drugs could cause numerous adverse health effects on aquatic life, even at very low concentrations. Therefore, it is necessary to pay attention on the behaviors of PhACs in wastewater/water treatment processes. The efficiency of currently treatment technologies for removing drug residues in wastewater/water is summarized in Chapter 5.

Chapter 5 TREATMENT TECHNOLOGIES FOR REMOVING PHARMARCEUTICALLY ACTIVE COMPOUNDS IN WATER AND WASTEWATER

There are limited reports available regarding treatment technologies for removing PhACs from water systems. In this project, a literature review was done aimed at PhACs removal efficiency of current treatment techniques.

5.1 Wastewater Technology

5.1.1 Conventional Municipal Treatment System

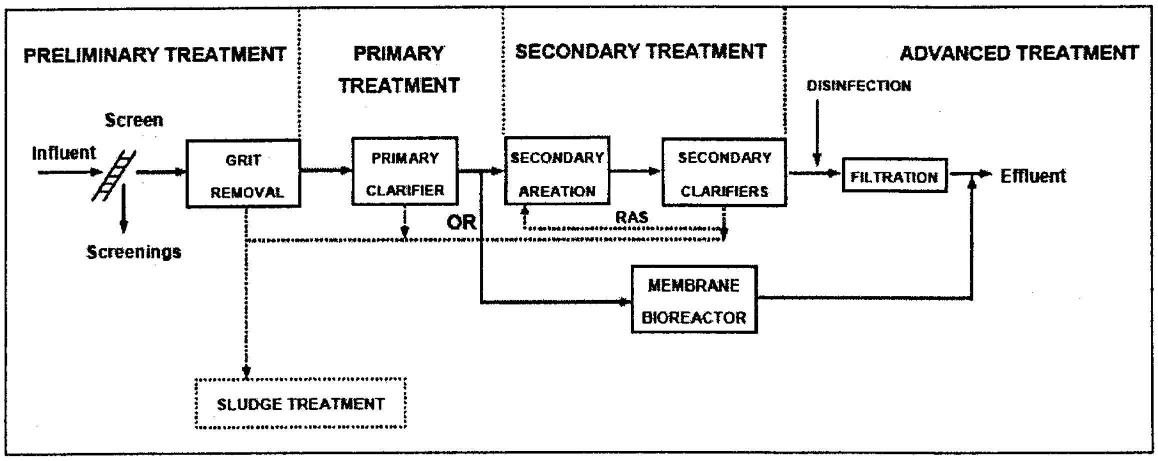


Figure 5-1 A flow scheme of conventional municipal treatment systems

Sewage treatment is a multi-stage process to renovate wastewater before it reenters a body of water, or applied to the land or reused. Generally, conventional wastewater treatment process includes five steps; preliminary, primary, secondary, advanced treatment and sludge treatment, as shown in Figure 5-1. Preliminary treatment, used to screen out, grind up, or separate debris, is the first step in wastewater treatment plants. Primary process is the second step in reduction and separation of greases and settleable solids from wastewater. Wastewater is held in settling tank or clarifiers for several hours allowing the particles to settle to the bottom and the greases to float to the top. The solids drawn off the bottom and skimmed off the top receive further treatment as sludge. The clarified wastewater flows on to secondary wastewater treatment, which usually is a biological treatment process to substantially degrade the biological content of the sewage such as human waste, food waste, soaps and detergent. After secondary treatment, the effluent may be discharged, depending on the discharge limits. In some cases, advanced treatment is required to remove nutrients or further reduction of organic and solid content in an effluent. Advanced treatment may also involve physical-chemical separation techniques such as adsorption, flocculation/precipitation, membranes for advanced filtration, ion exchange, and reverse osmosis. In various combinations, these processes can achieve any desired degree of pollution control. As wastewater is purified to higher and higher degrees by such advanced treatment processes, the treated effluents can be reused for urban, landscape, and agricultural irrigation, industrial cooling and processing, recreational uses and ground water recharge, and even indirect augmentation of drinking water supplies.

Sludges accumulated in the wastewater treatment process must be treated and disposed of in a safe and effective manner. The goals of sludge treatment are to stabilize the sludge, reduce odors, and reduce volume, kill disease causing organisms.

5.1.2 Removal Efficiency in Conventional Treatment Systems

Results from occurrence surveys (Sedlak et al. 2005, Clara et al. 2005 and Nakada et al. 2006) indicated that PhACs can be more or less removed in each single treatment process during sewage treatment plant. Among them, primary and secondary treatment processes as well as sludge treatment play roles in the removal of PhACs.

5.1.2.1 Primary Treatment

During primary treatment processes, sedimentation is used to reduce pollutants in wastewater. Due to the limitation of the hydrophilicity of PhACs, the removal efficiency of PhACs of various groups is low or zero by primary treatment, even with the best conditions (e.g., high temperature) (Carballa et al. 2004, 2005). For example, ibuprofen no significant elimination of ibuprofen was found during primary treatment in a full scale STP by Carballa et al. (2004).

5.1.2.2 Secondary Treatment

So far, worldwide sewage treatment plants mostly adopted biological methods to achieve the removal of organic matter from municipal water, of which the activated sludge treatment is the commonly used biological technique.

The activated sludge system is a wastewater treatment method in which the carbonaceous organic matter of wastewater provides an energy source for the production of new cells for a mixed population of microorganisms in an aquatic aerobic environment. The microbes convert carbon into cell tissue and oxidized end products that include carbon dioxide and water. In addition, a limited number of microorganisms may exist in activated sludge that obtains energy by oxidizing ammonia nitrogen to nitrate nitrogen in the process known as nitrification.

Bacteria constitute the majority of microorganisms present in activated sludge. Bacteria that require organic compounds for their supply of carbon and energy (heterotrophic bacteria) predominate, whereas bacteria that use inorganic compounds for cell growth (autotrophic bacteria) occur in proportion to concentrations of carbon and nitrogen. Both aerobic and anaerobic bacteria may exist in the activated sludge, but the predominant species are facultative, able to live in either the presence of or lack of dissolved oxygen.

The success of an activated sludge process is dependent upon establishing a mixed community of microorganisms that will remove and consume organic waste material, that will aggregate and adhere in a process known as bioflocculation, and that will settle in such a manner to produce a concentrated sludge (return activated sludge, or RAS) for recycling. Therefore, biological degradation and sorption onto the excess sludge are the core mechanisms of removal of PhACs during wastewater secondary treatment of the activated sludge process.

1) Biological Degradation

Joss et al. (2006) simultaneously ran three batch experiments using activated sewage sludge originating from nutrient-eliminating municipal wastewater treatment plants to study the biological degradation of 25 PhACs at typical concentration levels of 3μg/L. The three batch experiments were 1) the control batch without sludge, 2) an experiment with diluted sludge, and 3) an experiment with diluted sludge and substrate (addition of primary effluent). Comparing the results of three batch experiments, the control experiments run without biologically active sludge did not achieve any removal of all selected PhACs; however, an exponential decrease of the concentration over time can be seen for all the compounds in the batch experiments with sludge.

According to the comparison of results from batch experiments (Joss et al. 2006), the removal of PhACs during activated sludge treatment is due to their interaction with the sludge. Additionally, pseudo first order kinetic demonstrated by Ternes et al. (2004) in the EU-POSEIDON project can describe the biological degradation of PhACs as follows.

$$\frac{dC_i}{dt} = k_{i,biol} \cdot SS \cdot C_i \tag{5.1}$$

Where:

C_i = soluble substance concentration of the compound i inside the reactor [μg/L]

$k_{i,biol}$ = kinetic constant for pseudo first order degradation [L/(gSS·d)]

SS = suspended solids concentration [gSS/L]

Since suspended solids concentration (SS) are usually assumed to be constant for short-term batch observations, the term "pseudo" is added to the definition; and the direct proportionality of the transformation rate to the soluble substance concentration (C_i) is visually addressed as the "first order". The expression of the reaction rate constant $k_{i,biol}$ in per suspended solids concentration implied that it not only depends on the degradability of each specific compound but also on the dilute sludge composition.

Combining their experimental data and using Eq. (5.1), Joss et al. (2006) draw conclusions similar to those of previous researchers, for example, Metcalfe et al. (2003), EU-POSEIDON (2004), Clara et al. (2005), Joss et al. (2005), Lindqvist et al. (2005), Tauxe-Wuersch et al. (2005) and Chen et al. (2006). Namely, the observed biological removal varies strongly from compound to compound, with no evident correlation to the compound structure. Moreover, PhACs can be divided into three classes according to their persistence in state of typical reactor configurations: no substantial removal ($k_{i,biol}$ <0.1 L/(gSS·d)); partial removal (0.1< $k_{i,biol}$ <10 L/(gSS·d)); transformation by greater than 90% ($k_{i,biol}$ ≥10 L/(gSS·d)). For example, NSAID ibuprofen achieved notable removal rates of more than 90% and resulted in low-effluent concentrations at sludge age longer than 5 days, corresponding to a high $k_{i,biol}$ (≥10 L/(gSS·d)); antibiotics roxithromycin and sulfamethoxazole were seen partial and high variation removal of 0 to 90% at sludge ages longer than 5 days, corresponding to a $k_{i,biol}$ of 0.1 to 10 L/(gSS·d); whereas no remarkable biological transformation occurred for the antiepileptic carbamazepine, corresponding to a low $k_{i,biol}$ (<0.1 L/(gSS·d)).

Maurer et al. (2007) studied degradation rates of four β-blockers sotalol, atenolol, metoprolol and propranolol in sewage treatment plants. The pseudo-first-order degradation rate constants $k_{i,biol}$ in Eq. (5.1) were estimated to be 0.29, 0.69, 0.58 and 0.39 L/(gSS·d) for the four β-blockers mentioned above, respectively. The $k_{i,biol}$ values of 0.1 to 10 L/(gSS·d) means the four β-blockers are incompletely degraded during sewage treatment.

As for partially degradation of PhACs such as β-blockers and antibiotics, Ternes et al. (2004) explained that there are probably accuracy limitation for the experiments due to unaccounted influencing factors such as sludge loading and specific sludge composition. However, low $k_{i,biol}$ value (<0.1 L/(gSS·d)) lead to no significant degradation of PhACs like carbamazepine. Moreover, it could be clarified by the following sludge characteristics: (i) Biodiversity of the active biomass due to either differences in each species of micropollutants such as sludge age (Ternes et al., 2005) or the expressed enzymatic activity (Clara et al., 2005), (ii) Portion of active biomass within the total suspended solids (Ternes et al., 2005 and Joss et al., 2006), (iii) Floc size of the sludge for PhACs being well degraded (Joss et al., 2004 and 2005).

As a result, characteristic $k_{i,biol}$ value for each species of PhACs illustrates that biological degradation in municipal wastewater treatment contributes only to a limited extent to the overall load reduction of PhACs.

2) Sorption onto Sludge (Excess Sludge Removal)

During activated sludge treatment processes, sorption onto sludge is another one processing path for the removal of PhACs by derivating the compounds out of the water into activated sludge.

Ternes et al. (2004) assumed that the concentration of PhACs sorbed onto sludge ($C_{i,sorbed}$) is proportional to the concentration of PhACs in solution ($C_{i,soluble}$) for a compound i in equilibrium conditions as shown below.

$$C_{i,sorbed} = k_{d,i} \cdot C_{i,soluble} \tag{5.2}$$

Where:

$C_{i,sorbed}$ = concentration of the compound i sorbed onto sludge [μg/kg]

$k_{d,i}$ = sorption coefficient of the compound i [L/kg]

$C_{i,soluble}$ = soluble concentration of the compound i [μg/L]

According to equation (5.2), the sorption coefficient ($k_{d,i}$) is decided primarily by characteristics of the PhACs and of the sludge, therefore, the value of $k_{d,i}$ can represent sorption behavior of PhACs. Only those PhACs with high $k_{d,i}$ (>200 L/kg) values such as analgesic (diclofenac) and antibiotic (norfloxacin), showed a relevant process of sorption for PhACs elimination in EU-POSEIDON (2004).

In the studies of riddance of β-blockers in sewage treatment plants by Maurer et al. (2007), the $k_{d,i}$ values of four observed β-blockers sotalol, atenolol, metoprolol and propranolol were determined as less than 320 L/kg, it is seen that these β-blockers cannot significantly contribute to the overall elimination.

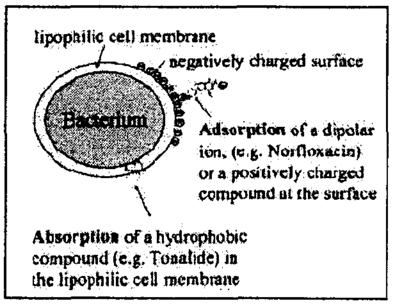


Figure 5-2 Absorption and adsorption mechanisms of PhACs on the sludge (Tonalide: personal care products, a synthetic polycyclic musk; Norfloxancin: abtibiotic) (Kujawa-Roeleveld et al., 2007)

All sorption experiments of PhACs involve two mechanisms absorption and adsorption (EU-POSEIDON 2004) as shown in Figure 5-2. When PhACs are mixed with sludge, the aliphatic and aromatic groups of the compound such as diclofenac came about hydrophobic interactions with the lipophilic cell membrane of the micro-organisms and the lipid fractions of the sludge and during this process, absorption played an important role to the sorption of PhACs onto sludge. On the other hand, for those charged molecules such as the fluoroquinolone antibiotics ciprofloxacin and norfloxacin, this can be attributed to specific sorption mechanism adsorption that seems to occur mainly due to the effects of electrostatic interactions between the positively charged amino group and the negatively charged surfaces of the suspended solids where the micro-organisms make up the main fraction. By this observation, the sorption onto suspended solids is not an effective mechanism for removal of PhACs except for hydrophobic and positively charged compounds (EU-POSEIDON 2004).

5.1.3 Advanced Treatment Systems

5.1.3.1 Membrane Bio-Reactors

The Membrane Bio-reactor (MBR) process is an emerging advanced wastewater treatment technology that has been successfully applied at an ever increasing number of locations around the world. In addition to their steady increase in number, MBR installations are also increasing in terms of scale.

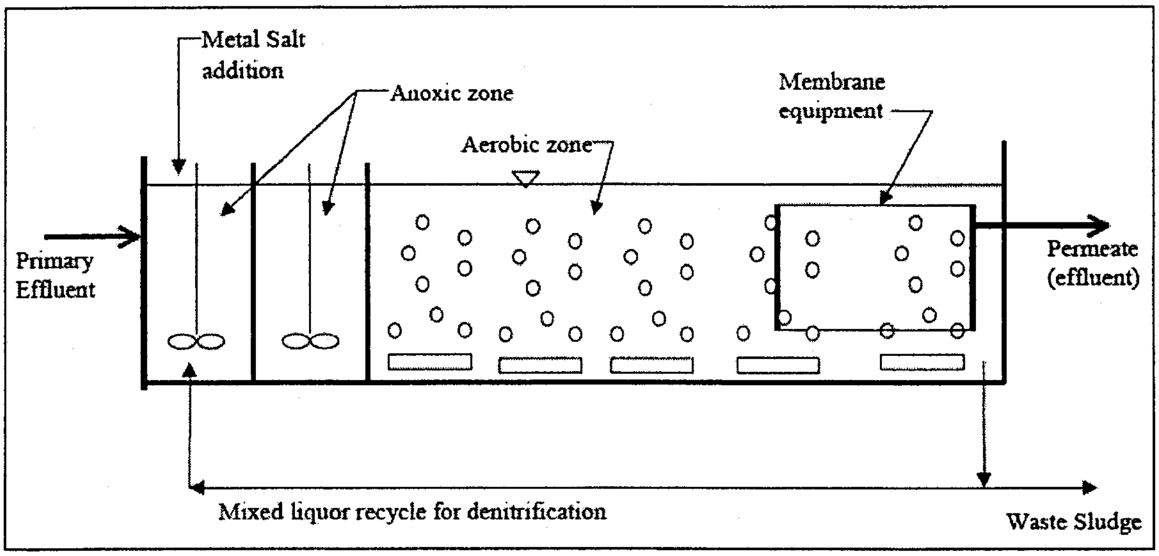


Figure 5-3 Typical schematic for membrane bioreactor systems (Internet-9, 2007)

The MBR process is a suspended growth activated sludge system that utilizes microporous membranes for solid/liquid separation in lieu of secondary clarifiers. The typical arrangement shown in Figure 5-3 includes submerged membranes in the aerated portion of the bioreactor, an anoxic zone and internal mixed liquor recycles. As a further alternative to Figure 5-3, some plants have used pressure membranes (rather than submerged membranes) external to the bioreactor.

Table 5-1 Mean removal of selected pharmaceuticals by the MBR and conventional activated sludge (CAS) processes (Radjenovic et al., 2007)

Compound	Elimination (%) in:	
	MBR[a]	CAS[b]
Analgesics and anti-inflammatory drugs		
Naproxen	99.3 (1.52)	85.1 (11.4)
Ketoprofen	91.9 (6.55)	51.5 (22.9)
Ibuprofen	99.8 (0.386)	82.5 (15.8)
Diclofenac	87.4 (14.1)	50.1 (20.1)
Indomethacin	46.6 (23.2)	23.4 (22.3)
Acetaminophen	99.6 (0.299)	98.4 (1.72)
Mefenamic acid	74.8 (20.1)	29.4 (32.3)
Propyphenazone	64.6 (13.3)	42.7 (19.0)
Anti-ulcer agents		
Ranitidine	95.0 (3.74)	42.2 (47.0)
Psychiatric drugs		
Paroxetine	89.7 (6.69)	90.6 (4.74)
Antiepileptic drugs		
Carbamazepine	No elimination[c]	No elimination
Antibiotics		
Ofloxacin	94.0 (6.51)	23.8 (23.5)
Sulfamethoxazole	60.5 (33.9)	55.6 (35.4)
Erythromycin	67.3 (16.1)	23.8 (29.2)
B-blockers		
Atenolol	65.5 (36.2)	No elimination
Metoprolol	58.7 (72.8)	No elimination
Diuretics		
Hydrochlorothiazide	66.3 (7.79)	76.3 (6.85)
Hypoglycaemic agents		
Glibenclamide	47.3 (20.1)	44.5 (19.1)
Lipid regulator and cholesterol lowering statin drugs		
Gemfibrozil	89.6 (23.3)	38.8 (16.9)
Bezafibrate	95.8 (8.66)	48.4 (33.8)
Clofibric acid	71.8 (30.9)	27.7 (46.9)
Pravastatin	90.8 (13.2)	61.8 (23.6)

[a,b] Values are averages, with relative standard deviations (%) in parentheses, for $n=10$[a] or $n=8$[b] samples

[c] Compounds were classified as "no elimination" if elimination was less than 10%

Compared to conventional activated sludge treatment, MBR is one novel approach to eliminate PhACs from wastewater and offers advantages such as long solids retention time (Clara et al., 2005) and low sludge load (Petrpvić et al., 2003).

Radjenovic et al. (2007) observed the behavior of several pharmaceutical products in different therapeutic categories (NSAIDs, antiepileptics and antibiotics, etc.) during wastewater treatment in a laboratory-scale MBR, and compared the results with those in a conventional activated sludge process. Through a two-month operation of the MBR, for most of the PhACs investigated, MBR treatment achieved ahigh and steady performance (removal rates >80%) compared to the conventional system (Table 5-1).

According to the experimental results of Radjenovic et al. (2007), such biodegradable PhACs as ketoprofen, diclofenac, bezafibrate, and gemfibrozil removal by the MBR system was very high and uniform (>90%) because of the smaller flock size of the sludge, which enhances mass transfer by diffusion and therefore increases elimination. Additionally, large amount of active biomass in suspended solids originating from MBR also improved the removal of this kind of PhACs.

In particular, Radjenovic et al. (2007) pointed out that substantially greater attenuation of diclofenac by the MBR was achieved with average removal efficiency of 87%, more than 50% in conventional activated sludge treatment. That is attributed to the longer solids retention time and greater adsorption potential of the MBR sludge compared to conventional activated sludge process. Unfortunately, antiepileptic carbamazepine was the most persistent drug and it passed untransformed through both the MBR and conventional systems. Other authors (Metcalfe et al. 2003, Clara et al. 2004, Joss et al. 2005) have reported similar results.

Although there are few papers reporting on the behavior of PhACs during MBR treatment, a fact that a complete removal could never be achieved by MBR even under optimum adaptation conditions for the rapid primary degradable PhACs was proven by many researchers (Kimura et al. 2004, Clara et al. 2005, Bernhard et al. 2006).

5.1.3.2 Others

There are many other advanced treatment techniques for treated wastewater such as membrane filtration, ozonation, advanced oxidation processes (AOPs) and disinfection. Because these techniques are also commonly used in drinking water treatment processes, the details about them will be discussed in the followed section.

5.2 Drinking Water Technology

5.2.1 Conventional Surface Water Treatment System

Water treatment originally focused on improving the aesthetic qualities of drinking water (EPA 2000). As early as 4000 B.C., water treatment methods such as filtering through charcoal and boiling were recommended by ancient Sanskrit and Greek writings to improve the taste and odor. Starting at the early 1800s, slow sand filtration was beginning to be used regularly in Europe, till to today; sort of filtration technologies have already been widely employed in worldwide water treatment plants. Behind the earliest water treatments, the turbidity concerns of people accelerated to the development of coagulation/flocculation techniques. Egyptians reportedly used the chemical alum to achieve coagulation at 1500 B.C. (EPA 2000).

In 1908 chlorine was used for the first time as a primary disinfectant of drinking water in Jersey City, New Jersey, the disinfection has become an indispensable in drinking water treatment processes, and the correlate technologies bloomed in water industries. According to a 1995 EPA survey, approximately 64 percent of community drinking water systems disinfect their water with chlorine. Almost all of the remaining surface water systems, and some of the remaining groundwater systems, use another type of disinfectant, such as ozone or chloramine.

Through the several-century growth, a classic flow diagram of a conventional surface water treatment plant was formed as shown in Figure 5-4. The basic treatment system includes screening, coagulation/flocculation, sedimentation, and filtration and post-disinfection is a traditionally used process.

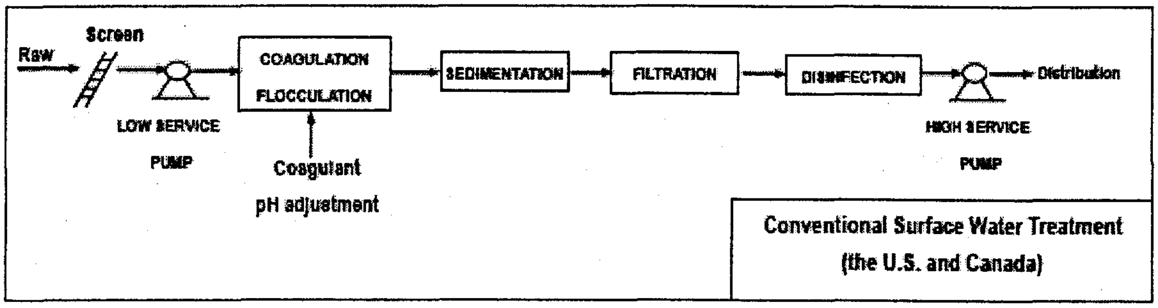


Figure 5-4 Schematic flow diagram of conventional surface water treatment plant in North America

5.2.2 Conventional Groundwater Treatment Plant

Contrasted to surface water, groundwaters usually have higher quality with respect to turbidity and microbiological contaminants such that disinfection may be the only required treatment, in some states disinfection may be even eliminated. Circumstances favoring this situation are that the aquifer has no direct connection to surface water and the well has been properly constructed so the aquifer cannot be contaminated at the well site (Letterman 2004). However, with the rapid development of kinds of industries such as mining and pharmacy, etc., much groundwater in cosmopolitan cities has been polluted to some extents by inorganic and organic substances. As a result, flocculation was frequently employed in the conventional groundwater treatment plant as shown in Figure 5-5.

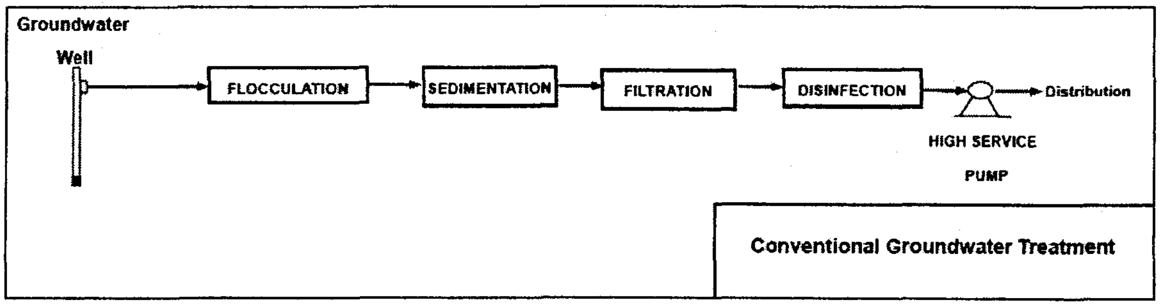


Figure 5-5 Schematic flow diagram of conventional groundwater treatment plant

5.2.3 Removal Efficiency in Conventional Drinking Water Systems

In conventional drinking water treatment process, the main removal mechanisms of PhACs involved are the coagulation/flocculation and disinfection.

5.2.3.1 Coagulation /Flocculation

Generally, such chemicals as metal salts or organic compounds are added into the wastewater to promote the agglomeration of suspended solids and colloids, thereby enhancing the removal of these particles; these processes are called coagulation-flocculation. A flotation process can be used as an alternative technique to separate finely suspended particles by their adhesion to the surface of rising bubbles, and has proved to be efficient for the removal of fat and other contaminants such as oils and biomolecules from water (Zouzoulis and Avranas 2000). By carrying out laboratory coagulation/flocculation studies (jar test), conventional coagulation/flocculation processes can accomplish minimal removal of some kinds of PhACs. These PhACs include analgesics diclofenac (Ternes et al. 2002) and naproxen (Boyd et al. 2003) with the exception of partial elimination (40-50%) of antibiotics (Adams et al. 2002). The low aqueous solubility and hydrophobic nature of the aforementioned PhACs and being partially removed may be an indicator for their removal potential (Westerhoff et al. 2005).

5.2.3.2 Disinfection

1) Chlorination

Chlorine is a strong oxidant used primarily as a disinfectant in drinking water treatment. While Sedlak et al. (2005) analyzed the removal efficiency of some kinds of μg/L-concentration PhACs including NSAIDs, β-blockers and antibiotics in many water treatment plants from the U.S.; they found elimination of certain PhACs such as diclofenac, ibuprofen, and propranol during chlorine disinfection of water effluent, especially when the water has been nitrified prior to disinfection. However, verification from the laboratory experiments through sampling before and after wastewater disinfection did not show any clear evidence for removal of these compounds. Therefore, further works is needed to prove the removal of the compounds during disinfection with chloramines.

When 0.5 and 6.0 mg/L of free chlorine (Cl_2) was applied to treated drinking water samples spiked with various pharmaceuticals (up to a concentration of 1μg/L) in laboratory tests, chlorine was found most effective to oxidize NSAIDs pyrazole derivatives phenazone, dimethylaminophrnazone and prpyphenazone with a 30 minutes contact time (Ternes et al., 2005). A complete oxidation of these compounds was achieved by a chlorine (Cl_2) dose of 0.5 mg/L were about 90%, 60% and 40%, respectively. The other selected pharmaceuticals, including caffeine, carbamazepine, diazepam, clofibric acid, ibuprofen and bezafibrate, were refractory to oxidation by chlorine.

Pinksron and Sedlak (2004) studied transformation of four β-blockers (atenolol, metoprolol, propranolol and nadolol) during chlorine disinfection. The β-blockers all exhibited a strong dependence of reactivity on pH. All four of the compounds exhibited an increase in reaction rates below pH 7. Except nadolol, three compounds (atenolol, metoprolol and propranolol) also showed an increased reaction rate above pH 8. The relative reactivity of these blockers was depended on their structure or function groups containing, for example, the β-blocker that does not exhibit an increase in reactivity at pH values above 8 (i.e., nadolol) contains a *tert*-butyl group (See Figure 5-6).

The difference in reactivity associated with this slight change in molecular structure is most likely attributable to the presence of an α-hydrogen (Figure 5-7) in atenolol, metoprolol, and propranolol (Pinksron and Sedlak, 2004). Chloramines that have hydrogen on the carbon α to the amine (Figure 5-7) have been shown to undergo a base-catalyzed decomposition reaction in which the hydrogen is abstracted and an imide (a functional group consisting of two carbonyl groups bound to a primary amine or ammonia) is formed. The imide then hydrolyzes, causing cleavage of the bond between the nitrogen and carbon and removal of the α-carbon and its substituents. An example of how this reaction would occur for propranolol can be seen in Figure 5-7.

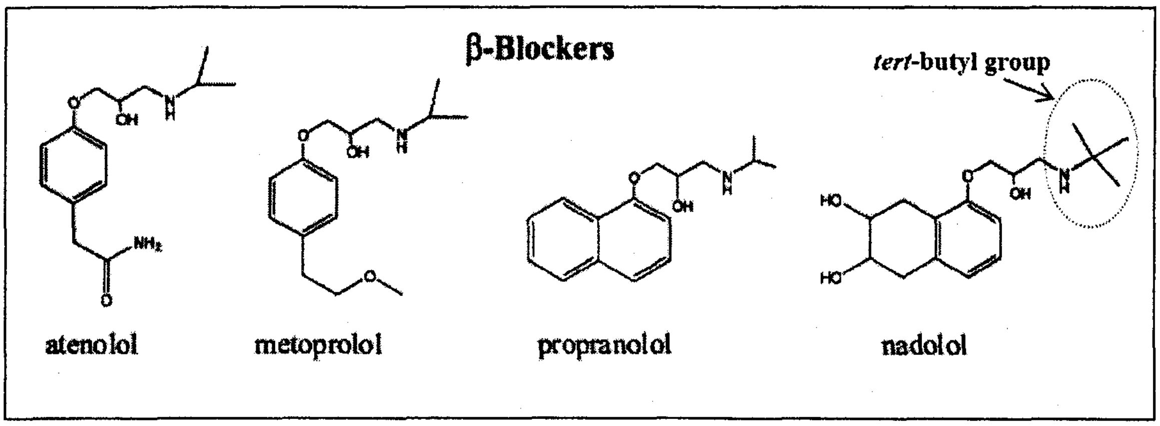
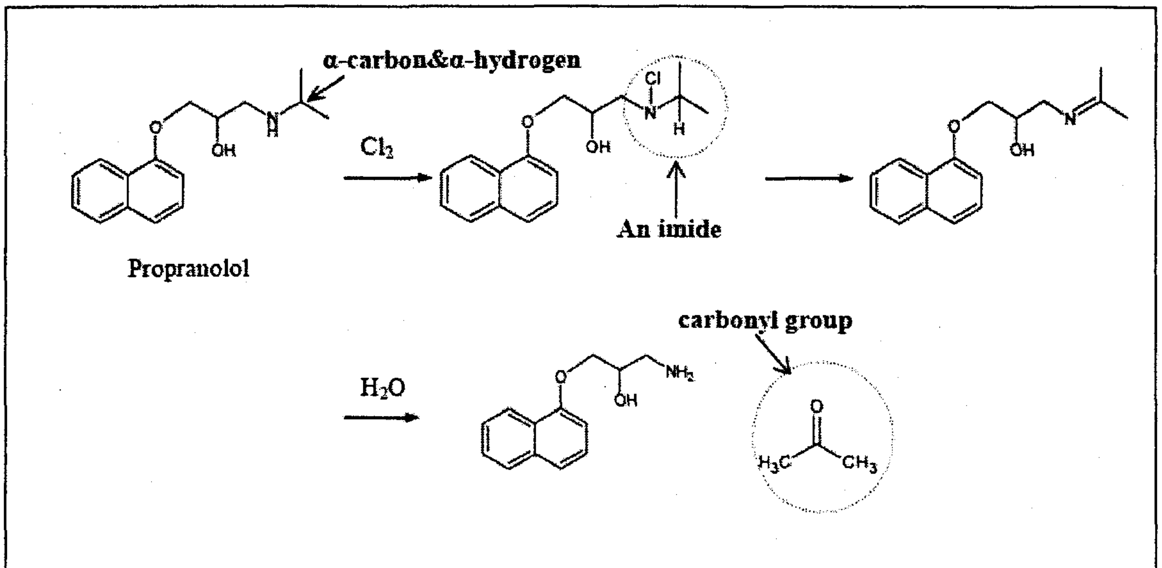


Figure 5-6 Structures of the β-blockers studied by Pinksron and Sedlak (2004)



The carbon atom adjacent to a carbonyl group is called the α-carbon.
The hydrogen atom adjacent to an α-carbon is called the α-hydogen.

Figure 5-7 Reactions between propranalol and chlorine

Moreover, effects of pH and chlorine dosage on the extent of acidic compound naproxen elimination were investigated by Boyd et al. (2005). In experiments, synthetic waters containing elevated concentrations of naproxen were oxidized by free chlorine at naproxen: chlorine molar ratios of 0.02 to 3.1 and pH values of 5 to 9. Firstly, results indicated that reactions between naproxen and chlorine were rapid and the reactions approached completion with 30 seconds for the experimental conditions. Results also demonstrate that naproxen was more readily transformed by contact with chlorine at pH 5.0, 99.7% of naproxen was removed after 15 minutes reaction. At neutral pH 7.0, about 99.2% of naproxen was eliminated in comparison to elevated pH 9.0, only 61.5% removal of naproxen was achieved at pH 9.0.

The extent of reaction of naproxen at pH 5.0 and 7.0 was attributed to the reactivity of free chlorine in this pH range. At low pH conditions, aqueous chlorine exists primarily as hypochlorous acid (HOCl), which favors the oxidation reaction with naproxen. Further, naproxen is a weak acid with pK_a of 4.4 (Cantabrana et al. 1995) and experimental conditions favored the anionic form of naproxen. As a consequence, at elevated pH 9.0, the predominant form of chlorine is hypochlorite ion (OCl^-) (Fair et al., 1948) and the reaction between the two anionic forms is less efficient compared to the low pH experiments. Because the formation of specific reaction products can vary depending on the characteristics of the water and treatment operation conditions, so far it is not clear how to identify the intermediated and chemical reaction end products. Additionally, the reaction kinetic of naproxen chlorination for arranging of water and wastewater treatment conditions is hard to understand. But, according to the study of Boyd et al. (2005), low pH conditions are likely to favor oxidation of naproxen by chlorination in water treatment systems.

Secondly, the experimental findings of Boyd et al.'s (2005) indicated that the rate and extent of reaction between naproxen and chlorine increased with greater chlorine dosages and a naproxen to chlorine molar ratio less than 3:1 is needed to remove naproxen from the aqueous phase in their batch experiments.

Overall, only a few PhACs tend to be transformed during chlorine disinfection (Glassmeyer and Shoemaker 2005 and Gibs et al. 2007). More research is needed to identify the specific intermediate and end transformation products and the characterizing mechanisms of PhACs' chlorination reactions for a range of water treatment conditions as well as natural aquatic environments such as pH and TOC (Ternes et al., 2005; Boyd et al., 2005). Furthermore, just as discussed previously, the examples of naproxen and β-blockers implied that the removal efficiency of PhACs by chlorination is not only relied on aquatic environments (e.g. pH), but also depends on the nature (e.g. structure and pK_a) of target PhACs. That means these conditions limit the applications of chlorination on removing the majority PhACs.

2) Chlorine Dioxide (ClO_2)

Since chlorine dioxide lowers potential of forming disinfection by products such as trihalomehtanes (THMs), it is an alternative to chlorine for disinfection of relatively high quality water including drinking water.

In order to predict the oxidation of PhACs under the given conditions, Ternes et al. (2005) carried out the deprotonated species of diclofenac, 17α-Ethinylestradiol, sulfamethoxazole as well as the neutral species of roxithromycin exhibit a high reactivity to chlorine dioxide. Most the remaining PhACs such as bezafibrate, carbamazepine, diazepam, ibuprofen, and iopromide, etc. did not react at an appreciable rate with chlorine dioxide.

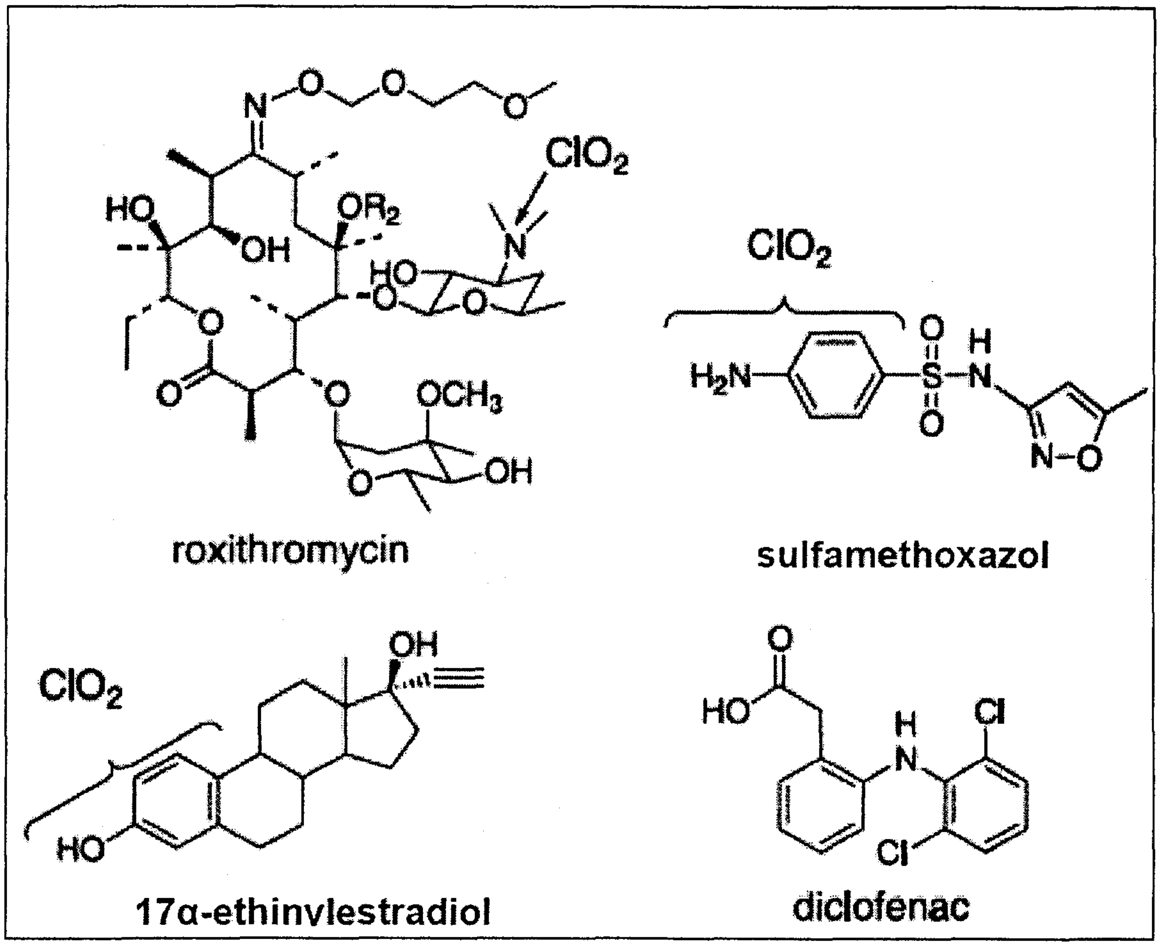


Figure 5-8 Chemical structures of reactive PhACs and sites of proposed ClO_2 attack. (Huber et al., 2005)

Huber et al. (2005) carried out a series of oxidation experiments of PhACs during water treatment including drinking water, surface water and groundwater treatment with chlorine dioxide and obtained similar results as Ternes et al. (2005). Chlorine dioxide was only effective to oxidize certain PhACs such as sulfonamide and macrolide antibiotics, and estrogens. In their work, diclofenac, 17α-Ethinylestradiol (EE2), sulfamethoxazole and roxithromycin showed an appreciable reactivity and exhibited relativly high reactivity at $pH \geq 7$. Because ClO_2 reacts very selectively with certain functional groups with high electron densities such as phenoxide ions and neutral tertiary amines (Hoigné and Bader 1994), the reactivity of the protonated forms of these functional groups is usually many orders of magnitude lower. Therefore, the pH-dependent reactivity of EE2 and roxithromycin, which exhibit a phenolic and a tertiary amino group, respectively, can be explained by the protonation state of their reactive functional groups. The pH dependency of the reactions also implies that ClO_2 attack actually takes place at the phenolic moiety of EE2 and at the tertiary amino group of roxithromycin (Figure 5-8). The reactions of diclofenac and sulfamthoxazole with ClO_2 may be contributed by the aniline group (C_6H_5-NH_2) they containing. Moreover, the deprotonation of the acidic nitrogen of the sulfonamide moiety enhances the reactivity of sulfamehtoxazole considerably.

Due to these specially pivotal function groups reacted with ClO_2 in PhACs, fewer compounds are efficiently removed during water treatment with chlorine dioxide compared to ozone with the exception of a little of PhACs stated previously.

5.2.4 Advanced Treatment

5.2.4.1 Ozonation

Ozonation as an advanced treatment technique is an interesting and representative chemical oxidation option. Ozonation of the treated wastewater has confirmed being a feasible polishing step for biologically treated wastewater with the potential of eliminating a wide variety of PhACs (Thernes et al. 2003, 2005). It is worthy to say that there is no indication that the by products of ozonation of PhACs are toxic (Ternes et al. 2005).

In drinking water treatment, ozonation showed high efficiency in oxidizing PhACs (Zwiener et al. 2000; Thernes et al. 2002 and Huber et al. 2003). Huber et al.

(2005) using ozone (O_3) treated municipal wastewater effluents in a pilot-scale plant. Selected pharmaceuticals macrolide and sulfonamide antibiotics, estrogens, and the diclofenac, naproxen, and indomethacin were spiked in the wastewater at realistic levels ranging from 0.5 to 5μg/L. The oxidization efficiencies of these drugs were more than 90% for O_3 doses greater than 2mg/L in all effluents. Compare to ClO_2, ozone exhibited higher removal ability of PhACs. Ozone reacts with the same functional groups as ClO_2, however, ozone also reacts with C=C double bonds and activated benzene rings. With respect to ozone, it has also to be taken into account that hydroxyl radicals formed due to ozone decay, can oxidize refractive PhACs to a substantial extent.

Unfortunately, ozonation were broadly applied in water treatment processes based on highly reactive radicals like phenol ring and the sulfur atom (Andreozzi et al. 2005 and Zwiener 2007), especially the hydroxyl radical (OH) (Vogna et al. 2004). During ozonation, micropollutants can be oxidized either by O_3 directly or by hydroxyl radicals (OH), which are formed as a consequence of O_3 decay. The two oxidants vary strongly in their reactivity. O_3 attacks very selectively certain functional groups, whereas OH is a nonselective oxidant that reacts very fast with a large number of moieties. Consequently, most OH is scavenged by the water matrix in wastewater (von Gunten 2003). Therefore, the oxidation of compounds that react slowly with O_3 is always less efficient than for compounds that react fast with O_3, even if the rate constants for the reaction with OH are nearly diffusion-controlled. This characteristic led to the presence in treated water of those compounds lacking susceptible functional groups such as ifosfamide, even at high ozone dosage (2.1mg/L) (McDowell et al. 2005). Otherwise, the oxidation reactions of certain PhACs were controlled by aquatic conditions. For example, the solubility of diclofenac is very limited at low pH, on the other hand, it could form foam at pH greater than 6. These prevented the investigation of ozonation in a broader range (Vogna et al. 2004).

5.2.4.2 Advanced Oxidation Processes

Advanced oxidation process (AOP) is considered as a good choice in order to treat the hazardous non-biodegradable pollutants including pharmaceuticals in water and wastewater (Andreozzi et al. 1999, Oppenländer 2003 and Pérez-Estrada et al. 2005). The AOPs are characterized by a variety of radical reactions that involve combinations of chemical agents (e.g., ozone (O_3), hydrogen peroxide (H_2O_2), transition metals, and metal oxides) and auxiliary energy sources (e.g., ultraviolet-visible (UV-Vis) radiation, electronic current, γ-radiation, and ultrasound). Examples of AOPs include O_3/H_2O_2, O_3/ UV, O_3/H_2O_2/ UV, H_2O_2/ UV, Fenton (Fe^{2+}/H_2O_2).

Hydroxyl radicals (OH) which are produced in AOP process are very reactive and play an important role in the mineralization of the pharmaceutical compounds in the final stage (Pérez-Estrada et al., 2005). However, other radical and active oxygen species such as superoxide radical anions ($O_2^{\bullet}-$), hydroperoxyl radicals ($HO_2^{\bullet}$), triplet oxygen ($^3O_2^{\bullet}$), and organic peroxyl radicals ($R-O-O^{\bullet}$) are also involved (Oppenländer, 2003).

Zwiener and Frimmel (2000) reported that ozonation (1 mg/L applied ozone) alone was not sufficient to convert 2 µg/L ibuprofen (NSAID) in distilled water, resulting in only 12% conversion in 10 min. However, increasing the ozone dose and adding hydrogen peroxide (O_3/H_2O_2 AOP) greately improved the ibuprofen conversion. They also investigated the effect of water matrix on the degraditon of ibuprofen using a river water sample. The presence of hydroxyl radical scavenger, bicarbonate ion and natural DOC, in the river water significantly reduced the extent of ibuprofen conversion by O_3/H_2O_2 AOP, implying the importance of hydroxyl radical reaction. In the river water, they noted that the treatment with 5.0 mg/L applied ozone and 1.8 mg/L hydrogen peroxide was sufficient to convert 2 μg/L ibuprofen in 10 min at pH 7.5 and 10 °C (Zwiener and Frimmel, 2000).

Andreozzi et al. (2004) investigated H_2O_2/UV and TiO_2 photo-catalysis for the decontamination of an aqueous pharmaceutica mixture containing ofloxacin, as well as five other drugs, namely diclofenac, propranolol, clofibric acid, sulfamethoxazole and carbamazepine. It was shown that H_2O_2/UV treatment ($[H_2O_2]_0$=5-10 mM, a low-pressure Hg lamp, at 254 nm, 2.51×10^{-6} Einstein·s^{-1}) was sufficient for the complete conversion of propranolol (β-blocker) and ofloxacin (antibiotic) at pH 7.4 and 25°C, whereas TiO_2/hv process was less effective.

Degradation of clofibric acid (lipid regulator) by ozone can be enhanced by adding hydrogen peroxide (i.e., O_3/H_2O_2 AOP) (Zwiener and Frimmel, 2000) or elevating pH (Andreozzi et al. 2003). These two approaches indicate the importance of hydroxyl radical reactions for the degradation of this drug metabolite.

Vogna et al. (2004) reported the degradation of carbamazepine by H_2O_2/UV AOP. Carbamazepine could be converted rapidly by H_2O_2/UV treatment; about 4.7 mg/L carbamazepine was completely converted within 4 min of treatment with 170 mg/L H_2O_2 and UV irradiation at 254 nm using a 17 W low-pressure Hg lamp (2.7×10^{-6} Einstein·s^{-1}) at pH 5.0 (Vogna et al. 2004). Recently, the carbamazepine degradation by TiO_2 photocatalysis was investigated in a series of study (Doll and Frimmel 2004, 2005). Doll and Frimmel (2004) showed that as compared with other persistent pharmaceuticals including clofibric acid, iomeprol, iopromide, carbamazepine could be rapidly degraded by TiO_2/hv process. More than 90% of initial carbamazepine (4.2 mg/L) was converted in nine minutes by treatment using 100 mg/L (TiO_2) (P25) and a 1000 –W xenon short-arc lamp as a source of simulated solar UV rays (1.35×10^4 Einstein$\cdot m^2\cdot s^{-1}$, $\lambda< 400$ nm) at pH 6.5 (Doll and Frimmel 2005). A successful pilot-scale investigation of the continuous treatment of carbamazepine by the TiO_2/hv process was reported using a cross-flow microfiltration to retain catalyst (Doll and Frimmel 2005). The degradation of carbamazepine was strongly inhibited in the presence of natural organic matter (NOM) in a lake water sample by competing for hydroxyl radicals, as well as by deactivating TiO_2 catalyst surface by adsorption (Doll and Frimmel 2005).

The advantages of AOPs processes include complete mineralization of PhACs, production of less harmful and more biodegradable by-products, and ability to handle fluctuating flow rates and compositions (Zhou and Smith 2001). However, the performance of AOP is affected by the presence of other water and wastewater constituents, such as NOM, dissolved and suspended solids, and alkalinity, as well as by water pH and temperature (Oppenländer 2003). For example, suspended solids and color can hinder photochemical reactions by light scattering and absorption and may impair the performance of photochemical AOPs, such as O_3/ UV, H_2O_2/ UV, photo – Fenton, and TiO_2/hv processes. Carbonate, bicarbonate, and chloride ions, as well as some natural organic compounds are known to act as radical scavengers. These compounds compete with target pollutants for hydroxyl radiacals; therefore, their presence increases oxidant demands and lowers the treatment efficiency. In addition, the costs of materials and equipment, as well as energy requirements and efficiency must be taken into account when assessing the overall performance of AOPs (Legrini et al. 1993 and Oppenländer 2003)

5.2.4.3 Adsorption on Activated Carbon

Activated carbon is a well-known process for removing various organic contaminants and organic carbon in general. Activated carbon is either applied as a powdered feed or in a granular form in packed bed filters. Granular activated carbon (GAC) is used at many water treatment plants in the U.S. and Canada (EPA 2000). The GAC can be employed as a replacement for anthracite media in conventional filters, thus providing both adsorption and filtration. Alternatively, GAC can be applied post-conventional filtration as an adsorbent bed (Moore et al. 2001, 2003). Estimated two thirds of all surface water treatment plants in the U.S. and Canada have the capability to feed powdered activated carbon (PAC) intermittently (EPA 2000). One of the more common submissions of PAC is for controlling tastes and odors such as geosmin and methylisoborneol (MIB) (Knappe et al. 1998). Recently, researchers have developed tailored activated carbons for improving geosmin and MIB removal (Nowack et al. 2004, Rangel-Mendez and Cannon 2005), and the work herein has appraised how this activated carbon also enhances the removal of PhACs. Studies about activated carbon efficiency for PhACs removal were performed both in lab scale experiments (Ternes et al. 2002, 2005 and Westerhoff et al. 2005) and full-scale utilities (Snyder et al. 2007).

Ternes et al. (2002, 2005) carried out adsorption of PhACs on GAC and on PAC in pilot-scale experiments. According to their experimental results, the octanol-water partition coefficient (K_{ow}) has proved useful for predicting removal efficiency.

Antiepileptic compounds (e.g. carbamazepine, diazepam) with rather high K_{ow} values ($10^{2.4}$ to $10^{6.4}$) are very easily adsorbable (<0.2 mg/L AC for 99% removal). Additionally, medium absorbability was observed for charged PhACs or compounds with lower K_{ow} values ($10^{-2.3}$ to $10^{2.7}$) such as ibuprofen, roxythromycin, sulfamenthoxazole and iopromide and showed a behavior similar to that of atrazine. Adsorption of charged PhACs on activated carbon is much lower than would be expected from the K_{ow} values of the protonated PhACs. This is also reflected by the low breakthrough volumes of clofibric acid in an activated carbon filter (17 m^3/kg activated carbon) compared with carbamazepine (70 m^3/kg activated carbon).

The study of Westrhoff et al.'s (2005) further demonstrate the affinity between K_{ow} values and the percentage adsorption of PhACs on activated carbon. Relatively higher removal, between 75 and 100%, has been observed in batch experiments with 5 mg/L activated carbon and four hours of contact time for PhACs with K_{ow} values higher than 10^3 (e.g. carbamazepine). Exceptions from this correlation include N-heterocyclic compounds (e.g. pentoxifylline and trimethoprim); because of specific interactions of the protonated compounds with activated carbon, removal efficiencies are higher for these PhACs than might be expected from their K_{ow} values. In contrast, those functional groups like carboxyl groups in PhACs much lowered the counterpart (e.g. clofibric acid, ibuprofen and diclofenac) adsorption efficiency on activated carbon, because in water they are to some extent dissociated and, therefore, negatively charged.

Snyder et al. (2007) evaluated activated carbon at bench-scale using rapid small scale column tests and at two full-scale utilities. In their study, both PAC and GAC were capable of removing nearly all compounds evaluated by greater than 90%. However, activated carbon efficacy is greatly reduced by the presence of natural organic matter which competes for binding sites and can block pores within the activated carbon structure. PAC offers the advantage of providing fresh carbon continuously since it is fed as a new product and is not recycled through the treatment process. Since PAC is added to the plant dynamically as a feed "chemical", it can be used seasonally when risk of trace organics may be greater (e.g., low-flow events). The ability of PAC to remove organic contaminants depends upon PAC dose and contact time, as well as the molecular structure and behavior of the contaminant of interest. GAC also was found to be highly-effective; however, water soluble contaminants can breakthrough the GAC much more rapidly than strongly bound hydrophobic contaminants. In two full-scale GAC applications evaluated, vastly different results were observed (Snyder et al. 2007). At a drinking water facility with on-site and regular regeneration, removal of trace organics occurring in source water was efficiently improved. In contrast, a facility with relatively high levels of TOC using GAC without regular replacement/regeneration provided very little removal. These data collectively show that activated carbon in both powdered and granular forms has great potential for the removal of trace organic contaminants; however, PAC dose and GAC regeneration/replacement will be critical for excellent removal (Snyder et al. 2007).

Generally, activated carbon is a powerful process to remove most PhACs. Whereas adsorption on activated carbon depends on the non-polar character of uncharged compounds which do not have functional groups or N-heterocyclic structural groups, a number of PhACs such as iodinated contrast media iopromide and the antibiotic sulfamethoxazole showed insufficient affinity to activated carbon as mentioned previously. In addition to the molecular structures of PhACs, activated carbon dosage or regeneration and the react time as well as the treated water conditions also influence to some extent the adsorption ability of activated carbon for PhACs. Additionally, due to the introduction of carbon, the disposal of spent carbon may lead to some practice problems such as complicated operation and extra post treatment costs.

5.2.4.4 Membrane Filtration

Implementation of membranes in water treatment is steadly increasing. It is well-known that pressure-driven membranes are capable of removing microbial constituents without increasing disinfection by-products, thereby allowing compliance with the rules promulgated in response to the 1986 Surface Water Treatment Rule Amendments (Adham et al. 2005). Based on the pore size of membrance, there are four main membrane types from larger size microfiltration following by ultrafiltration and nanofiltration to smaller pore size reverse osmosis. Recently, data have become available which demonstrate that membrane technology can be highly effective for the removal of PhACs (Kiso et al. 2001, Van der Bruggen et al. 2001, Drewes et al. 2002, Yoon et al. 2002, Schafer et al. 2003, Nghiem et al. 2005 and 2006).

1) Microfitration /Ultrafiltration

Among multitude types of membrane filtration, microfiltration/ultrafiltration (MF/UF) systems are strongly recommended for reducing micro-pollutants when there are space limitations and/or variable feed water quality. However, microfiltration (Sedlak et al. 2005, Snyer et al. 2007) and ultrafiltration (Snyer et al. 2007,Yoon et al. 2007) membranes have little value for the direct removal of the vast majority of PhACs by sieving.

2) Nanofiltration

Nanofiltration (NF) is an important technology for water reuse. Complete or near complete removal of a wide range of contaminants in secondary effluents can be achieved with NF membranes (Van der Bruggen et al. 2003). As a result, the rejection ability to PhACs have attracted many researchers (Kiso et al. 2001, 2002, Nghiem et al. 2004, 2005, Yoon et al. 2007).

Kiso et al. (200, 2002) tested strong hydrophobic PhACs including aromatic pesticides, non-phenylic pesticides, and alkyl phthalates by nanofiltration (NF) membranes and concluded that PhACs rejection was correlated significantly with molecular width and size in addition to compound hydrophobicity. They observed that rejection of non-dissociated PhACs were insensitive to feed water pH and removal increased linearly with the molecular weight and molecular width. The rejection efficiency, however, also correlates with the concentration of PhACs, which necessitates more effort at lower concentrations.

Nghiem et al. (2004, 2005) investigated the retention mechanisms of four classes PhACs antibiotics (sulfamethoxazole), antiepileptic (carbamazepine) and anti-inflammatory (ibuprofen) by NF membranes. Laboratory scale experiments were carried out with two well characterized NF membranes, with the goal of relating pharmaceutical retention behavior to membrane characteristics, physicochemical properties of the pharmaceutical molecules, and solution chemistry. Results show that retention of PhACs by a tight NF membrane is dominated by steric (size) exclusion, whereas both electrostatic repulsion and steric exclusion govern the retention of ionizable PhACs by a loose NF membrane. In the latter case, speciation of PhACs may lead to a dramatic change in retention as a function of pH, with much greater retention observed for ionized, negatively charged PhACs. For uncharged pharmaceutical species, intrinsic physicochemical properties of the pharmaceutical molecules can substantially affect their retention. In its neutral form, ibuprofen adsorbs considerably to the membrane because of its relatively high hydrophobicity. Similarly, polarity (represented by the dipole moment) can influence the separation of molecules that are cylindrical in shape because they can be directed to approach the membrane pores head-on due to attractive interaction between the molecule polar centers and fixed charged groups on the membrane surface. This phenomenon is probably inherent for high dipole moment organic compounds, and the governing retention mechanism remains steric in nature.

Yoon et al. (2007) evaluated initial interactions between 27 PhACs and NF membranes having different compounds in various waters using a dead-end stirred-cell filtration system. Eight of 27 PhACs showed 100% recovery based on the mass balance for NF membrane tests. Among the compounds showing less than 100% recovery, the compounds (androstenedione, oxybenzone, progesterone, testosterone, triclosan) having K_{ow} of greater than $10^{2.8}$ exhibited typically less than 40% recovery except gemfibrozil and ibuprofen. However, the other compounds ($K_{ow} < 10^{2.8}$) showed higher recovery (>75%). For the compounds, a general trend appears to exist with higher mass recovery at lower K_{ow}. These results indicate that observed retention for the relatively hydrophobic compounds based on their K_{ow} was significantly governed by adsorption.

Overall, nanafiltration are really powerful processes to remove PhACs. However, the removal efficiency of PhACs is relative to many factors such as the character of PhACs (i.e., K_{ow}, hydrophobicity and molecule size) and membranes (i.e., density). Further, for negatively charged PhACs rejection efficiencies were higher than for antiepileptics, because antiepileptics belonging to neutral pharmaceutical compounds tend to be adsorbed on the membrane materials (Zwiener, 2007). Consequently, these relative factors limited the application of nanofiltration membranes for removal of the majority PhACs.

3) Reverse Osmosis

In contrast to MF/UF and NF, reverse osmosis (RO) membranes were capable of removing almost all PhACs (e.g. NSAIDs, beta-blockers and lipid regulators) (Sedlak et al., 2005).

Kimura et al. (2003) explored the rejection of PhACs by RO membranes as a function of their physicochemical properties and initial feed water concentration. Experimental results indicated that negatively charged compounds could be rejected very effectively (i.e., >90%) regardless of other physicochemical properties of the tested compounds due to electrostatic exclusion. No time-dependency was observed for rejection of charged compounds. In contrast, rejection of non-charged compounds was generally lower (<90% except for one case) and influenced mainly by the molecular size of the compounds. Kimura et al. (2003) also observed clear time-dependency for rejection of non-charged compounds, attributable to compound adsorption on the membrane. It was demonstrated that feed water concentration influenced rejection efficiency of the membrane. Experiments conducted at a low ng/L concentration range resulted in lower rejection efficiency as compared to experiments conducted at a μg/L range, suggesting the need to conduct experiments at the relevant concentration of interest.

Snyder et al. (2007) investigated the removal of a broad range of representative PhACs during RO membranes processes in dynamic flow-through pilot skids or in operational full-scale treatment plants. In this work, findings indicated that RO membranes were capable of removing nearly all compounds that were investigated to levels less than method reporting limits. However, trace levels of some contaminants like antibiotics (Sedlak et al., 2005) were still detectable in RO permeates. The compounds that breached RO membranes were not consistent, and no clear relationship between molecular structure and membrane breaching could be established.

Just as discussed above, current waste-/drinking water treatment technologies neither conventional wastewater/water treatment processes nor advanced treatment techniques can achieve ideal or complete removal of PhACs in water systems. Due to some kinds of affect factors like compound structure and aquatic pH value as mentioned previously as well as the treatment process employed, activated sludge (type and age), environmental temperature, light and matrix effect (O'Brien and Dietrich 2004, Carballa et al. 2004), there are always certain PhACs present in the treated water after the corresponding treatment processes. For example, the pharmaceutical removal rate of NSAIDs is rather low (Petrovic et al. 2003), because their high water solubility and poor degradability. Consequently sewage effluents are one of the main sources of NSAIDs and their secondary products, and they can reach groundwater as well as drinking water (Ternes 2002, Reddersen et al. 2002).

Membrane filtration seems a better technique compared to other currently technologies. However, there are still some problems, for example, certain concentrated products would field during filtration processing, and there are not any technologies to further treat these condensates. Additionally, MF/UF is commonly used technique at present in water industries. As mentioned previously, MF/UF has little value to remove PhACs in wastewater or water. Therefore, the novel advanced treatment technologies for elimination of drug residues are desired to be developed. In next chapter, freeze concentration as a lately developed technology will be primarily introdued.

Chapter 6 FREEZE CONCENTRATION

When ice is crystallized from an aqueous solution, the ice crystal is essentially built up by pure water, leaving the solutes in the remaining liquid phase. This process of separation of the dissolved impurities is named the freeze concentration (Rodríguez et al. 2000)

6.1 Freezing of Water

At the standard conditions of temperature (0°C) and pressure (101.32 kPa), three phases of water stay in the stable equilibrium. Generally, temperature and pressure determine the phases of water: liquid, ice or vapor phase. During phase change processes, water must give off heat in order to pass it from the liquid to the solid state. The heat is the internal energy of water and is called the latent heat of fusion. Homogeneous, heterogeneous, or a combination of them is comprised of the mechanism of ice nucleation (Franks 1982). Homogeneous nucleation is the self-nucleation of water under deeply supercooled (water is cooled to a temperature below the freezing point without freezing). Hobbs (1974) reported that homogeneous nucleation can not occur for extremely pure water until it is supercooled to near -40°C. Conversely, heterogeneous nucleation must depend on a foreign particle, which is larger then 10 nm (Franks 1982), making an ice embryo grow clinging to it. The heat of fusion is extracted by conduction through the ice that has already formed on the foreign particle. The unfrozen water possesses high cooling efficiency than any region of convex curvature, and a smooth interface is restored prior to freezing of the convex region, thus the water-ice interface tends to remain flat (Chalmers 1959).

Irrespective of the nucleation process, an ice embryo firstly forms and grows step by step till it reaches a critical size, when the freezing process predominates; the ice crystal will continue to grow by adding water molecules to its crystal structure, as bricks are filled into the construction of walls (Chalmers 1959). In short, ice is a solid consisting of a crystallographic arrangement of water molecules (Glen 1974). Moreover, the purely electrostatic attraction between counter charged molecules strongly bonded these water molecules, which results in the great regular and symmetrical structure of an ice crystal (Glen 1974).

6.2 Impurity Separation during Freezing

Freeze concentration totally is a physical process, and ice formed during this process has highly organized structure as previously mentioned. Therefore, when freezing occurs, if solute concentration is not very low and the freezing process is enough slow (Chalmers 1959), so that the impurities and water molecules separate, each going their own way; each ice crystal continues to grow as long as water molecules are available. And all other impurities and solid particles, due to the strong attraction between water molecules (Glen 1974), cannot incorporate into the ice crystal lattice and are kept ahead of the boundaries of the ice crystal where they become compressed or dehydrated (Chalmers 1959), which resulting in pure water obtained (Halde 1979). Consequently, the concentration of soluble impurities in the liquid phase is higher than in the solid phase (ice). If the ice front advances at a rate too rapid for the solute diffusion process, a concentration gradient will be created.

In fact, impurity separation process during freezing is complicated, especially for diversiform solutes and solution in practice. In sections to follow, different impurity separations during freezing are discussed based on various solutes (inorganic or organic) and different conditions (simple or complex solution).

6.2.1 Rejection of Inorganic Impurities in a Simple Solution

Traditionally, most of the earth and the universe are inorganic (free of C-H containing species). When considering inorganic chemistry and life, it is useful to recall that many species in nature are not compounds but are ions. Sodium, chloride, and phosphate are essential for life as are some inorganic molecules such as carbonic acid, nitrogen, carbon dioxide, water and oxygen (Internet-10, 2007). In this section, the behavior of inorganic impurities in a simple solution (i.e. synthetic water, non-mixing solution of inorganic and organic) during freeze concentration will be firstly discussed.

Trace inorganic impurities cause specific changes in the physical and chemical properties of the phase boundary of growing ice during freezing process (Gross 1967). The populations of ion and valence defects play the role in most of these effects. For salts, during freezing of slightly impure water, the charge separation during the phase charge at the ice/water phase boundary resulting into some preferential ions are incorporated into the ice; for certain salts, the ice attains negative charge, for others, positive. When a very dilute solution of certain salts freezes rapidly, a strong potential difference is established between the solid and liquid phases (Internet-11, 2007). This mechanism was defined *Workman-Reynolds effect.* Gross (1967) using an apparatus achieving constant freezing rates systematically observed *Workman-Reynolds effect* of various simple inorganic solutions. According to the characteristics of the ion and charge transfer reactions, Gross (1967) divided all inorganic ionic solutes into three groups listed in Table 6-1.

Table 6-1 Different behavior of various inorganic solutes in a simple solution

Group	Inorganic	Behavior at Ice/Water interface
I	Alkali halides and ammonium fluoride	Negative ions are preferentially incorporated into the ice; the solution electrode shows a positive potential; freezing potentials are strongly depended on concentration and freezing rate
II	Ammonium salt (except the fluoride) and lead salts	Positive ions are preferentially incorporated into the ice, but their number is always small; freezing potentials are high; charge transfer is always small
III	Halogen acids and carbonate-free bases of alkali metals	No charge separation takes place; incorporation for the acids decreases from the hydrofluoric to the iodic

In the literature review of Gross (1967), the authors presented that Workman and Reynolds conceived the phase boundary structure as a double layer formed by ions selectively adsorbed at the ice surface, layers of oriented water dipoles, and a diffuse ionic layer in the liquid. The thickness of this double layer would be of the order of hundreds of Angstrom units. Therefore, the freezing potential phenomenon must introduce from at least three processes: differential adsorption at the phase boundary, differential incorporation into the ice structure, and differential diffusion into the liquid away from the ice/water interface, of which differential incorporation plays the dominant role. Gross (1967) summarized the experimental results done by many others with such Group III substances as HF, CsF and KF and found that samples grown from dilute fluoride solutions (10^{-3} M or less) at low freezing raters (5 microns a second or less) behave like samples from dilute acid solutions because of differential ion incorporation that replace part of the cations by protons in the ice structure. Summary about conductivity changes of doped with miscellaneous impurities of Group I and II as a function of concentration at -10°C indicated that the salts among all solutes including LiOH, NaOH, NH_4OH, LiF, NaF, LiCl, NaCl, KCl, RbCl, CsCl, and NH_4Cl did not obtain a clear concentration dependence of the conductivity. However, for NH_4OH and NaOH, it was found that there was significant dependence of conductivity. Additionally, through investigation of Pruppacher (1967) for the effect of miscellaneous inorganic compounds, the reduction of the freezing rate increased the concentrations of the impurity ions in the liquid phase.

6.2.2 Rejection of Organic Impurities in a Simple Solution

In contrast to inorganic, the mechanism of rejection of organic impurities from a simple solution seems different because the organic dissociation potential; molecular size or weight; or nature, location and number of substituent groups did not affect recovery of organic substances during freeze concentration (Baker 1966). Baker (1966, 1967, 1969, 1970) did a systematic investigation of the process of freeze concentration of organic materials in simple aqueous solution. The effect of various factors including physical, chemical and mechanical parameters has been determined. All experimental studies focused many organic chemicals including phenol and substituted phenols, volatile fatty acids and acetophenone. Single-stage and cascades of single-stage freezing steps were used to recover quantitatively trace amounts of these solutes. Results indicated that physical factors including initial sample volume and mixing rate did not show influence on the recovery efficiency. However, geometric mechanical factors limited the final volume to which concentration could be made without loss in efficiency. In experiments of Baker (1966), if the final volume is more than a limited final volume (30mL), essentially complete recovery of organic material can not be achieved with single-stage freezing. In addition, Baker (1967) further pointed out that mixing has no effect in distilled water and the absence of inorganic solutes, but when inorganic solutes were present, freeze concentration efficiency of organic solutes was increased with increased mixing.

Many chemical factors such as pH, inorganic content and ionic strength were also investigated for the effect of freeze concentration efficiency of organic solutes. Initial pH did not influence organic recovery from distilled water (Baker, 1967). Addition of salts to distilled water solutions or increasing of ionic strength resulted in impaired efficiency of freeze concentration of organic solutes, especially when concentrations of total dissolved salt content exceeded 10^{-4} mg/L (Baker, 1967). Moreover, Baker (1967) explained the importance of ionic strength affecting organic recovery, because ice morphology may be changed with formation of dendritic surfaces and incorporation of solute-rich concentrate in the forming ice phase.

According to investigations of Baker (1966, 1967, 1969, 1970), ice washing was proven important for even high complete organic solute recovery. For instance, washing of ice formed from deionized water solution gave essentially complete organic solute recovery over the entire volume concentration range. Although washing of ice formed form solutions containing dissolved solids did not achieve complete recovery, it indeed improved organic solute recovery (Baker, 1969).

Liu et al. (1997, 1999) proposed and tested progressive freeze concentration of liquid foods including glucose, blue dextran and tomato juice. Progressive freeze concentration is a method of freeze concentration based on a concept completely different from the conventional method of freeze concentration (suspension crystallization) being used for concentration of liquid food. The difference between the two methods is discussed in detail in section 6.3. According to their experimental results, a high speed stirring at the ice/solution interface and a low ice crystal growth rate resulted in the enhanced ice purity and the high concentration ratio between unfrozen liquid and mother solution. Moreover, through adding the ice-nucleating protein (0.01-0.1 wt%) into the mixture solution of glucose (5 wt%) and/or blue dextran (0.005 wt%), it was proven that the ice-nucleating protein can suppress the initial supercooling of solution and effectively improved the concentration efficiency of the organic solutes during freezing (Liu et al. 1997). Initial concentration of solutes showed that in the investigation of Liu et al. (1999) with aspect to progressive freeze concentration of tomato juice, influence the concentration efficiency. A diluted system seems to much suit for progressive freeze concentration into high concentrated products. This conclusion also was reported in a similar research by Miyawaki et al. (2005).

6.2.3 Rejection of Impurities in a Complex solution

Freeze concentration of organic solute in complex solution including mineralized waters and industrial wastewaters was also studied by Baker (1967, 1970). At the same experimental conditions, pH effect on recovery of organic solute in complex solution was different compared to simple solution with an example of *m*-cresol in a mineralized tap water. Single-stage freeze concentration efficiency of *m*-cresol increased as pH was decreased from 10 to 3 at initial and final volume ratios exceeding 5:1 but no effect on distilled water (Baker 1967, 1970). Additionally, there was no evidence possible of the cation-ligand complex effect on freeze concentration efficiency of organic solutes between pH 3 and 10. The individual components were not selectively separated from complex solution (Baker 1967).

Yang et al. (1992) used freeze concentration to obtain concentrated and stable dye solution, because there is neither evaporatiom nor sublimation the solvent out of the solution below dyes' melting point. Utilizing simple experimental equipment involving a beaker and a freezer, the concentration of 500% and 13,000% of dyes solution were achieved using one step and three repetitive freezing cycles' concentration, respectively. It is worthy to point out that the recovery of the dye after one freezing cycle can reach 75% for only the dye in the concentrated solution, and the remaining 25% dye can be recuperated by freezing the melted ice from the first freezing, then further increase the recovery. Moreover, through comparing dye adsorption and color yield on cotton fabric between the dye solutions before (original) and after freeze concentration, no momentous difference was found in both of them, thus proved that freeze concentration did not alter the visual absorbance characteristics of the dyes, namely, the dye solution after freeze concentration keeps the same dyeing competence as the original solution (Yang et al. 1992).

Additionally, Yang et al. (1992) found that the solubility of the dye influenced the recovery. Because the attractive forces between dyes and water prohibit the freezing process, concentrated abilities of dyes with higher water solubility are not as well as lower than the water solubility dyes as shown in Figure 6-1.

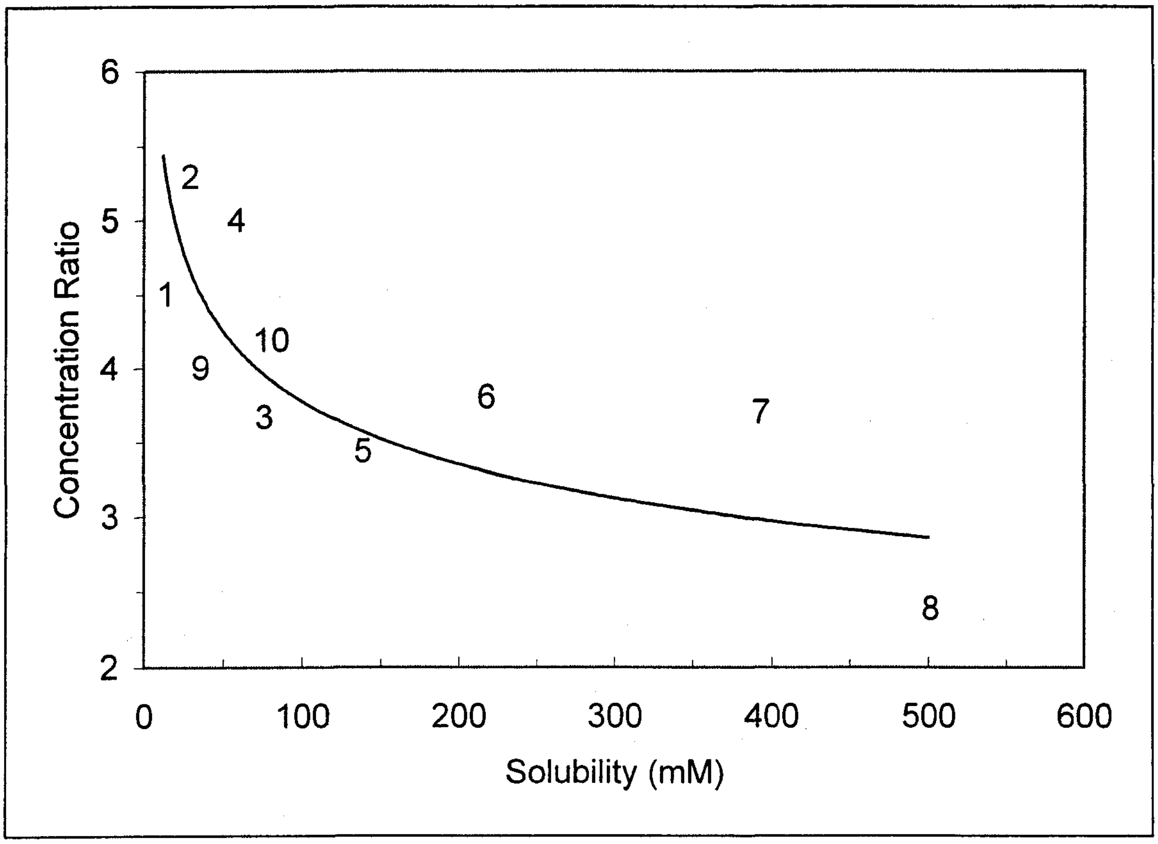


Figure 6-1 Relationship between solubility and efficiency of freeze concentration. The numbers in the graph represent ten different dyes (Yang et al., 1992).

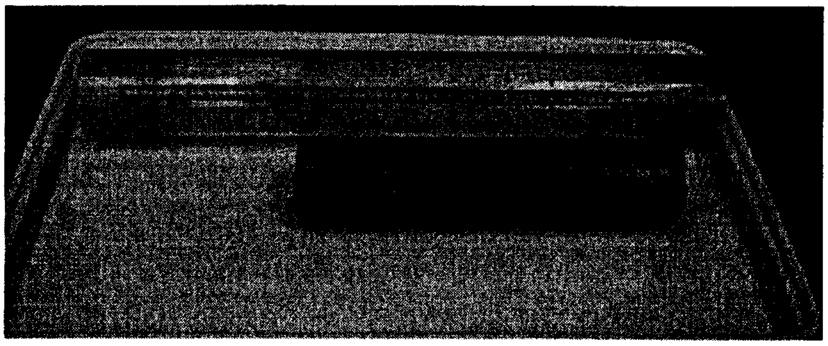
$$\text{Concentration ratio} = \frac{\text{concentration after freezing}}{\text{initial concentration}}$$

Initial concentration = 100 mg/L; initial nonfrozen solution volume = 60 mL. After freezing, total nonfrozen solution volume = 5 to 10 mL (Yang et al., 1992).

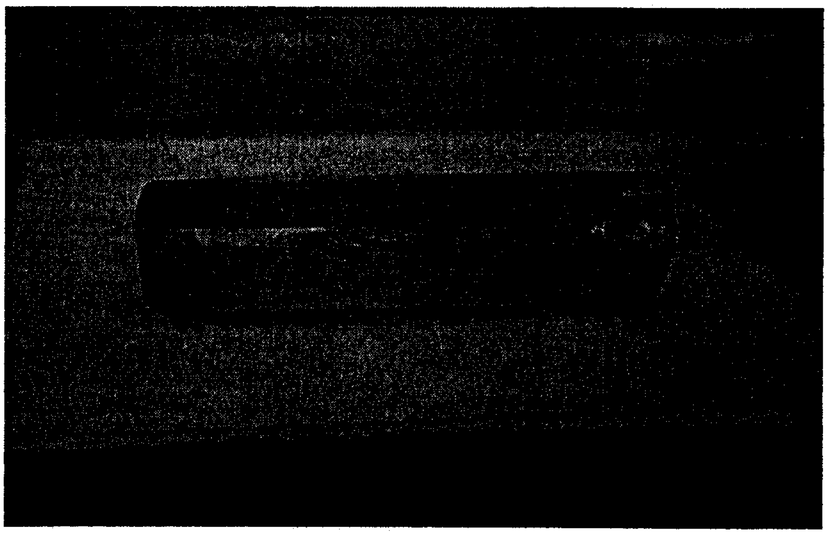
Table 6-2 Removal efficiency of freeze concentration for different wastewaters (Lorain et al., 2000)

Wastewater type		Initial concentration C_i (mg/l)	Purified phase C_S (mg/l)	Freezing ratio R_F (%)	Efficiency E (%)
Urban	After primary settling	270	49	64	88.3
	After secondary settling	65	6	72	93.3
Vinaigrette	Raw	2865	834	57	83
	After UF	2000	78	48	98
Mayonnaise	Raw	955	82.5	70	94
	After UF	460	14	58	98
Cutting oil	After UF	3000	85	60	98

Lorain et al. (2000) conducted freeze concentration experiments for complex wastewaters using a glycerin solution as cooling medium, and all experiments accompanied with rotation speed of 80rpm to achieve a good mixing of the liquid phase. Experimental results indicated different removal efficiency of soluble pollutants in different wastewaters (Table 6-2). Additionally, because all soluble impurities were removed in the unfrozen liquid phase, it implied non-selectivity of freeze concentration for all impurities.



a. Ice cylinder produced at high freezing rate (11.7 mm/hr)



b. Ice cylinder produced at low freezing rate (2.1 mm/hr)

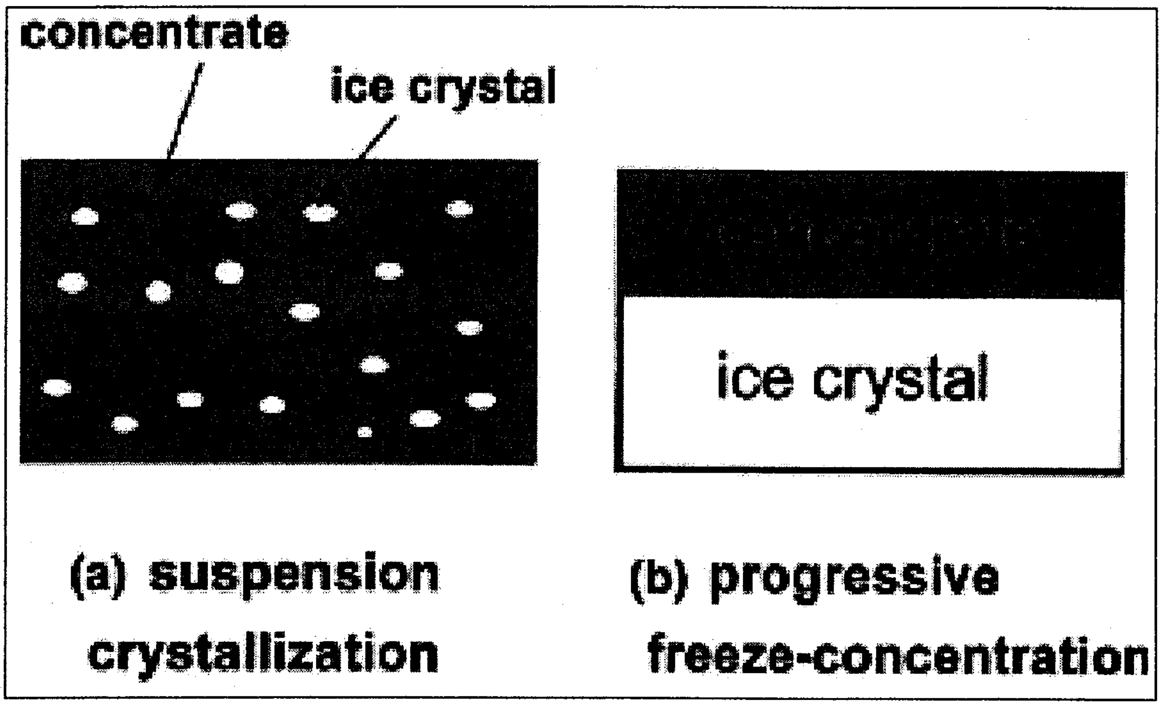
Figure 6-2 Visual comparison of ice cylinder color and clarity (Martel et al., 2002). (Freezing direction is left to right. Original samples are the same)

A typical Army industrial waste (also called "pinkwater") was conducted freeze concentration processes to remove such hazard substances as TNT (2,4,6-trinitrotoluene), RDX (hexahydro - 1,3,- trinitro - 1,3,5 - triazine), HMX (octahydro - 1,3,,7 - tetranitro - 1,3,,7 - tetrazocine), TNB (1,3, - trinitrobenzene) and the degradation products 2 - Am - DNT (a - amino - 4,6 - dinitrotoluene) and 4 - Am - DNT (4 - amino - 2,6 - dinitrotoluene) in pilot-scale by Martel et al. (2002). The effects of freezing rate (from 2.1 to 11.7 mm/hr) were primarily examined. Generally, either concentrated efficiency of impurities or quality of the melt ice depends on the freezing rate, and freezing rate as low as 1.9 mm/hr (got from the liner line of best fit) would be required to high-quality melt ice. Even through visual observations (Figure 6-2), it is proven that the slower freezing rates products a clearer ice than fast freezing rate. The clear ice suggested the high rejection efficiency of impurities from ice crystals during freezing processes. However, the author figured out the low freezing rate of 1.9 mm/hr is hard to achieve using conventional freezing equipment; therefore, a multi-stage freezing at a higher freezing rate was given as an alternative.

6.3Application of Freeze Concentration in Water and Wastewater Purification

Shapiro (1961) first applied freeze concentration technique to concentrate organic compounds as a potentially useful pre-analytical procedure. In past several decades, freeze concentration has been developed and applied in many fields including chemical concentration (Yang et al. 1992) and liquid food industry (Deshpande et al. 1984, Van Pelt. and Swinkels 1986, Anon 1989). Among these applications, liquid food industry include a variety of products such as fruits juices (Bayindirli et al. 1993), coffee and tea extracts, beer and wine, vinegar, and dairy products (Hartel and Espinel 1993).

Actual implementation of freeze concentration for sea water desalination was never implemented on a large scale (Spiegler 1966, Ziering 1973); however, the potential of freeze concentration was investigated thoroughly, especially for the wastewater treatment. When most of the wastewater is frozen, a large amount of clean water and concentrated wastewater should yield (Heist 1981). Because of the high concentrated rate of five to ten times of the residual wastewater, It is rather easy to further treat the condensate by certain concomitant methods such as burning upon drying, recovering useful components (Iammartino 1975) or using other wastewater treatment systems. Test applications so far have included remediation of hazardous waste lagoons, concentrations of deep mine reject water, recovery of process materials from ammunition plant wastes, and by-product recovery from organic chemical (Anon 1989).



(a) suspension crystallization

(b) progressive freeze-concentration

Figure 6-3 Two methods for freeze concentration (Miyawaki et al., 2005)

With the development and increasing applications of this technology, freeze concentration includes two practical methods: suspension crystallization and progressive freeze concentration as shown in Figure 6-3. Moreover, the differences between the two methods are tabulated in Table 6-3. Suspension crystallization for freeze concentration is a more commonly used method before the 1990's.

Table 6-3 The differences of suspension crystallization processes and progressive freeze concentration (Wynn, 1992)

Suspension crystallization	**Progressive freeze concentration**
	Crystal growth
Crystals freely suspended in melt.	Crystals grown as a layer on heat-transfer surface.
Crystals and melt at the same temperature.	Crystals are colder than melt.
Driving force is super saturation.	Driving force is temperature gradient.
Large inter facial area.	Moderate interfacial area.
Poor interfacial turbulence.	High interfacial turbulence.
Low mass-transfer rates.	Moderate mass-transfer rates.
	Phase separation
Large interfacial area.	Moderate interfacial area.
Separation by filters, centrifuges, or decanters.	Separation by gravity draining.
Driving force is the solid/liquid density difference.	Driving force is the liquid/gas density difference.
Solid/liquid separation is difficult.	Solid/liquid separation is easy.
Product can be washed to improve purity.	Product can be washed or sweated to improve purity.
Product is in solid form.	Product is in liquid form.

During suspension crystallization processing as shown in Figure 6-3(a), the crystals are freely suspended in a melt. Such processes depend on density difference to promote countercurrent transport of crystals and melt. Ice crystals can grow large by Ostwald Ripening, an observed phenomenon in solid solutions which describes the evolution of an inhomogenous structure over time (Internet-12, 2007). In the Ostwald ripening mechanism (Huige and Thijssen, 1972), larger crystals grow from those of smaller size which have a higher solubility than the larger ones. In the process, many small crystals were formed initially but slowly disappeared, except for a few that grew larger, at the expense of small crystals. The smaller crystals act as fuel for the growth of bigger crystals. Based on Ostwald Ripening, the freeze concentration began to be applied to concentrate coffee extract, fruits juice (Braddock & Marcy 1985, Deshpande et al. 1982), dairy products (Hartel & Espinel 1993), and so on, and has been lately adopted in hazardous wastewater treatment (Holt 1999, Lemmer et al. 2001). However, the complicated freeze concentration system is required due to the size limitation of ice crystals, which is composed of surface-scraper heat-exchanger for generation of seed ice, recrystallization vessel for ice crystal growth, and washing tower for separation of ice crystals. Certainly, all of the equipment made freeze concentration process more expensive than the other conventional methods of concentration such as reverse osmosis and evaporation (Rodríguez et al. 2000). Such factors as high expense and complicated operational systems have limited the applications of freeze concentration.

In order to improve the practical application of freeze concentration, the progressive freeze concentration as shown in Figure 6-3(b) is developed in which a crystal layer is grown on a heat-transfer surface (Bae et al. 1994 and Liu et al. 1997). It is one method of melt crystallization (Atwood 1973, Gilbert 1991), which has been employed mainly to purify organic materials. Historically, the progressive freeze concentration was proposed as a small-scale concentration technique for a pre-analytical purposes, which is applicable to both aqueous and organic solutions (Matthews and

Coggeshall 1959, Shapiro 1961, Baker 1963). Impurities in organic solvent were effectively concentrated by this method (Matthews and Coggeshall 1959). In this method, phase separation to facilitate countercurrent contacting is accomplished by gravity draining and functions irrespective of density differences. In other words, since a single large ice crystal, instead of many small ice crystals in the suspension crystallization method, is formed and grow on the cooling surface in a crystallization vessel. It easily achieves the separation between the ice crystal and the mother solution, which leads to the simple freeze concentration system which is distinctly lower in cost than the suspension crystallization.

Halde (1979) had frozen different kinds of sludges and found that separation is difficult and depends on many experimental factors such as the mixing rate, the size, and the ability of the impurities to settle. The limiting factor is particle diffusion, which is often slower than the speed of the advancing solid interface. In this case impurity entrapment tends to occur. Halde (1979) also suggested that non-soluble compound treatment is difficult. On the other hand, the freeze concentration seems to be more efficient for soluble compound separation.

Radial freezing with stirring was introduced to treat wastewater by Gay et al. (2003). The sky-scraping purification rates of up to 99.97% were attained due to the application of stirring. Moreover, the qualities of treated water can reach drinking water standards.

Gao et al. (2003, 2004) used two spray freezing operations: partial and complete freezing, equipment similar to an irrigation system (spray guns or sprinklers), to treat industrial wastewaters such as pulp mill effluent and oil sands tailings pond water.

Although the reduction efficiencies of organic and inorganic contaminants were different and usually greater than 60% impurity removal in the spray ice was achieved when the freezing degree is 70% of the total volume of the sprayed water. And, during spray ice production, partially frozen wastewater obtained higher impurity removal efficiency compared with the completely frozen wastewater. Therefore, partially freezing is necessary to get higher quality recovered water from spray wastewater. Moreover, due to the simple operation as well as the economical and technical feasibility, freeze concentration became an alternative wastewater treatment technology in cold regions.

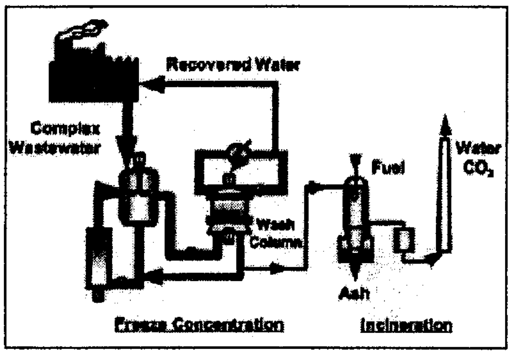


Figure 6-4 Freeze concentration/incineration flow diagram (Niro Ltd., 1999)

Except to the aforementioned laboratory-scale investigations, two full-scale wastewater treatment plants have adopted freeze concentration processes. The first one founded in 1997 is an Asian chemical plant with almost a 2 MW load. A combination of freeze concentration followed by incineration is used by the plant. Figure 6-4 illustrates the freeze concentration/incineration processes. The freezing process takes place in surface-scraped heat exchangers with some ice seed in the recrystallizer vessels. Because of the ice crystal shape and counter current washing with by-product water, 100% pure spherical crystals grow in the recrystallizers which are separated from the concentrated liquid in wash columns. Finally, ultra pure water can be attained from the melted ice and the non-diluted concentrated to be incinerated (Niro Ltd. 1999).

In 1999, another unit with 3.5 MW load was started up in Europe. As for treatment processes, the two plants have no significant differences, but the European complex uses more recrystallization units (five in series) and wash columns (seven in parallel) than that of the Asian plant (Niro Ltd. 1999).

According to practical operation of the two plants, for caustic wash water, Asian and European plant can achieve 18 and 34 million ton per hour water recovery, respectively, with <50 part per million total dissolved solids in recovered water (Niro Ltd. 1999). Additionally, through comparing the total operational costs for the various systems at the Asian plant (Table 6-4), freeze concentration/incineration was proven to be viable economic approach compared to incineration especially in the presence of volatile organic compounds.

Table 6-4 Estimates of the total operating costs for the various systems at the Asian plant (Niro Ltd., 1999)

	Approximate cost per m^3 feed
Thermal wet air oxidation & bio-treatment (both types)	\$30-45
Catalytic wet air oxidation & bio-treatment	\$35-50
Direct incineration	\$90-125
Evaporation & incineration (total stream)	\$70-95
Freeze concentration & incineration	\$35-50

As shown in Table 6-4, the freeze concentration and incineration costs are significantly lower than direct incineration and evaporation/incineration, and similar to any of the feasible oxidation steps and bio-treatment. However, the freeze concentration and incineration demonstrate three noteworthy advantages over the bio-treatment systems the drug concentration in water sample. So far, there are no standard methods to detect drug residues in water and it is still in the research stage. Some popular analytical methods for drug residues are summarized in Chapter 7.

Chapter 7 ANALYTICAL METHODS

It is a real challenge to analyze PhACs in the environment, especially the low-concentration drug residues in aquatic environment. Although recently several methods have been developed for the determination of pharmaceuticals and their metabolites in the lower nanogram per liter range, the amounts of accepted method for analysis of these residual drugs are still limited in their available (Boyd et al. 2003). Certain approaches such as gas chromatography mass spectrometry (GC-MS), high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) were frequently adopted by many researchers: Koutsouba et al. (2003), Rodríguez et al. (2004), Lin et al. (2005), Sedlak et al. (2005), Bendetta et al. (1995) Blanchflowe et al. (1997), Zwiener and Frimmel (2004), Marchese et al. (2003), Gómez et al. (2006), Lee et al. (2007), Hamscher et al. (2002), Hilton and Thomas (2003), and Cahill et al. (2004).

All approaches are based on two primary chromatography (GC and LC) accompanying with different detectors such as MS and tandem MS. The choice of GC and LC is usually relied on the physicochemical characteristics of the target PhACs (Boyd et al. 2003). For example, most GC methods must incorporate a derivatization step to overcome the thermolabile property of many polar pharmaceuticals and their metabolites, but no this problem if using LC due to it does not need a derivatization step (Cha et al. 2005).

Detection limits achieved by these analytical methods such as GC-MS, HPLC and LC-MS vary depending on sample origin and the objectives of a specific application. They cover a wide range from ng/L to μg/L (Sacher et al. 2001, Metcalfe et al. 2003, Lee et al. 2003, 2005, Lindqvist et al. 2005, Chia and Huang 2005). The complexity of a sample matrix may complicate the sample preparation procedure and cause high levels of interference in the final extract, which can make the proper identification and quantification of target parameters difficult. A traditional way to reduce the effects of both background and potential interferences on the accuracy of the quantification process of target analytes is to use in tandem or some times even triple mass spectrometry techniques. The advantages of these methods, in comparison to single MS, are increased sensitivity and greater selectivity. This approach also allows method detection limits (MDLs) to be achieved in the range from 0.1 to 1 g/L even for such samples as municipal wastewater or solid matrices (Verenitch et al. 2006). Therefore, numerous experimental batches extended from three foundation methods (GC-MS, HPLC and LC-MS) are prevailing in pharmaceuticals analysis works, i.e., GC-MS/MS, HPLC-MS, HPLC-MS/MS and LC-MS/MS.

In the present study, the most advanced aspects of current GC-MS, HPLC and LC-MS as well as their expansion methodologies for PhACs analysis are summarized in the following sections.

7.1 Extraction Processes

Because most PhACs are usually present at very low concentrations (<μg/L as described in Chapter 3) in samples for complex aquatic environmental, nearly all techniques of analysis of PhACs in environmental matrices are applied after clean-up and enrichment steps except for PhACs to be persistent in the environment for them to be detected (Robinson et al. 2007). Owing to the development of new generations of sorbent materials, solid-phase extraction (SPE) or solid-phase microextraction (SPME) other than liquid extraction (LE) have became popular extraction processes in water studies (Robinson et al. 2007).

The use of different sorbents in the extraction cartridge ensures more complete retention and better reproducibility of PhACs than liquid/liquid extraction. And the other the problems associated with liquid/liquid extraction, such as use of expensive, breakable specialty glassware, and disposal of large quantities of organic solvents can be prevented with SPE. In summary, SPE is more efficient than liquid/liquid extraction, yields quantitative extractions that are easy to perform, is rapid, and can be automated. Solvent use and lab time are reduced using SPE to pre-treat target PhACs solutions (Internet-13 2007).

On the other hand, such advantages as less required sample volume, solvent-free, and easily automated, which allows high enrichment factors in the concentration of organic compounds in aqueous matrices (Huppert et al. 1998, Rodríguez et al. 2004), demonstrate the distinctly predominance of SPME over SPE during gas chromatography mass spectrometry (GC-MS) analysis of PhACs .

Certainly, SPE can be coupled on-line with the LC-MS systems, allowing the transfer of the whole extract into the chromatographic system, thus either reducing the sample volume that has to be extracted or increasing the method sensitivity. Recently Quintana et al. (2006) presented an automated, on-line renewable, solid-phase extraction-liquid chromatography method and achieved recoveries of >88% of certain NSAIDs including ketoprofen. Such automated online SPE-LC-MS systems are highly attractive due to their high sensitivity and high reproducibility with almost complete elimination of the risk of contamination.

7.2 Gas Chromatography-Mass Spectrometry (GC-MS)

7.2.1 Description

Gas chromatography-mass spectroscopy (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Gas chromatography uses the difference in the chemical properties between different molecules in a mixture to separate the components of a mixture, and mass spectroscopy characterizes each of the components individually by breaking each molecule into ionized fragments and detecting these specific fragments for different molecules using their mass to charge ratio (Figure 7-1).

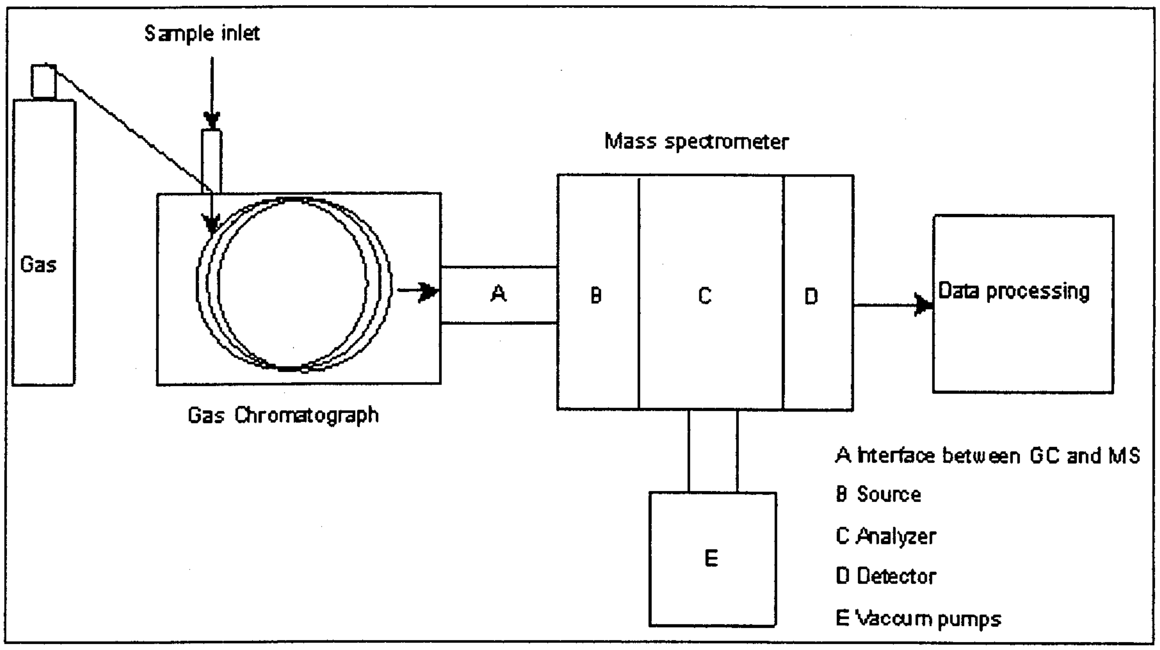


Figure 7-1 Scheme of typical GC-MS instruments. (Internet-14, 2007)

The primary goal of chemical analysis of GC-MS is to identify a substance by comparing the relative concentrations among the atomic masses in the generated spectrum to a spectrum library to see if its characteristics are present for some sample in the library. Another analysis application is to measure the peaks in relation to one another, with the tallest peak receiving 100% of the value, and the others receiving proportionate values, with all values above 3% being accounted for. The parent peak normally indicates the total mass of the unknown compound. This value can then be used to fit a chemical formula containing the various elements assumed to be present in the compound. The isotope pattern in the spectrum, which is unique for elements having many isotopes, can also be used to identify the presence of various elements. Once a chemical formula has been matched to the spectrum, the molecular structure and bonding can be identified, and needs to be consistent with a substance of the characteristics recorded by GC/MS. The fitting is normally done automatically by programmers which come with the machine, given a list of the elements which could be present in the sample.

A "full spectrum" analysis considers all the "peaks" within a spectrum. However, selective ion monitoring (SIM) which looks only at a few characteristic peaks associated with a candidate substance, can also be done. This is done on the assumption that at a given retention time, a set of ions is characteristic of a certain compound. This is a fast and efficient analysis, especially if one has some prior information about a sample or are looking for a specific compound. When the amount of information collected about the ions in a given gas chromatographic peak is reduced, the sensitivity of the analysis goes up. So, SIM analysis allows a smaller quantity of a compound to be detected and measured, but the degree of certainty about the identity of that compound is reduced.

7.2.2 Application in Detecting PhACs

Among the existing literatures cited in this chapter, the main applications of GC-MS are determination of acidic drugs on aquatic environment. Medicinal-class drugs including NSAIDs and lipid regulators are also called acidic drugs due to their specific chemical characteristics such as polarity, hydrophilicity and pK_a around 4.0 (Verenitch et al. 2006, Farré et al. 2007) based on their chemical structures containing carboxylic moieties and one or two phenolic hydroxyl groups (Ternes 2001). These acidic drugs can be determined at low-ng/L concentrations means of GC-MS or GC-MS/MS after pre-treatments like extraction (Chia and Huang 200, Verenitch et al. 2006). However, a derivatization step must be performed before gas chromatography analysis of acidic drugs in order to increase the volatility, decrease the polarity of these analytes, and in some cases to improve their detection e.g., pentafluorobenzyl bromide (PFBBr) permits analytes to be determined by electron-capture detection or electron-capture MS (Koutsouba et al. 2003).

In addition, according to different derivatization methodologies and matrices of acidic pharmaceuticals, the limits of quantification (LOQs) of GC-MS or GC-MS/MS varied from ~1 ng/L to high ng/L (Ternes et al. 1998, Buser et al. 1998, 1999, Koutsouba et al. 2003, Rodríguez et al. 2004, Lin et al. 2005). For instance, as listed in Table 7-1, LOQs down to 10 ng/L were achieved in sewage treatment plant effluents as well as in surface water using GC-MS, and ~1 ng/L using GC-MS/MS (Ternes et al. 1998). Moreover, some examples of derivatization pathways used in acidic drugs analysis are shown in Figure 7-2.

Table 7-1 LOQ for different matrices (Ternes et al. 1998)

Analytes	LOQ in STP[a] GC-MS (ng/L)	LOQ in SW[b] GC-MS (ng/L)	LOQ in DW[c] GC-MS/ MS (ng/L)
Acetylsalicylic acid (A.S.A)	50	10	10
Clofibric acid	50	5	1
Ibuprofen	50	5	1
Gemfibrozil	50	5	5
Fenoprofen	50	5	5
Ketoprofen	50	5	5
Diclofenac	50	5	1
Fenofibric acid	50	5	5
Bezafibrate	250	5	25
Indometacine	50	5	5
Naproxen	50	10	N.A[d]
Tolfenamic acid	50	20	N.A[d]
Meclofenamic acid	50	20	N.A[d]

[a] Sewage treatment plant (STP) effluent.

[b] Surface water.

[c] Drinking water.

[d] Not analyzed.

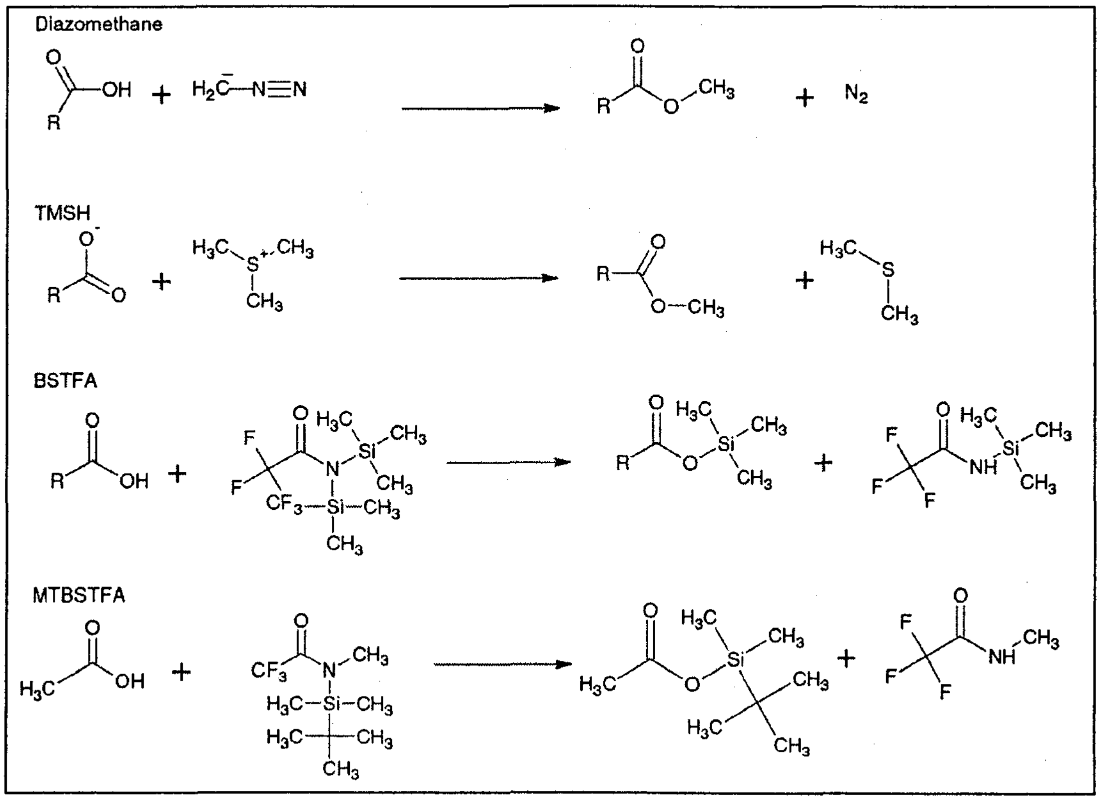


Figure 7-2 Examples of derivatization pathways for GC-MS analysi of NSAIDs.

TMSH: trimethylsulfonium hydroxide;
BSTFA: *bis* (trimethylsilyl)trifluoroacetamide;
MSTFA:N-methyl-N-(trimethylsilyl)trifluoroacetamide (Farré et al. 2007).

Among the available alkylating reagents, diazomethane poses high reactivity toward most acidic groups, thus became the most common derivatization reagent for analysis of acidic pharmaceuticals (Buser et al. 1998, Öllers et al. 2001, Tixier et al. 2003). SPE-GC/MS achieved detection limits of 0.1~10 ng/L for various NSAIDs and lipid regulators analysis in different matrices (i.e., drinking water, surface water and wastewater) (Buser et al. 1999, Öllers et al. 2001). However, the toxicity, carcinogenicity and explosiveness of diazomethane made alternative derivatization procedures must be developed like alkyl halides. Pentafluorobenzyl bromide (PFBBr) is a good example. Many authors, for instance Reddersen et al. (2002) and Koutsouba et al. (2003), derivatized polar pharmaceuticals using PFBBr coupling with other reagents such as triethylamine and N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) as catalyst and followed by GC-MS with limits of detection of 1 to 10 ng/L.

On-column or in-port alkylation can be accomplished by tetramethylammonium hydroxide (TMAH) or trimethylsulfonium hydroxide (TMSH) and other alkyl analogs, which undergo pyrolytic decomposition at the GC injection temperature, donating alkyl groups (methyl groups in these cases) and yielding trimethylamine or dimethylsulfide, respectively, as decomposition by-products (Zapf and Stan 1999). While TMAH may damage the analytical column, TMSH is less reactive. On-column derivatizations of NSAIDs were reported by Jux et al. (2002) using both trimethylsulfonium hydroxide (TMSH) and trimethylanilinium hydroxide (TMAH) in methanol; however, with TMSH small molecular by-products are not detected as they remain in the solvent peak of the chromatogram. TMAH produces aniline as a by-product which can interfere with the analysis. Methyl chloromethanoate is another derivatization agent worthy of mentioning (Weigel et al. 2002, Bendz et al. 2005).

On-line derivatization in the injection port and using a large volume sample injection with tetrabutylammonium (TBA) salts has been reported to be a rapid and simple alternative to conventional derivatization methods for pharmaceutical residues (Lin et al. 2005). Nevertheless, the most widely used alternatives to diazomethane are silylating agents. There are two main classes of silylating agents: those producing trimethylsilyl (TMS) derivatives and those producing tert-butyldimethylsilyl (TBDMS) derivatives. TMS derivatives can be produced by a wide variety of reagents, like N,O-bis (trimethylsilyl)trifluoroacetamide (BSTFA) and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). TBDMSderivatives are prepared by reaction with N-methyl-N- (tert-butyldimethysilyl)trifluoroacetamide (MTBSTFA). At present, the majority of derivatizations applied in NSAID analysis are based on alkylsilyl reagents such as MTBSTFA and MSTFA (Kosjek et al. 2005). The resulting TBDMS derivatives have greater thermal and hydrolytic stability (e.g., they can tolerate about 1% water (Rodríguez et al. 2005)) compared to TMS derivatives; furthermore, EI-MS spectra of TBDMS derivatives are normally characterized by a base peak corresponding to the loss of the tert-butyl group from the molecular ion, which when added to their higher molecular mass improves chromatographic separation and MS detection (Woo and Kim 1999). However, the drawback of TBDMS reagents compared to TMS reagents is their lower reactivity. Rodríguez et al. (2003) described the determination of five NSAIDs (ibuprofen, naproxen, ketoprofen, tolfenamic and diclofenac) in sewage using MTBSTFA as derivatization agent followed by GC-MS with quantification limits for sewage water ranging from 20 to 50 ng/L. Recently, Lee et al. (2005) reported the determination of phenols and acidic pharmaceuticals including salicylic acid, ibuprofen, naproxen and diclofenac using derivatization by MTBSTFA with 1% tert-butyldimethylsilyl (TBDMS).

GC-MS also can used to detect β-blockers and β-sympathomimetics, but similar to acidic drugs, an efficient derivatization is essential, because both β-blockers and β-sympathomimetics contain a secondary aminoethanol structure as well as several hydroxyl groups, and these functional groups lead to their relatively high polarity. Additionally, due to their structures of multiple-function groups, the derivatization processes commonly consist of two steps in order to get the complete and stable derivatives of both aminoethanol and hydroxyl groups (Ternes et al. 1998).

Ternes et al. (1998) adopted a method including SPE and derivatization coupling with GC-MS detection and achieved detection down to 5 ng/L of 11 betablockers and β-sympathomimetics, at the same time; they got exceeding 70% recovery. The use of an acidic pH would be appropriate for the protonation of acidic phenolic hydroxyl groups, but is disadvantageous for the desired deprotonation of the secondary amino moieties. Because of enhanced sorption effects of the betablockers, presumably caused by the secondary amino group, losses by sorption on active glass surfaces as soon as they are dissolved in aqueous matrices should be avoided. For instance, the usage of glass vials for the final phosphate buffer extraction volume leads to reduced recoveries of the betablockers, while in PTFE vials or in organic solvents no sorption losses were observed (Ternes et al. 1998).

Neutral pharmaceuticals such as the most used antiepileptic carbamazepine are referred to some different compounds from distinct medicinal classes which contain no acidic functional groups and, hence, can be enriched at neutral pH in the reversed phase and sorbents can usually be analyzed by GC-MS without derivatization. Ternes et al. (1998) also observed many neutral drugs and got in excess of 70% recovery as well as LOQs of 20-50 n/L and 100-250 ng/l in drinking water and STP effluents, respectively. However, the determination of certain neutral drugs such as phenazon, carbamazepine, cyclophosphamide, ifosfamide and pentoxifylline was frequently subject to interference by organic co-extractants in real samples of rivers and sewage effluents.

7.3 High Performance Liquid Chromatography (HPLC)

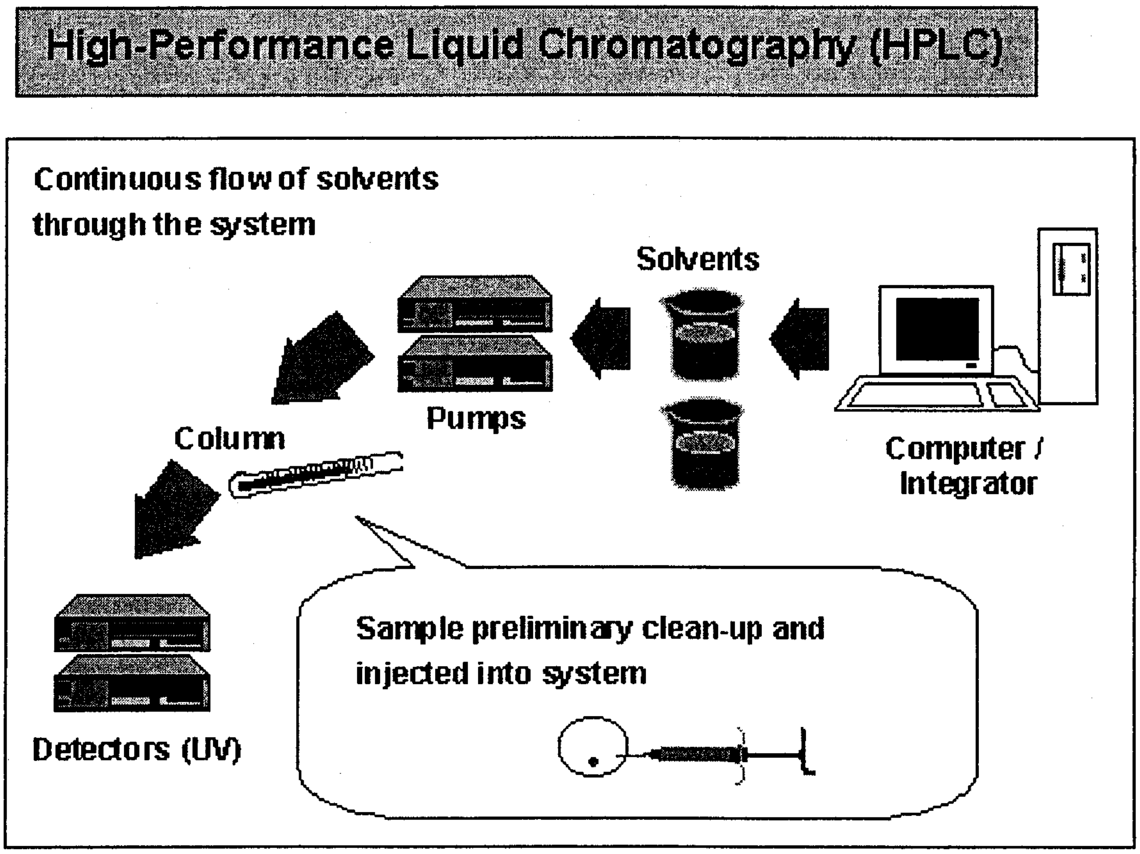


Figure 7-3 Scheme of typical HPLC instruments (Internet-16, 2007)

In contrast to GC, HPLC is a form of liquid chromatography to separate compounds that are dissolved in solution. The work flow line of HPLC is similar with GC-MS and includes separating components, analyzing molecules and finally printing to a spectrum. Typical HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector as shown in Figure 7-3.

Compounds are separated by injecting the analyte onto the column under the high pressure pump. The different components in the mixture pass through the column and are retarded by specific chemical or physical interactions with the stationary phase as they traverse the length of the column. The amount of retardation depends on the nature of the analyte, stationary phase and mobile phase composition. The retention time at which a specific analyte elutes (comes out of the end of the column) is considered a reasonably unique identifying characteristic of a given analyte. The detector serves as the "eyes" of the HPLC system to attain the analysis results. Finally, these analytial results are transmitted to the computer and are recorded in the form of chromatogram. (Internet-15, 2007).

As discussed previously, GC-MS is one of the most common techniques (Weigel et al. 2004, Quintana et al. 2004). However, some of drug compounds cannot be determined by GC without derivatization, because they are thermally unstable, polar or ionic (Görög 2007). In our experimental processes, derivatization step makes the sample preparation laborious and time-consuming, increases the possibility of contamination and errors, and may lead to degradation of labile compounds. Therefore, the introduction and rapid spread of HPLC undoubtedly demonstrate importance for the determination of involatile PhACs such as antibiotics (Meyer et al. 2000) and distinct advantages for those polar compounds over GC-MS (Weigel et al. 2004, Nikolai et al. 2006). In HPLC technique, the common detectors companying with LC techniques include MS and ultraviolet (UV)-detector. Application of HPLC system with UV detection and appropriate preconcentration step allows developing simple and sensitive procedure for the analysis of pharmaceuticals (Görög 2007). However, the detect limits of HPLC-UV for PhACs are at the μg/L-level (Anna et al. 2007).

During the past decade, LC-MS via atmospheric pressure ionisation (API) interfaces, especially electrospray ionization (ESI), have become the analytical techniques of choice for the determination of polar environmental pollutants including PhACs (Reemtsma 2003, Zwiener and Frimmel 2004). However, one drawback of the electrospray MS technique during measurements of environmental samples is the signal suppression effect, which has a negative influence on the reproducibility and accuracy of the analyses (Ternes 2001). Since matrix components can be rather variable between samples—in nature and in amount—their effects are difficult to predict. The mechanism of interference during the electrospray ionization process is still not clear (King et al. 2000).

The use of internal standards may compensate matrix effects. However, these effects can strongly depend upon the chromatographic retention time and more than one internal standard may be needed (King et al. 2000). Therefore, the pretreatments like SPE are usually essential prior to the LC-MS analysis in order to reduce the matrix effects in complex samples.

Chromatographic separation is another important process in reducing ion suppression. Although separation is not necessary using MS/MS detection, a complete chromatographic separation improves detectability and reproducibility. LC-MS/MS detection including time-of-flight (TOF) and quadrupole time-of-flight (Q-TOF) have showed many advantages in practice. For instance, for more polar compounds, the ubiquitous betablockers atenolol or sotalol could only be termined by LC-ES/MS/MS, and for the detection of neutral drugs, the detect limits of LC-ES/MS/MS were improved to 10 ng/L from hundreds of ng/L of GC-MS, and independent from the water matrics (Ternes et al. 1998).

The main advantages of Q-TOF-MS are the availability of full-scan mass spectra throughout each chromatogram (Marchese et al. 2003). However, one of the most important drawbacks of LC-TOF/MS for quantitative measurements of environmental contaminants is the effective linear dynamic range (typically two to three orders of magnitude) which is significantly lower than the quadrupole instruments dynamic range (typically >4 orders of magnitude). Marchese et al. (2003) compared QqQ instrument and a hybrid Q-TOF-MS for the determination of analgesics in water. The quantity limits obtained for the TOF experiment were approximately three to five times greater than those obtained using a QqQ-MS operating in MRM mode. Limits of quantification (LOQs) estimated were less than 3 ng/L for each analyte. The between-day precision and linear dynamic range results of the method with the QTOF analyzer were similar to those obtained using QqQ (MRM) analysis. Petrovic et al. (2003) developed a new multiresidue method using ultra-performance liquid chromatography (UPLC) Q-TOF-MS for screening and confirmation of 29 pharmaceutical including NSAIDS. UPLC uses columns packed with 1.7-µm particles and enables elution of sample components in much narrower, more concentrated bands, thus permitting very good separation of all compounds in 10 min. The detection limits (MDLs) reported by Petrovic et al. (2003) ranged from 10 to 500 ng/L for wastewater samples.

Table 7-2 summarizes the main applications of liquid chromatography coupled to the different mass spectrometric techniques. In general, although LC-based methods can achieve lower detection limits than GC-based methods, LC-based methods belong to lately developing techniques thus they still cannot completely instead of GC-MS or GC-MS/MS in drugs analysis.

Table 7-2 LC-based methods used for the quantitative determination of NSAIDs in water samples (Farré et al. 2007)

Compounds	Matrix	Pre-treatment	Extraction procedure	Elution solvents	LC-separation		MS system	LOD (ng/L)
					Column	Mobile phase		
NSAIDs	Surface, drinking and wastewater	No pre-treatment for surface and wastewater; $Na_2S_2O_3$ addition for drinking water	SPE	MeOH/ TBACl	C18	MeOH/aq. ammonium formiate	QqQ ESI	Nr
NSAIDs	Surface and wastewater	Acidified pH=2–2.5	SPE	MeOH	Phenyl-hexyl	MeOH/aq. Hac (ion par TRBA)	QqQ ESI	LOQ 0.15–2.5 surfacewater
NSAIDs	Surface, drinking and wastewater	Acidified pH=2–2.5	SPE	MeOH	C18 end-capped	MeOH/water	QqQ ESI	LOQ 7.5–75
Multiresidue method including NSAIDs	Surface, drinking and ground water	Acidified pH=3	SPE	MeOH/ ammonia	C18	Aq. NH_4Ac/ MeOH	QqQ-ESI Q-TOF ESI	LOQ 5–25
Multiresidue method including NSAIDs	River, drinking and wastewater	None	SPE	MeOH	C18	Aq. NH_4Ac/ MeOH	QqQ	5–50
Multiresidue method including NSAIDs	Wastewater	Ultrafiltration; acidified pH=2–2.5	SPE	MeOH	C18	MeOH/aq. HAc (ion par TRBA)	QqQ-ESI	LOQ 0.15–2.5 surface water
Multiresidue method including NSAIDs	Wastewater	Filtration	SPE	MeOH	Waters ACQUITY C18 1.7 μm	MeOH/water	Q-TOF ESI	10–500 ng/L
Multiresidue method including NSAIDs	Hospital effluent wastewaters	None	SPE	MeOH	C18 End-capped	MeOH/water	QqQ ESI	7–47
Multiresidue method including NSAIDs	Surface and wastewater	None	SPE	MeOH	C18 End-capped	MeOH/water	QqQ ESI	1–10 in surface water; 3–160 in wastewater

Chapter 8 MATERIALS AND METHODS

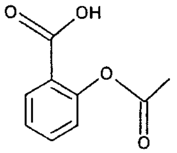
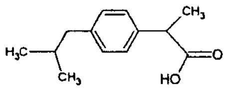
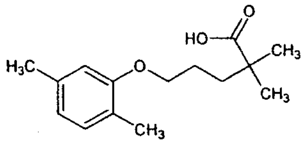
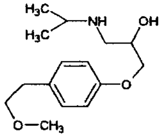
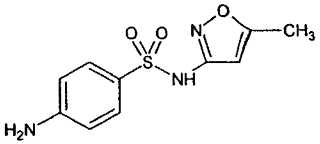
8.1 Materials

8.1.1 Drugs Selected for Experimentation

Acidic drugs including NSAIDs and lipid regulators are mainly medicinal classes, and are frequently found at high concentrations in water systems as discussed in Chapter 2 and 3. In this study, we selected three acidic drugs including acetylsalicylic acid, ibuprofen and gemfibrozil, as well as one beta-blocker metoprolol and one antibiotic sulfamethoxazole as our primary objects. All drugs studied in this thesis are listed in Table 8-1 and were purchased from Sigma-Aldrich Chemicals Canada.

Each individual stock solution of each drug at 100μg/mL was prepared in acetonitrile for the preparation of calibration standards and freezing original samples. But for a mixture of multiple drugs (each at 50μg/mL), acetone was used as a solvent. Mthyl *tert*-butylether (MTBE) was used to extract the drug in the solution. Sodium sulfate removed any residual water in MTBE extract. These solution, acetonitrile, acetone, MTBE and sodium sulfate, were obtained from Sigma-Aldrich Chemicals Canada. The silylating agent, N,O-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS), in 0.1-mL ampoules, was obtained from Supelc. A solvent, *iso*-octane, for the derivatives of GC-MS analysis was also gotten from Sigma-Aldrich Chemicals Canada. Solid-phase extraction (SPE) cartridges (Oasis® HLB, 6mL, 0.2 g, part no. WAT 106202), were purchased from Waters.

Table 8-1 List of the selected drugs

Name	Acetylsalicylic Acid (A.S.A.)	Ibuprofen (IBP)	Gemfibrozil (GFB)	Metoprolol	Sulfamethoxazole
Application	Analgesic	Anti-inflammatory	Lipid regulator	Beta-blocker	Antibiotic
Structure					
Formula	$C_9H_8O_4$	$C_{13}H_{18}O_2$	$C_{15}H_{22}O_3$	$C_{15}H_{25}NO_3$	$C_{10}H_{11}N_3O_3S$
Molecular wight (MW)	180.2	206.3	250.3	267.4	254.3
Solubility in water (g/L)	1.0	2.1×10^{-2}			
pK_a	3.5	4.9-5.7	4.7		
$\log K_{ow}$		3.5-4.5	4.77		
$\log K_d$ (K_d in L/kg water)		0.9			
OH group	1	0	0	1	0
COOH group	1	1	1	0	0
Characteristic ions, m/z	195, 210	263, 278	201, 194, 322		
References	Internet-17, 2007 Lee et al. 2003 Yoon et al. 2007	Lee et al. 2003 Lindqvist et al. 2005 Carballa et al. 2005	Yoon et al. 2007	Internet-18, 2007	Internet-19, 2007

Note:

pK_a: acid dissociation constant, is an equilibrium constant for the dissociation of a weak acid.
K_{ow}: the octanol/water partition coefficient.
Kow = Concentration in octanol phase / Concentration in aqueous phase

8.1.2 Water Sample Preparation

Low concentration (10ng/L, 50 ng/L and 100 ng/L) water samples (single drug solution) for freeze concentration were prepared based on following steps.

Step 1: Each individual stock solution of each drug at 100 µg/mL was prepared in acetonitrile for the preparation of calibration standards.

Step 2: Based on predicted drug concentration in feed water sample, spiking 100 µg/mL stock solution of needed volumes in distilled water was prepared. For very low initial concentration such as 10 ng/L and 50 ng/L, the stock solution was diluted a few times in acetonitrile.

Water samples containing high drug concentration ($\geq$ 5 mg/L) included single drug sample and five drugs mixed samples. Individual drug was firstly dissolved in 1 mL acetone, and then distilled water was added to preferred sample volume. On the other hand, five drugs simultaneously mixed in 5 mL acetone first and finally the 5 mL solution was spiked into distilled water to get predicted sample.

8.2 Sample Analysis

8.2.1 pH

pH analyses of all samples were performed with Thermo Orion Model 710A+ pH meter in accordance with the section 4500-H^+ B of Standard Methods (1998). The pH meter had a relative accuracy of ± 0.001 pH units. All determinations were conducted in triplicates.

8.2.2 Conductivity

Conductivity analysis was determined in accordance with the section 2510 B of Standard Methods (1998). The conductivity measurement instrument used was advanced conductivity meter (Thermo Orion 162A). All determinations were conducted in triplicates.

8.2.3 Total Organic Carbon

Total organic carbon (TOC) samples were analyzed by using Dohrmann TOC Analyzer following the method described in section 5310 C of Standard Methods (1998). TOC test was done for the samples containing high drug concentration, of which were original samples never passed through freeze concentration and other sample prepared in two-stage freeze concentration. All determinations were conducted in duplicates.

8.2.4 Gas Chromatography-Mass Spectrometry

The concentration in the feed water, the liquid-phase, and the solid-phase (melted ice) concentrations were analyzed in GC-MS, and are denoted as C_o, C_L and C_S, respectively. The separated efficiency (E) is referred to as the mass of impurity removed, and can be considered as the ratio between the quantity of the pollutant entrapped in the solid phase (ice) and the total quantity of pollutant as estimated by the equation (8.1). The recovery efficiency (R) can be considered as the ratio between the quantity of the pollutant entrapped in the liquid phase and the total quantity of pollutant as estimated by the equation (8.2).The freezing ratio R_F (freezing degree) is obtained from the equation (8.2).

$$E = (1 - \frac{C_S \times V_S}{C_o \times V_o}) \times 100\% \quad (8\text{-}1)$$

$$R = \frac{C_L \times V_L}{C_o \times V_o} \times 100\% \quad (8\text{-}2)$$

$$R_F = \frac{V_S}{V_o} \times 100\% = (1 - \frac{V_L}{V_o}) \times 100\% \quad (8\text{-}3)$$

Where:

E = separated efficiency;

C_s = drug concentration in the solid –phase sample (melted ice);

V_s = volume of the melted ice;

C_o = drug concentration in the feed water;

V_o = volume of the feed water;

R = recovery efficiency;

C_L = drug concentration in the unfrozen liquid;

V_L = volume of the unfrozen liquid

R_F = freezing ratio.

8.2.4.1 Sample Preparation

For the three acidic drugs (ASA, IBU and GEM), the method described by Lee et al. (2003) was used as a reference in method. Each set of samples which included blank and duplicate samples, was prepared in a batch in the lab at Lakehead University. Following by solid phase extraction including adjusting the samples pH, passing the samples through the extraction columns, drying the samples, resuspending the samples and derivatization of the samples. Finally, the derivatized drugs were analyzed by GC-MS.

1) Solid phase extraction

A 6- mL, 200-mg Oasis® HLB was conditioned by elution with 5 mL of methanol, followed by 10 mL of water at pH 2. Via a siphon tube, each sample was applied to the extraction cartridge using a 1-L glass separatory funnel. A flow rate of 5 mL/min was maintained by adjusting the system vacuum. When the extraction was complete, the cartridge was dried under full vacuum for 1 minute. Then, the cartridge was eluted with two 2.5-mL aliquots of methanol to remove the adsorbed drugs. The methanol extract was evaporated to about 300 μL and was back extracted by 5 mL of methyle *tert*-butylether (MTBE) in 2-, 1-, 1- and 1-mL fractions. After adding much anhydrous sodium sulfate to remove any residual water, the MTBE extract was evaporated to 50μL and putted into a 1.8 mL GC autosampler vial.

2) Derivatization

The addition of the trimethylsilyl (TMS) group(s) to selected drugs (polar compounds) confers thermal and chemical stability in addition to enhanced volatility (Blau and Halket 1993). One hundred microlitres of N,O-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was added to the MTBE extract and maintained at 60 °C for 20 minutes to form the trimethylsilyl (TMS) derivatives. Finally, the derivatized sample was adjusted to 1.0 mL in *iso*-octane for GC/MS analysis.

3) GC-MS Operation

GC-MS is the primary analysis of drugs used for human therapy (excluding antibiotics) (Sedlak et al., 2005). GC-MS analyses of these pharmaceuticals as their TMS derivatives were performed with a CP 3800 gas chromatograph equipped with a VARIAN CP-8400 automatic liquid sampler and a VARIAN 1200 Mass Selective detector. Separation of the derivatives was achieved with a 30m×0.25 mm I.D., 0.25 μm film thickness, fused silica capillary column (VF-5MS) using the following temperature program: initial temperature 50 °C, 1-min hold, oven temperature ramps 30 °C/min from 50 to 150 °C, and 10 °C/min from 150 to 280 °C. The oven temperature was kept at 280 °C for 6 minutes. A constant carrier (helium) flow of 1 mL/min was maintained. The injector temperature was 250 °C. Samples in 1-μL aliquots were injected in the splitless mode with a splitless time of 1 minute.

For quantitative analyses, selected ion monitoring (SIM) was used and the characteristic ions chosen for each derivative are listed in Table 8-1.

8.3 Experimental Procedures (Unidirectional Downward Freezing)

The synthetic water samples were partially frozen, and 80% ice and 20% water were produced under different experimental conditions. Unidirectional downward beaker freezing experiments were carried out in a cold room at -7°C and -15°C, which were the warm and colder temperatures of the cold room. The dimensions of the cold room are 2,362.2 mm (L), 2,362.2 mm(W) and 2,286.0 mm(H). The temperature fluctuation of the cold room was low according to frequent monitoring of the temperature. As shown in Figure 8-1, plastic beakers (1000mL) were used for the freezing, because glass beakers might break down at low temperatures. Beakers were insulated except the top so that the freezing would proceed unidirectional from top to bottom.

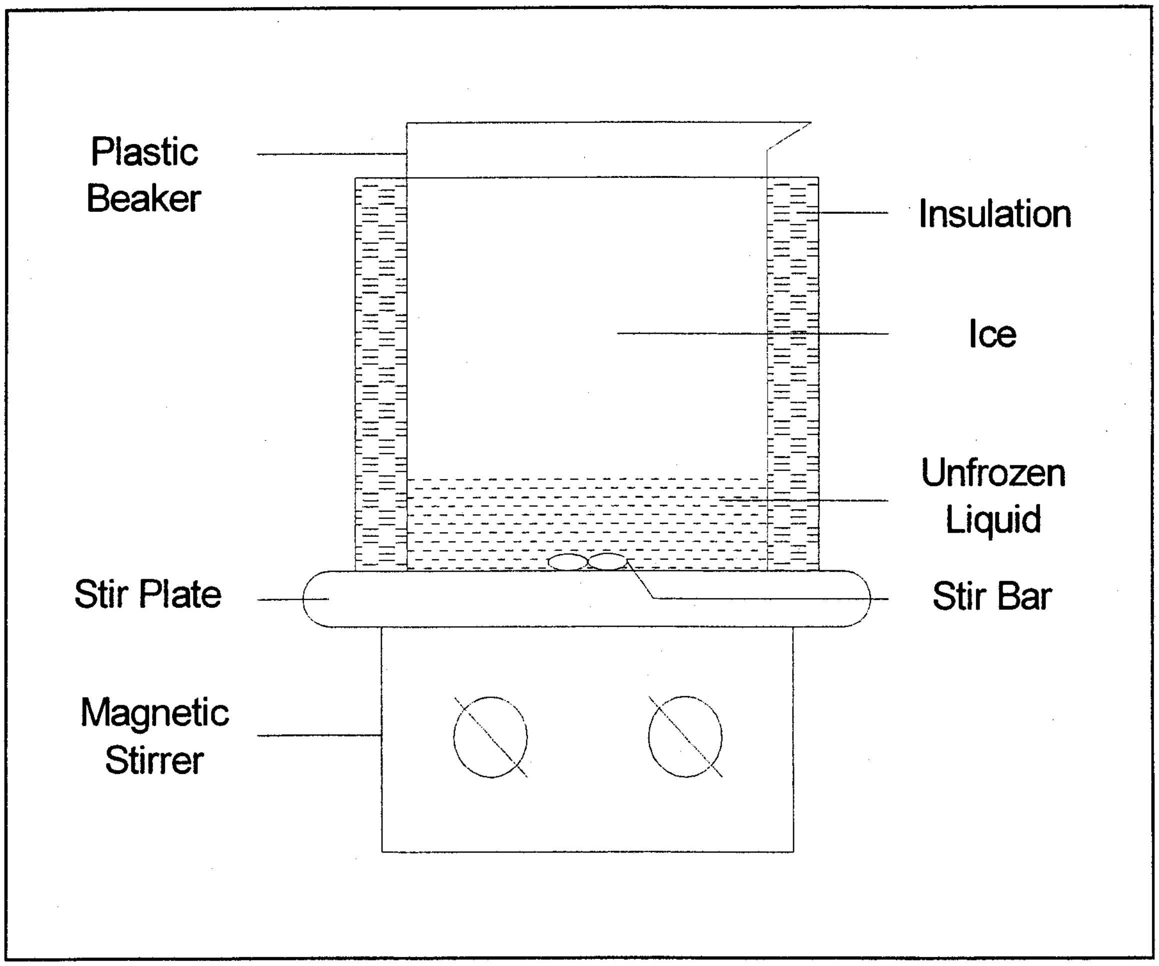


Figure 8-1 Unidirectional downward freezing equipment

Sample volumes of 1000 mL were used for 80% partial freezing. Samples were pre-cooled to near 0°C in the refrigerator before starting the beaker freezing test. Time required for 80% partial freezing of 1000mL at -7°C and -15°C were around 35 hours and 19 hours, respectively. The upper portion of the beaker was the frozen portion (ice) and the lower portion of the beaker was the unfrozen portion (unfrozen liquid).

The partial freezing of the samples was carried out in the beakers with stirring. A magnetic Fisher Stirrer (mini stirrer) was used for stirring operations. The stirrer was placed inside the cold room. To obtain the same extent of stirring for all the runs, the speed (300 rmp) dial was fixed at 5 and a same magnetic bar of 24 mm length.

When the desired volume was frozen (as judged by experience), the freezing was stopped, the unfrozen contents were sucked out from the plastic beakers by syringe and poured into an appropriate graduated cylinder. The volume of the concentrated solution is measured and recorded. The ice was melted and recovered. The partial concentrated liquid and the melted ice were subjected to the pH and conductivity analysis immediately and others were stored in sample bottles in side a refrigerator waiting for GC-MS sample preparation to get quantification of PhACs.

For low feed water concentration of selected drugs, the first freezing stage began with a prepared drug concentration of 10, 50, or 100 ng/L, the second freezing stage used only the concentrated drug solution from the first cycle. Therefore, we can evaluate the recovery of drugs by twice freezing cycles. However, for high initial concentration of selected drugs, the second cycle freezing is different with that of the low initial concentration of selected drugs. The melted ice from the first cycle was used to perform the second cycle in order to examine the remove efficiency of drugs by freeze concentration.

Chapter 9 RESULTS AND DISCUSSIONS

Synthetic water was used for unidirectional downward freezing experiments. The results from these experiments are presented and discussed in this chapter.

9.1 GC-MS Results

Because GC-MS is very sensitive analytical equipment, at least three calibration standards containing different drug concentrations had been simultaneously analyzed with the samples. Because GC-MS cannot stand water, each individual standard for each drug was prepared in acetonitrile. All standards also went through the same preparation processes as water samples. According to the standard curve, the concentration of water samples can be calculated. An example for each drug is presented in sections to follow.

9.1.1 Acetylsalicylic Acid Responses of GC-MS

As introduced in Section 7.2, GC-MS using full scan MS can determine unknown compounds/impurities in a sample. However, in SIM certain specific ion fragments are entered into the instrument method and only those mass fragments will be detected by the mass spectrometer. The benefits of using SIM analysis is that the typical detection limit can be lowered since the instrument is only looking at a handful of fragments (e.g., three fragments) during each scan and therefore more scans can take place each second. In this study, a high concentration (>0.2 ppm) drug calibration standard solution was first analyzed in full scan mode to determine the retention time and the mass fragment fingerprint before detecting relatively low-concentration experimental sample by SIM instrument method. As shown in Figure 9-1, a full-scan chromatogram plot of 1 mg/L of A.S.A, a huge peak was displayed at the retention time of 7.9 minutes in a typical broad range of mass fragments (m/z 40 to m/z 400) to monitor. The peak was confirmed as TMS derivative of aspirin (Figure 9-2) by comparing the mass fragment to the data library in the computer program.

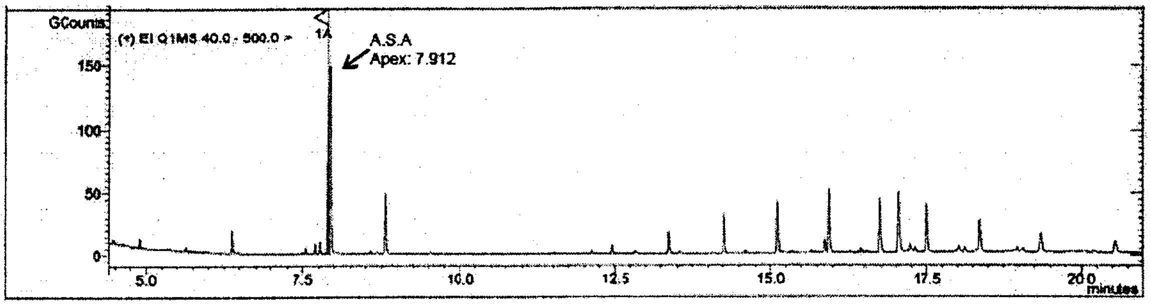


Figure 9-1 Full-scan chromatogram plot of 1ppm A.S.A. standard

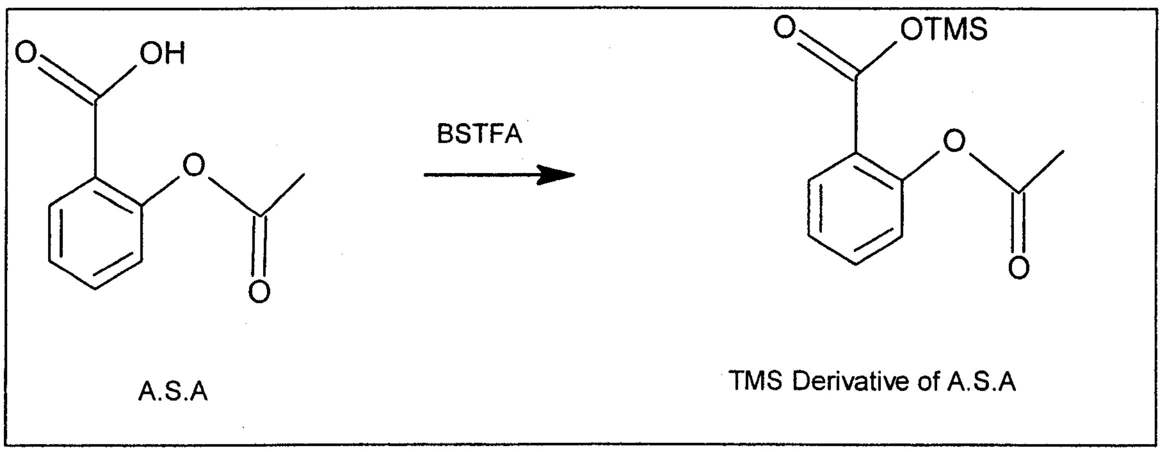


Figure 9-2 Derivatization of A.S.A.

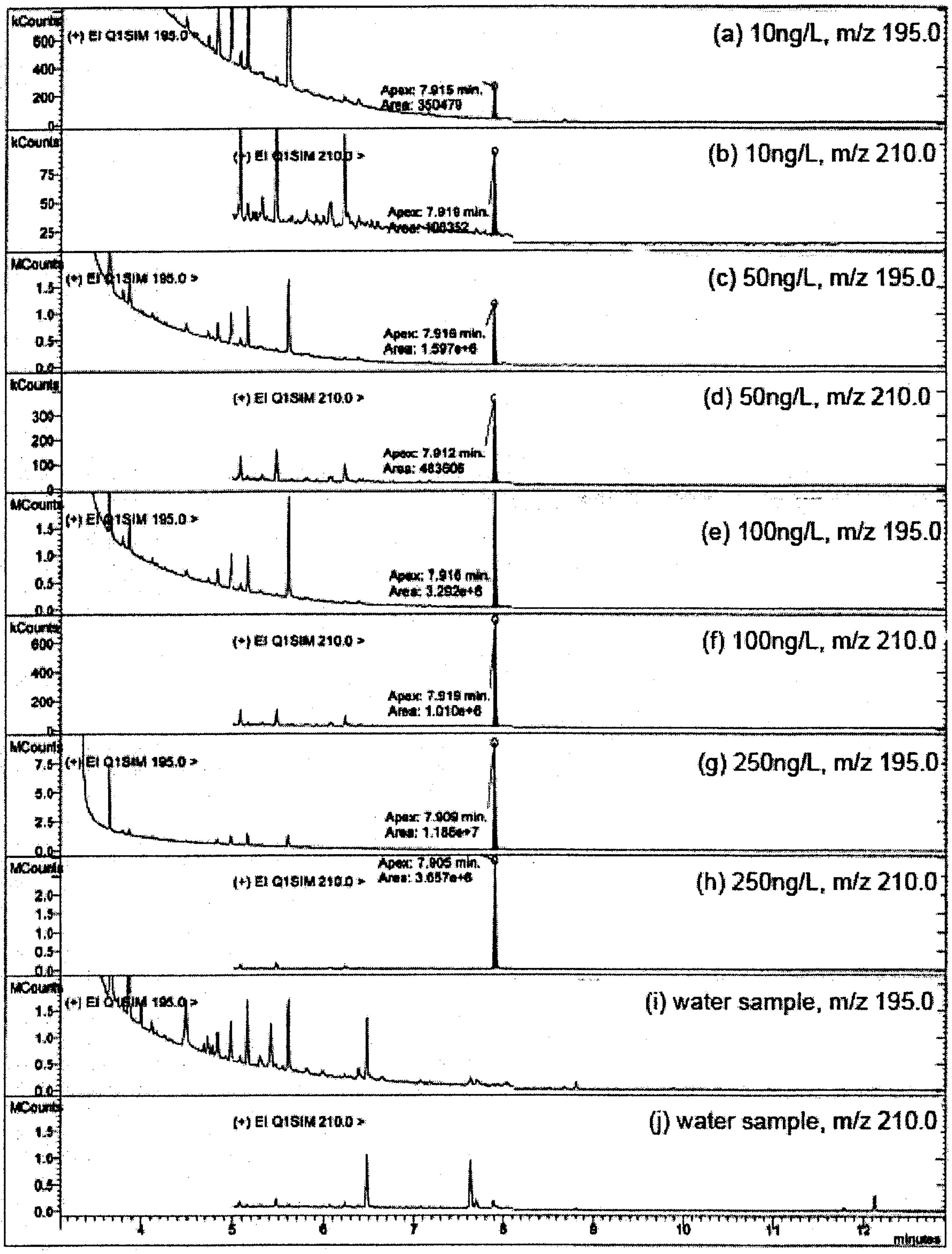


Figure 9-3 Chromatogram plots of typical A.S.A. external calibration standards and unfrozen liquid from two-stage freezing

Through full scan for a high-concentration test solution, a retention time of A.S.A. was known around 7.9 mins. Therefore, the area of peaks shown at the retention time can be read from SIM chromatogram plots. For instance, the responses of GC-MS four external calibration standards and a typical experimental sample were demonstrated in Figure 9-3. The four standards were prepared at the gradient concentrations of about 10, 50, 100 and 250 ng/L. The experimental sample is a two-stage unfrozen liquid from feed water containing A.S.A of 100ng/L. M/z ratios 195.0 and 210.0 were selected to quantification of each A.S.A test solution. At around 7.9 mins on the abscissa, except for the water sample, a series of area-increasing peaks appeared with the standard concentrations; therefore, peak area can be plotted with the correspondingly known A.S.A concentration in the standards. The trendlines for different m/z ratios were obtained by curve fitting (Figure 9-4) and the R^2 values are 0.9712 and 0.9721, respectively.

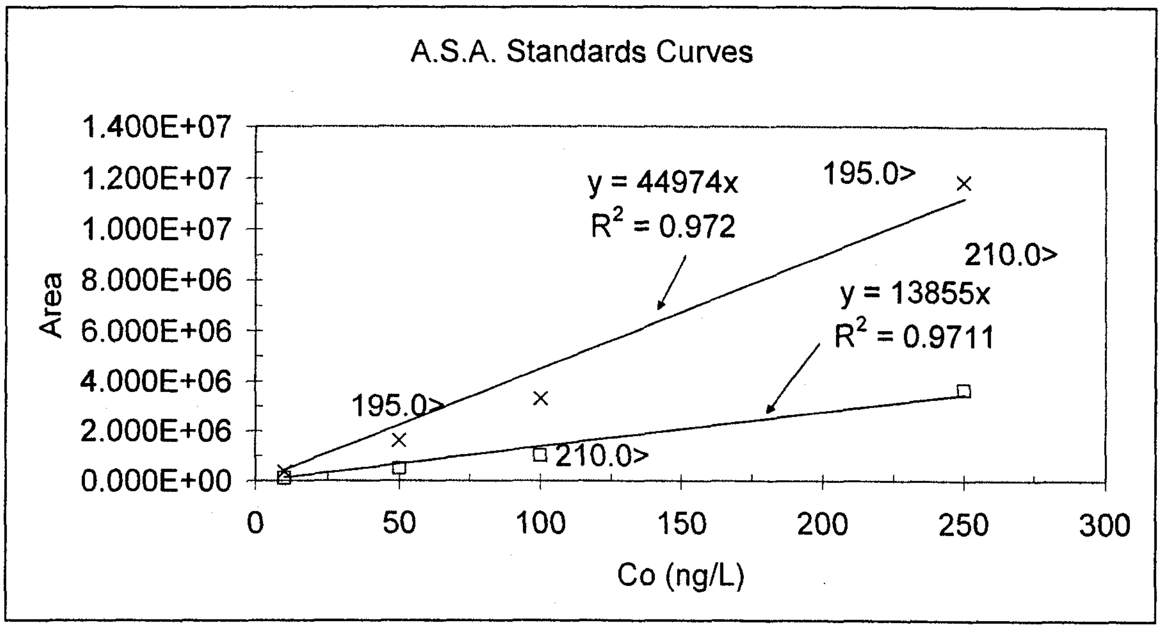


Figure 9-4 A.S.A standard curves from GC-MS responses

For the water sample shown in Figure 9-3, there was no significant peak shown at around 7.9 mins, which suggested no A.S.A in the sample, even the prepared feed water acetylsalicylic acid (A.S.A) concentration was as high as 100 ng/L, which was higher than that of the other drugs (ibuprofen and gemfibrozil) tested. Throughout experiments, hundreds of A.S.A solutions, including different concentrations of A.S.A in feed water as well as their corresponding unfrozen liquid and the ice after first-stage freezing and second-stage freezing were analyzed. In addition, the volume of samples was also increased from 400mL to 1000mL, but there is still no response shown in the chromatograph plot. Figure 9-3 (i) and (j) only showed a typical example of A.S.A solution which has theoretically highest concentration in all tested water samples. These results indicated that A.S.A easily degradation in aquatic environment and it is the primary reason leading to A.S.A water sample under detection limits in this study, which agreed with findings of other researchers. For instance, Verenitch et al. (2006) found the instability of A.S.A in aqueous matrices, especially, during the methylation process at high temperature in GC-MS analysis. Stable responses of the GC-MS for the external standards tested during the same time period indicated that the equipment was functioning well.

9.1.2 Ibuprofen Responses of GC-MS

Full spectrum of 1 mg/L of ibuprofen was shown in Figure 9-5, a peak was displayed at the retention time of 8.8 minutes in a typical broad range of mass fragments (m/z 40 to m/z 400). The peak was confirmed to be TMS derivative of ibuprofen (Figure 9-6) by comparing the mass fragment to the data library in the computer program.

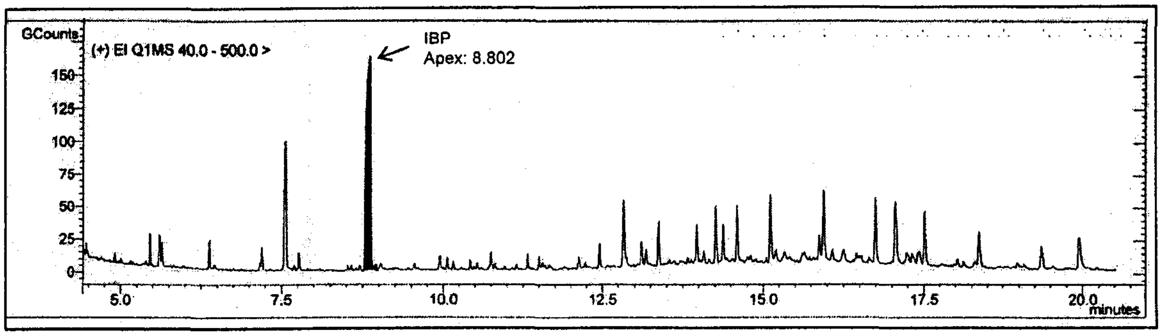


Figure 9-5 Full-scan chromatogram plot of 1ppm ibuprofen standard

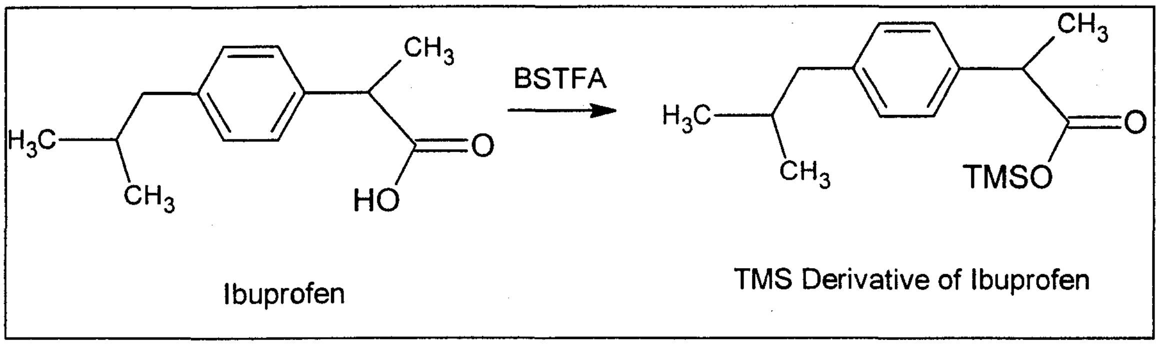


Figure 9-6 Derivatization of ibuprofen

Through full scan for a high-concentration test solution, a retention time of IBP was known around 8.8 mins. The responses of GC-MS to three external calibration standards are demonstrated in Figure 9-7. The three standards were prepared at the gradient concentrations of 10, 50 and 250 ng/L. M/z ratios 263.0 and 278.0 was selected for IBP quantification. Additionally, the standard curves for IBP are illustrated in Figure 9-8.

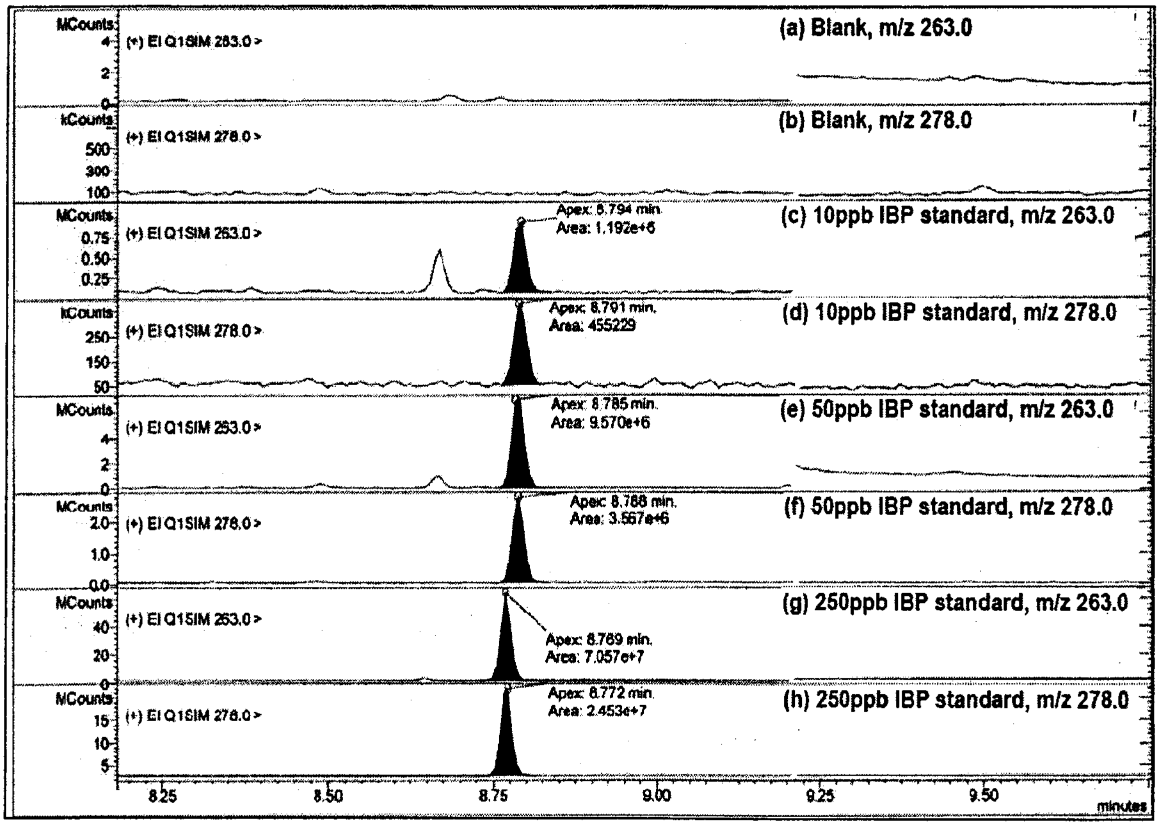


Figure 9-7 Chromatogram plots of typical IBP external calibration standards

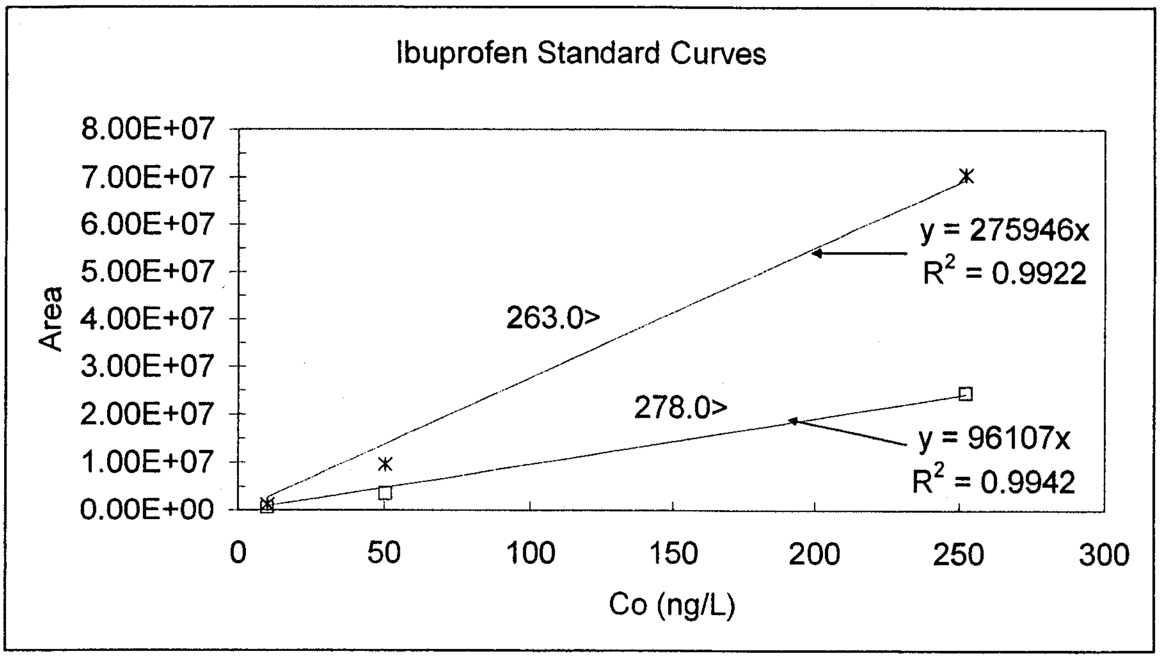


Figure 9-8 IBP standard curves from GC-MS responses

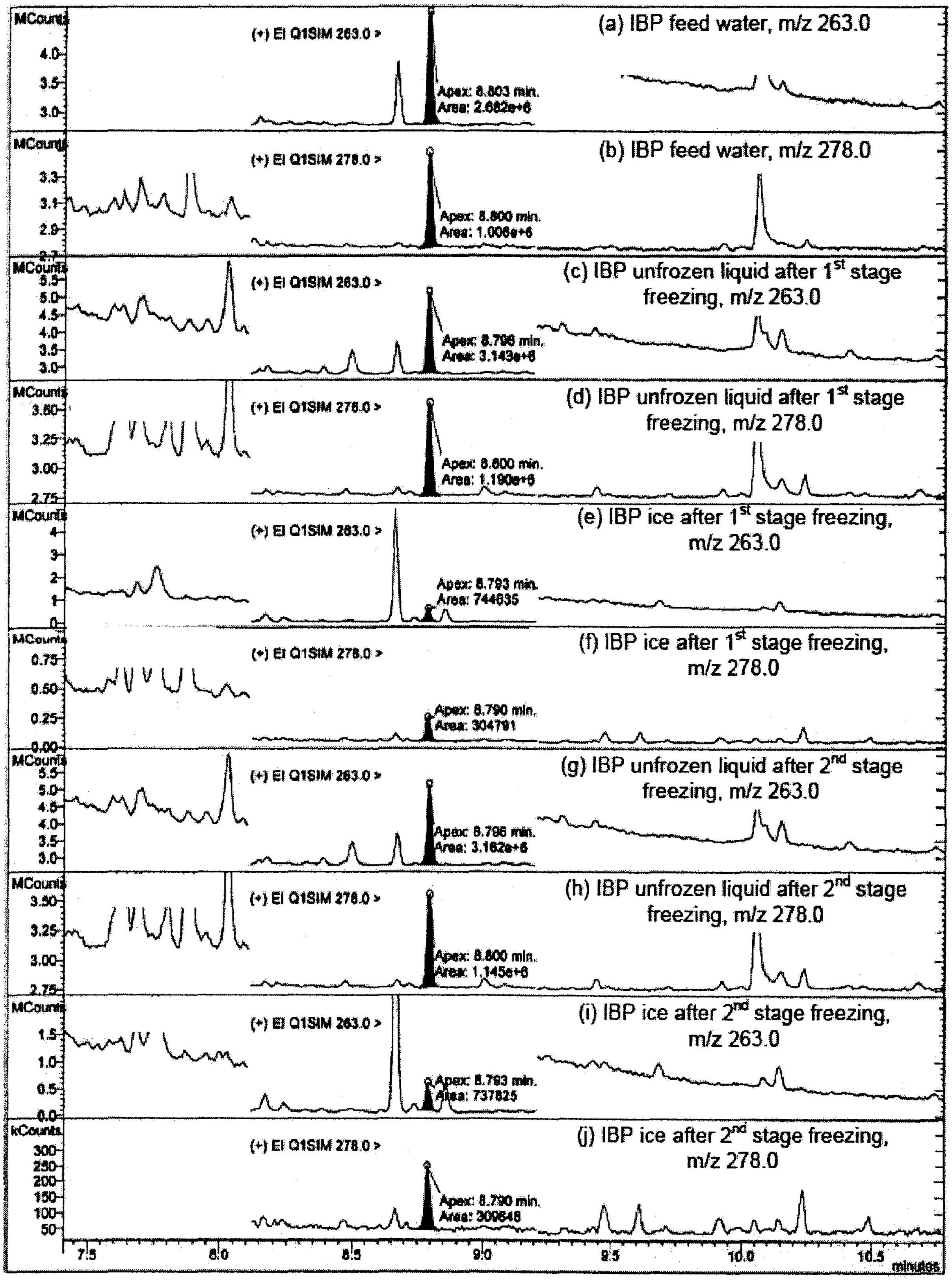


Figure 9-9 GC-MS responses of IBP samples

Figure 9-9 showed a set of responses of ibuprofen samples at two m/z ratios in SIM method. These samples from freeze concentration experiments included feed water, first stage freezing unfrozen liquid and ice, and second stage freezing unfrozen liquid and ice. One sample from each group was used as an example for presentation in this section. According to the responses shown in Figure 9-9 and equation: y=275946x for m/z 263.0 presented in Figure 9-8, the corresponding concentrations of these samples were calculated and the results were listed in Table 9-1.

Table 9-1 Calculation results of one ibuprofen freezing run

Sample [1]	Sample Volume (mL) [2]	GC-MS Response 263.0 [3]	GC-MS Response 278.0 [4]	Calculation Results (ng/L) 263.0 [5]	Calculation Results (ng/L) 278.0 [6]	Average concentration (ng/L) [7]
Feed water	1000	2.682E+6	1.006E+6	9.72	10.47	10.09
1st stage freezing unfrozen liquid	350	3.143E+6	1.190E+6	32.54	35.38	33.96
1st stage freezing ice	1000	744635	304791	2.70	3.17	2.93
2nd stage freezing unfrozen liquid	100	3.162E+06	1.145E+6	114.59	119.14	116.86
2nd stage freezing ice	300	737825	309648	8.91	10.74	9.83

The first column in Table 9-1 is the name of water sample. The detected sample volume was listed in Column 2. Because the responses of GC-MS demonstrate impurities abundance in 1mL concentrated sample from 1-L feed water, when the feed volume is not one liter, a volume ratio (1000 mL / real used volume in mL) must be taken account into the calculation of concentration. For example, based on the GC-MS responses in

Column 3, the concentration of 11.39 ng/L of 1st stage of freezing unfrozen liquid in default 1-L feed water was determined by using Eq. 9-2. However, the actual detected sample volume is 350mL, after given the volume ratio 1000mL/350mL, the real concentration of the unfrozen liquid is 32.54 ng/L. The same as the calculation of concentration at m/z 263.0, the concentration at m/z 278.0 is shown in the sixth column. Finally, the average concentration was calculated from values of two m/z ratios and was stated in final column.

9.1.3 Gemfibrozil Responses of GC-MS

Full spectrum of 0.2 mg/L of gemfibrozil is shown as Figure 9-10; a peak displayed at the retention time of 11.8 mins in a typical broad range of mass fragments (m/z 40 to m/z 400).

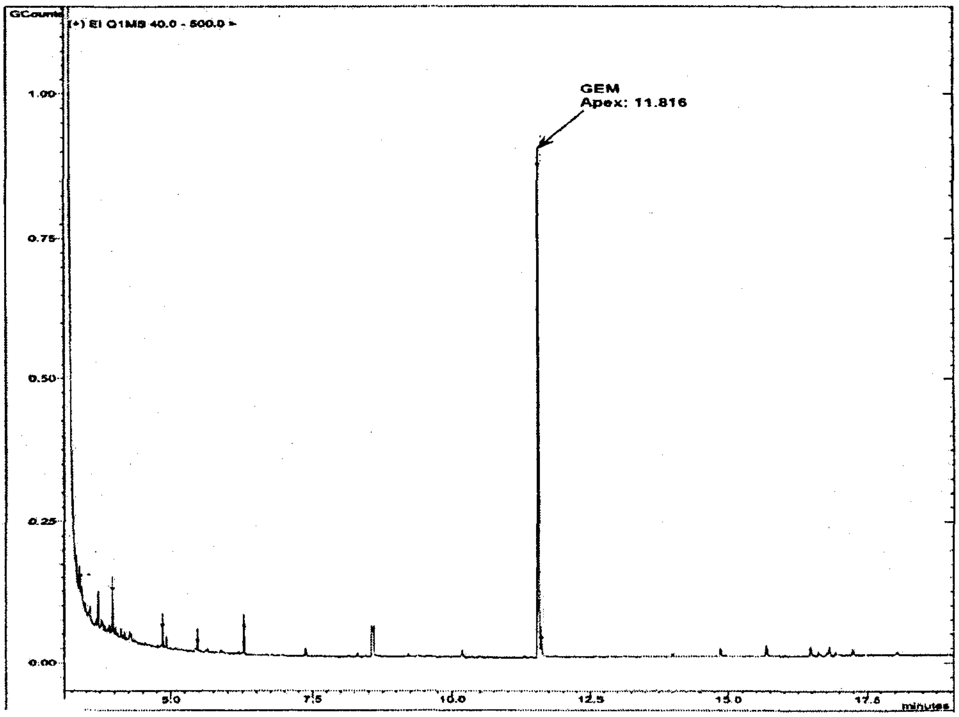


Figure 9-10 GC-MS full spectrum of 0.2 ppm GEM external standard

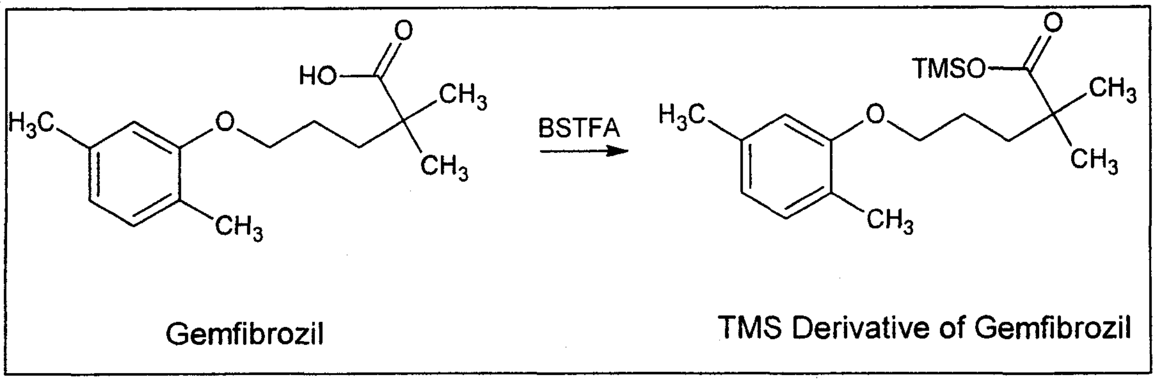


Figure 9-11 Derivatization of GEM

The peak shown in Figure 9-10 was confirmed as the TMS derivative of gemfibrozil (Figure 9-11) by comparing the mass fragment to the data library in the computer program.

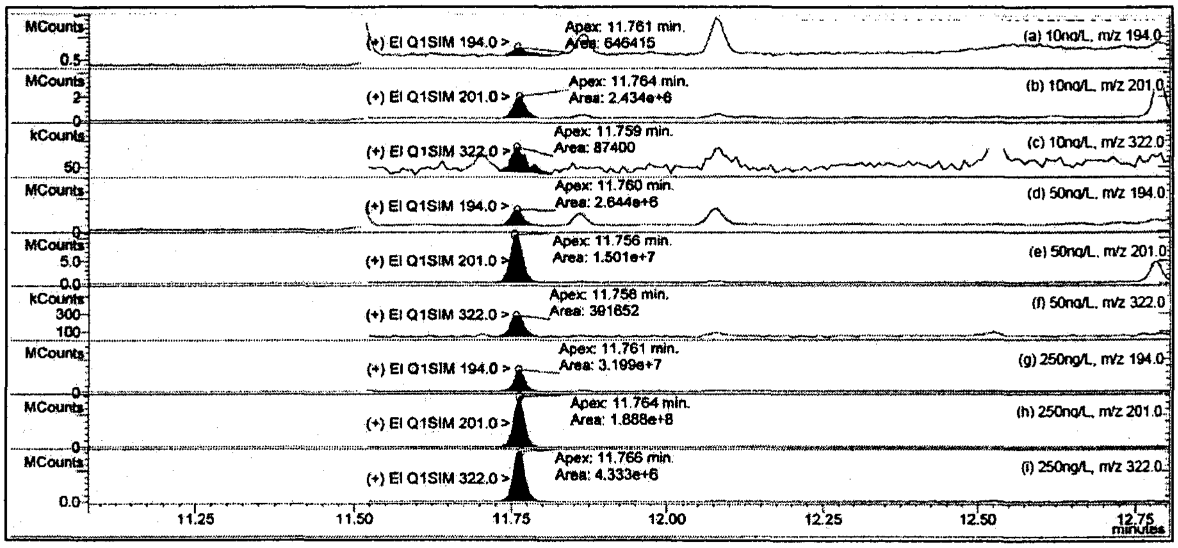


Figure 9-12 Chromatogram plots of typical GEM external calibration standards

The responses of GC-MS to three external calibration standards (10, 50 and 250 ng/L) are demonstrated in Figure 9-12. The selected m/z for quantification of each gemfibrozil tested solution was 194.0, 210.0 and 322.0. As shown in Figure 9-13, the R^2 values of the trendlines were 0.9759 and 0.9774, respectively.

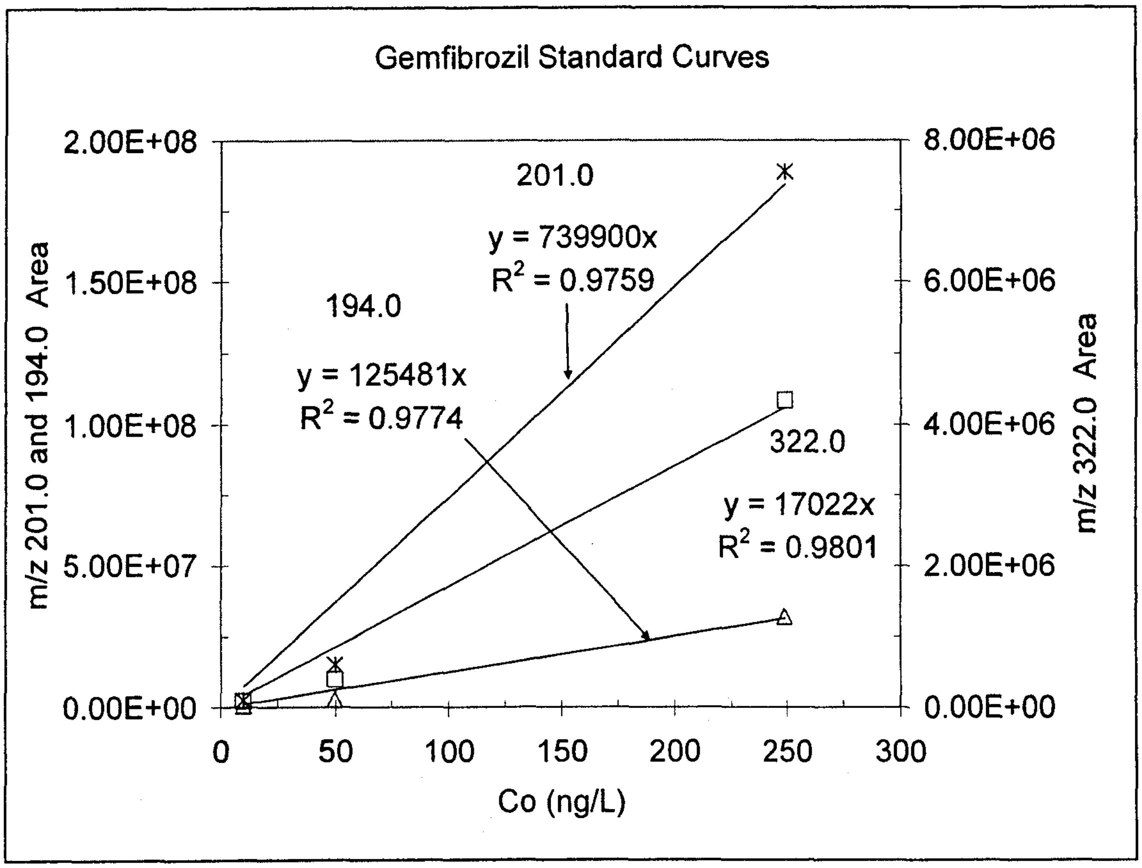


Figure 9-13 Gemfibrozil standard curves from GC-MS responses

As an example, Figure 9-14 and Figure 9-15 show a set of responses of gemfibrozil samples from freeze concentration experiments at three m/z ratios. These samples included feed water, first stage freezing unfrozen liquid and ice (Figure 9-14), second stage freezing unfrozen liquid and ice (Figure 9-15).

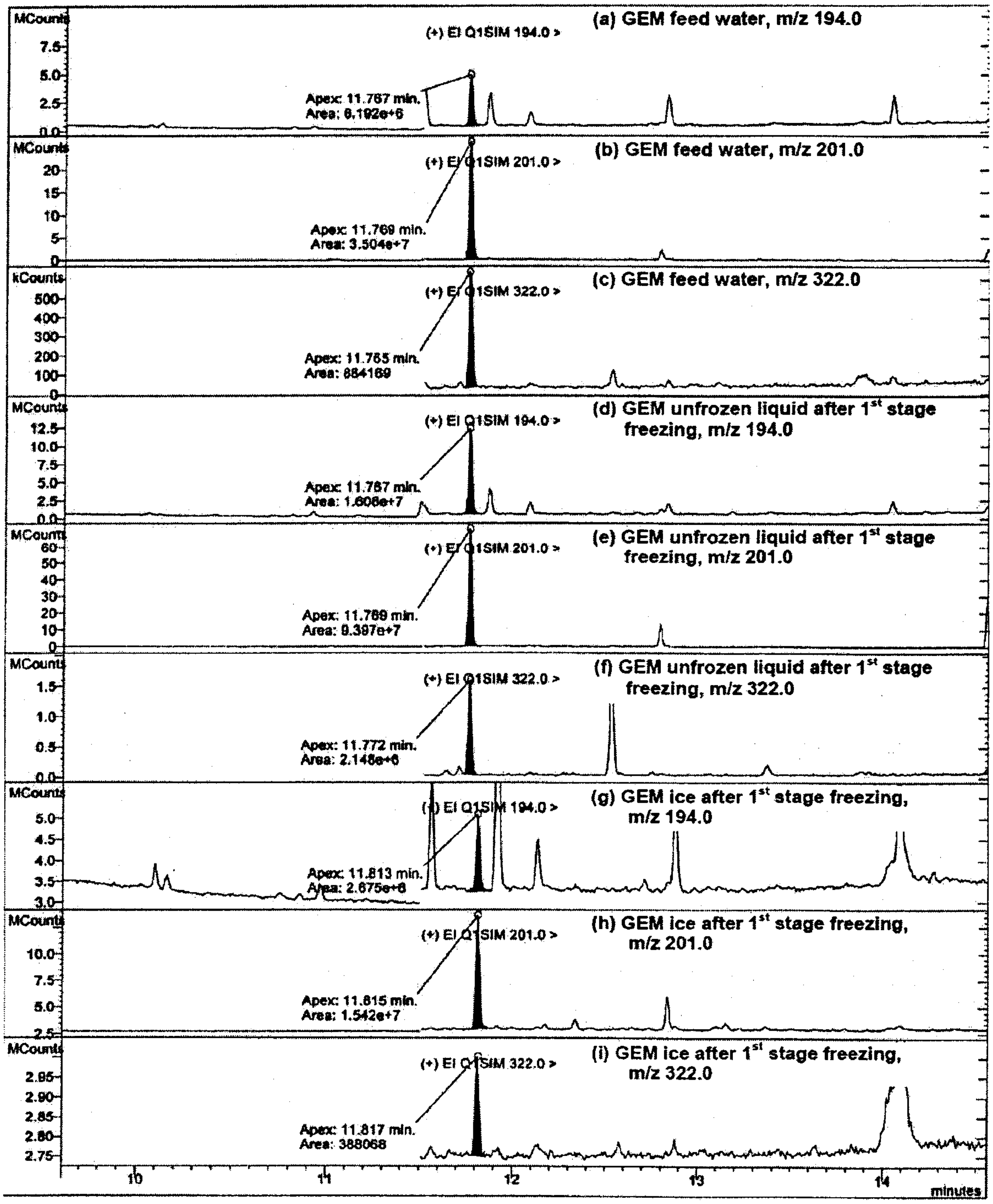


Figure 9-14 GC-MS responses of gemfibrozil feed water and samples after 1st stage freeze concentration

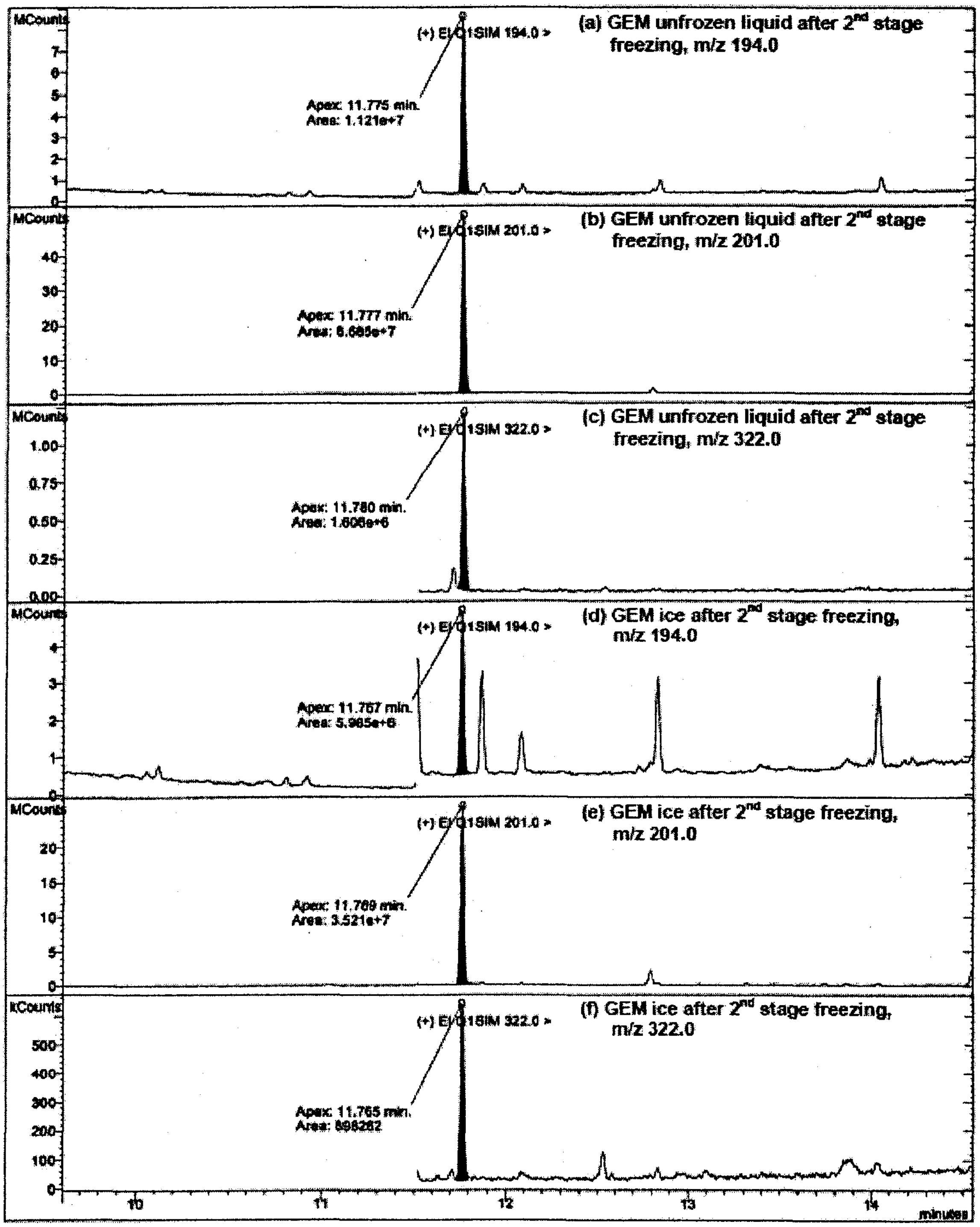


Figure 9-15 GC-MS responses of gemfibrozil samples after 2nd stage freeze concentration

According to the responses shown in Figure 9-14, Figure 9-15 and the standard curve in Figure 9-13, the corresponding concentrations of these samples were calculated and tabulated in Table 9-2. Similar calculation method for ibuprofen was used to determine the concentrations in various samples.

Table 9-2 Calculation results of one gemfibrozil freezing run

Sample	Sample Volume (mL)	GC-MS Response			Calculation Results (ng/L)			Average concentration (ng/L)
		194.0	210.0	322.0	194.0	210.0	322.0	
Feed water	1000	6.192E+6	3.504E+7	884169	49.35	47.36	51.94	49.55
1st stage freezing unfrozen liquid	550	1.608E+7	9.397+7	2.148E+6	232.99	230.92	229.44	231.12
1st stage freezing ice	1000	2.675E+6	1.542E+7	388068	21.32	20.84	22.80	21.65
2nd stage freezing unfrozen liquid	100	1.121E+7	6.685E+7	1.606E+6	893.36	903.50	943.48	913.45
2nd stage freezing ice	330	5.965E+6	3.521E+7	898262	144.05	144.20	159.91	149.39

9.1.4 Detection Limits of GC-MS

In the early testing of this study, the detection limits of GC-MS could reach 10 ng/L for the three acidic drugs. However, after some repair work of the broken parts of the instrument in February, 2007, the detection sensitivity of instrument was significant lowered, it was no longer able to detect concentrations of the drug at the 10 ng/L level or even much higher concentrations as illustrated in Figure 9-16.

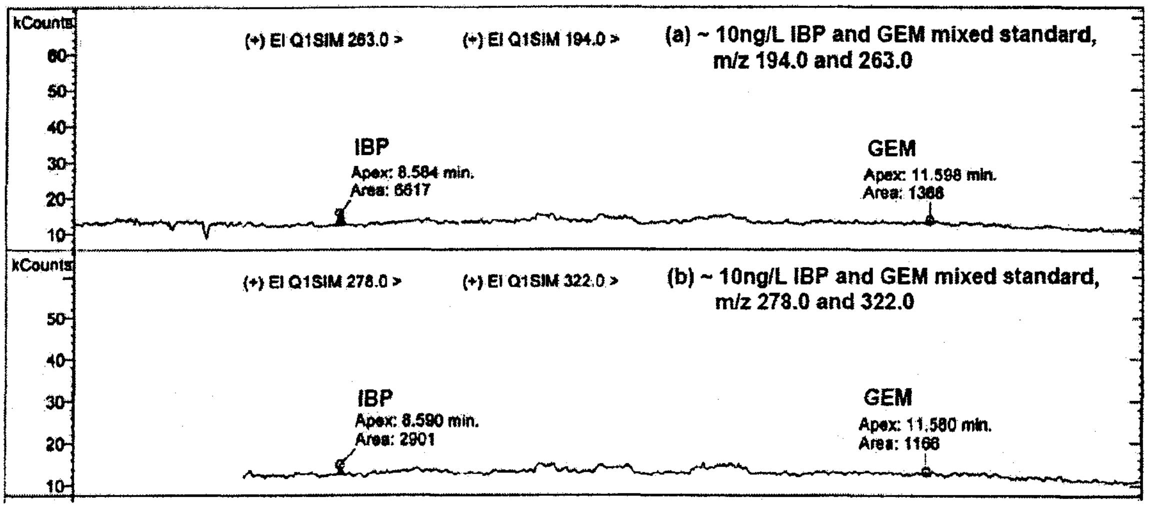


Figure 9-16 Chromatograph plots of 10 ng/L ibuprofen and gemfibrozil mixed external standard

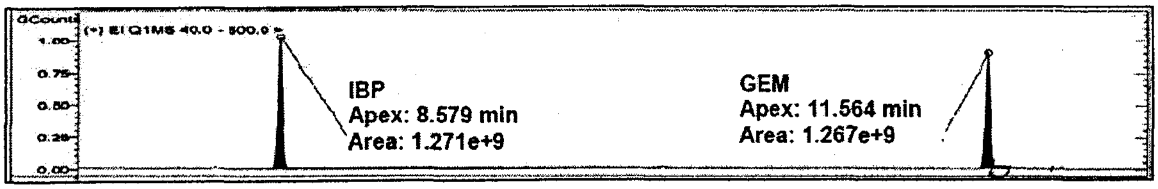


Figure 9-17 Full spectrum of 20 mg/L ibuprofen and gemfibrozil mixed external standard

There are some possible causes including the interference of the mixed drugs, and the instrument problem leading to the high detection limits. Firstly, a 20 mg/L mixed-drug standard was prepared for GC-MS analysis to find out the influence of ibuprofen on the analysis of gemfibrozil or vice versa. This result is shown in Figure 9-17 and it indicates the close responses for two drugs at the same concentration. For example, 20 ppm ibuprofen got an area of 1.271E+09, and the 20 ppm gemfibrozil attained the area of 1.267E+9 which have identical quantity dimension with area value of 20 ppm ibuprofen. In addition, the peaks of the two drugs were obviously found to be lower than that of individual drug shown in Figure 9-5 and Figure 9-10. For example, 1ppm ibuprofen standard (Figure 9-5) and 0.2 ppm gemfibrozil standard (Figure 9-10) show a peak greater than 150 GCounts (Giga counts: 10^9) and 0.9 GCounts, respectively; however, the peaks shown in Figure 9-17 was only about 1 GCounts for each drug in 20 mg/L mixed–drug standard, however, the trends of reflection of each other seems to be same.

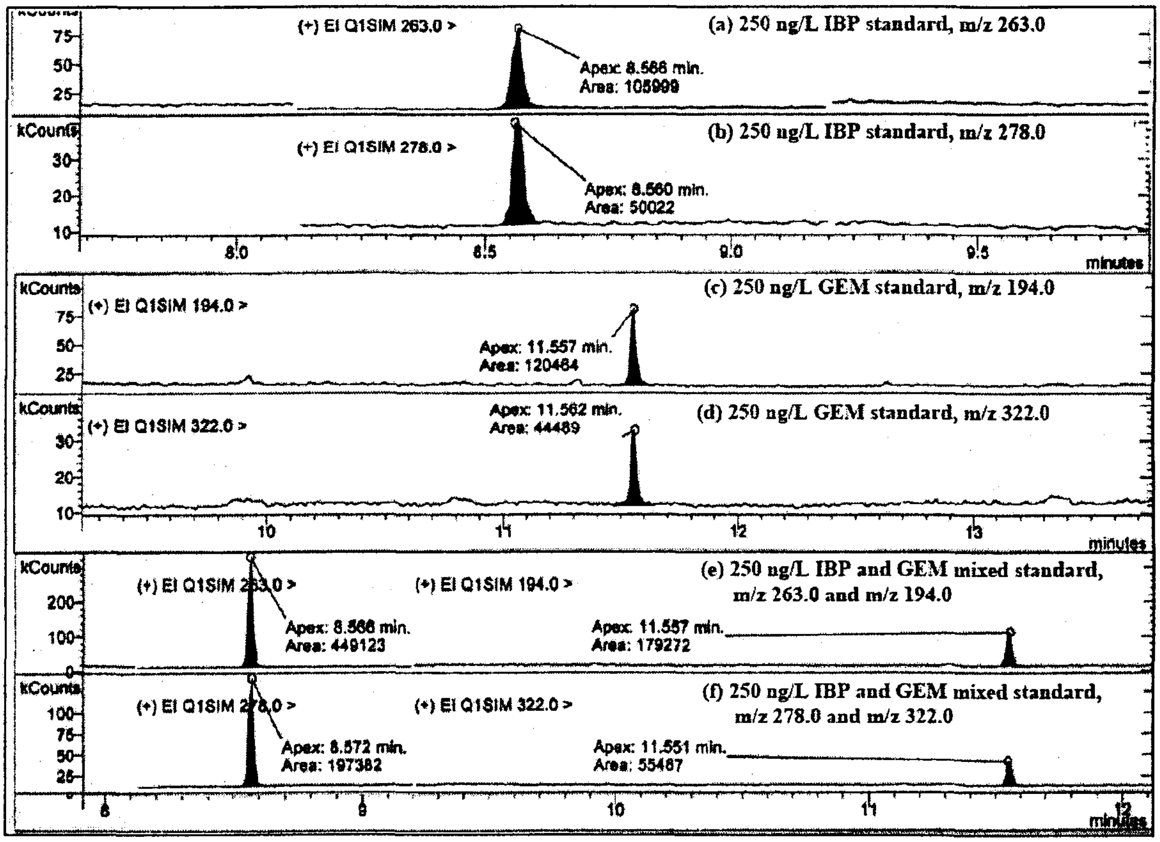


Figure 9-18 Chromatograph plots of ca. 250±8.2 ng/L individual ibuprofen and gemfibrozil as well as two drugs mixed external standards

A group of parallel tests was done to further investigate the influence direction (increasing or decreasing) of mixed drugs on individual drugs. Figure 9-18 shows the experimental results at two m/z ratios for each drug. No matter in single drug solution or the standard for the mixed drugs, selected drugs were prepared at around 250±8.2 ng/L concentration within one hour on the same day before the analysis of GC-MS.

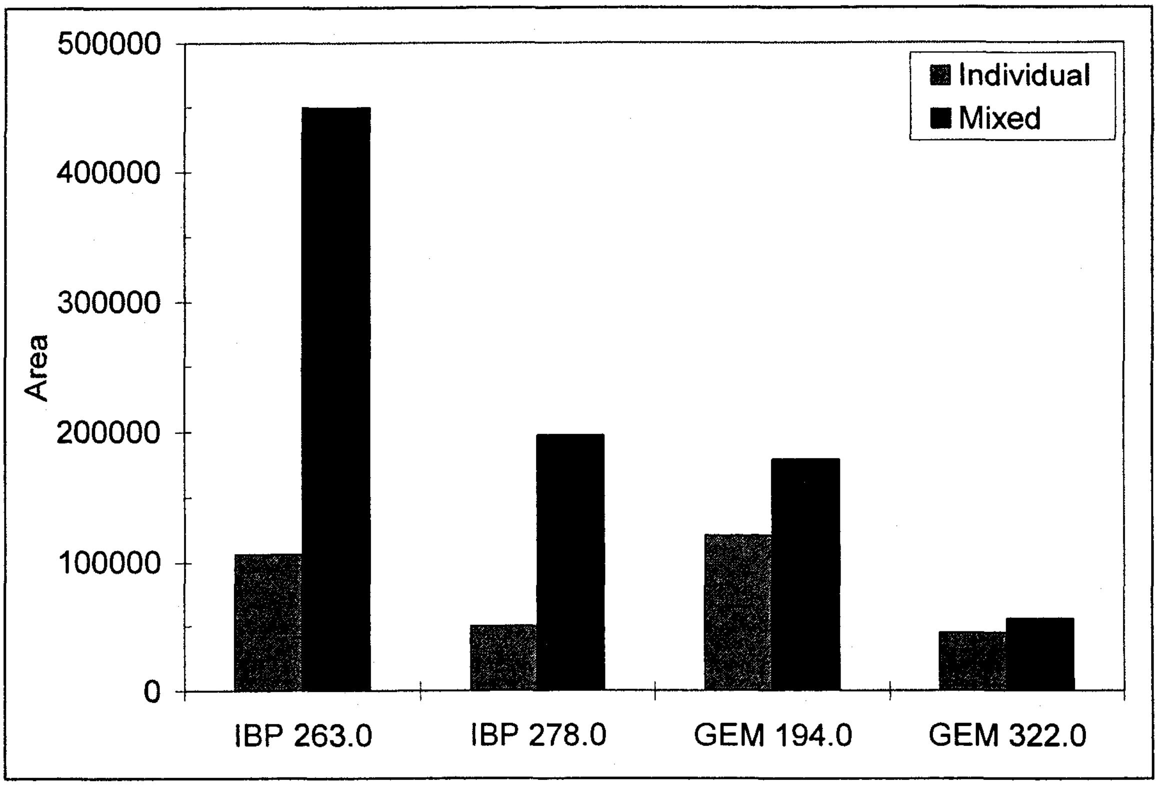


Figure 9-19 Comparison of GC-MS responses for individual ibuprofen, gemfibrozil and two drugs mixed standards at the same concentration of 250±8.2 ng/L.

Figure 9-19 illustrate obviously the increasing ibuprofen responses from the GC-MS, but for gemfibrozil looks a little bit increasing which may be leaded by the differences between prepared concentrations, because 246.0 ng/L gemfibrozil was in single gemfibrozil standard compared 258.0 ppt gemfibrozil in drug-mixed standard. The increasing responses of ibuprofen in matrix are hard to explain and were never mentioned by other studies.

Another reason for different response of the GC-MS can be attributed to the replacement of the part and contamination of GC-MS column (VF-5MS) by the samples, which consequently could have lowered the precision of the equipment. It is noted that equipment has not been functioning at the levels before the repair.

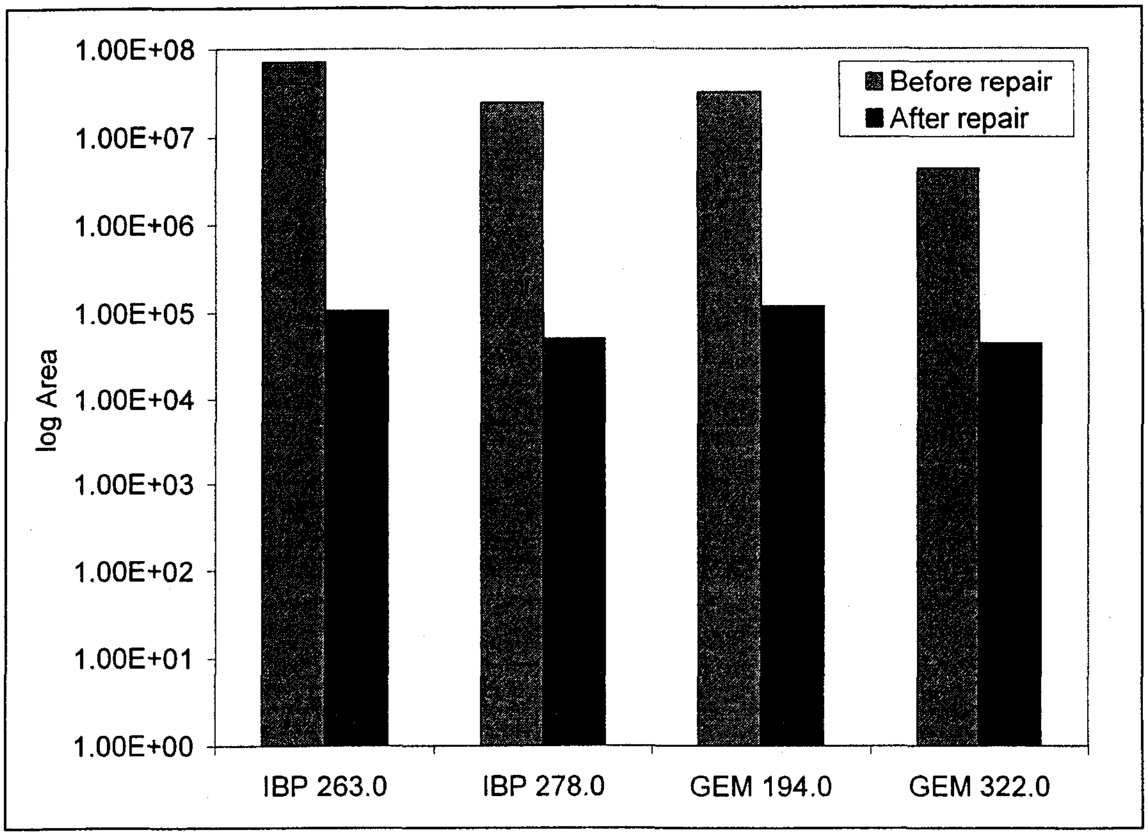


Figure 9-20 Response changes for 250 ±3.1 ng/L individual ibuprofen and gemfibrozil using fixed GC-MS compared to the old instrument used in the early studies.

Figure 9-20 shows the lower responses for individual ibuprofen and gemfibrozil of closed concentration from repaired machine compared to the old instrument.

Additionally, there are many other reasons for lowering the precision of the equipment. The plot of peak area with test date in

Figure 9-21 clearly demonstrates decreasing GC-MS responses of each drug in the sequential testing. Two possible explanations may lead to the decreases. One is that the derivatization reagent, BSTFA, as mentioned previously is a contamination for GC-MS and easily makes the deviant working of the instrument. Because the instrument used in this study is public at Lakehead University, the GC-MS running of different samples from other researches perhaps introduce unknown chemicals then influence the equipment. In general, multiple influences resulted in the unstable running of GC-MS.

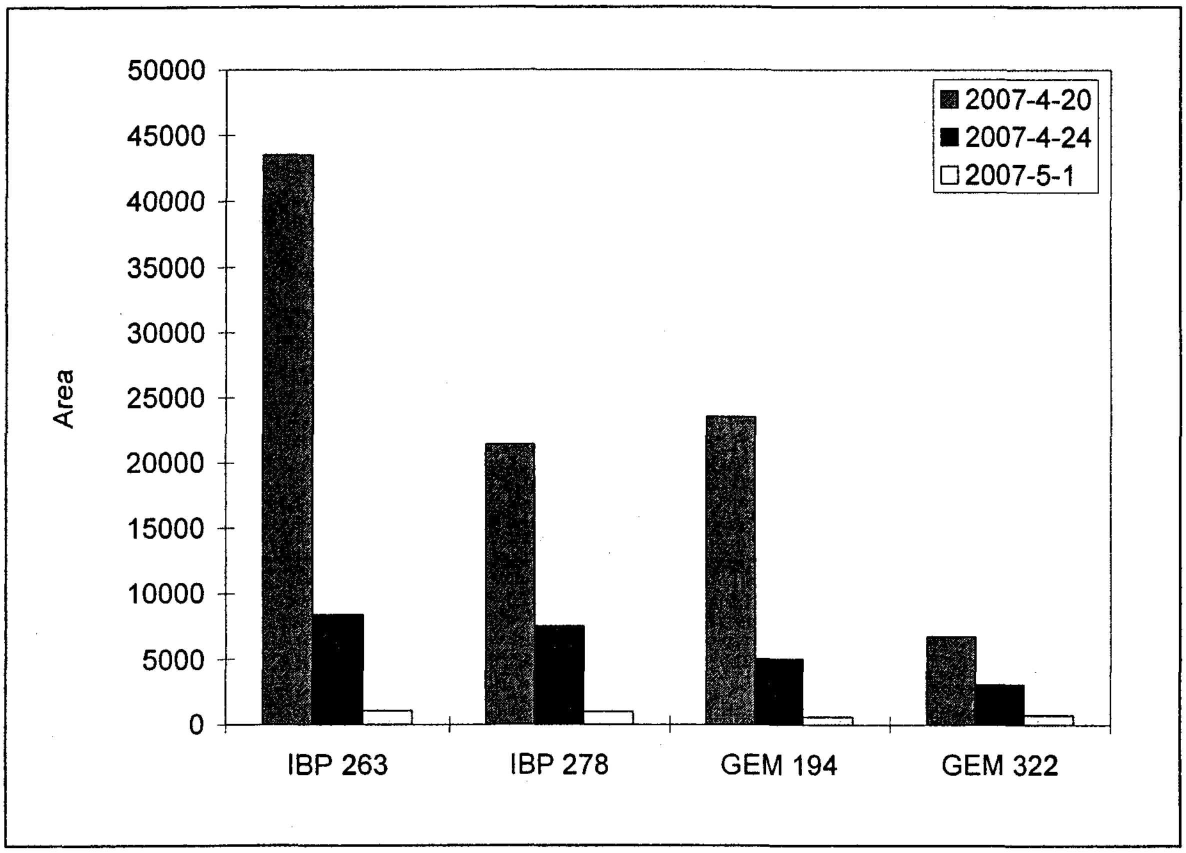


Figure 9-21 Inconsistent GC-MS responses for 10 ng/L individual ibuprofen and gemfibrozil solutions obtained

In an effort to get more accurate analytical results and quality control, in addition to the external calibration standards, an internal standard of 2,3-D of 10 mg/L for sample analysis was invoked. A typical SIM spectrum of drug-mixed standard solution with 10 mg/L internal standard is shown in Figure 9-22. In the figure, (a) and (b) shows the results of 20 ng/L drug-mixed standard at two m/z ratios (c) and (d) presents that of 200 ppb drug-mixed standard at two m/z ratios.

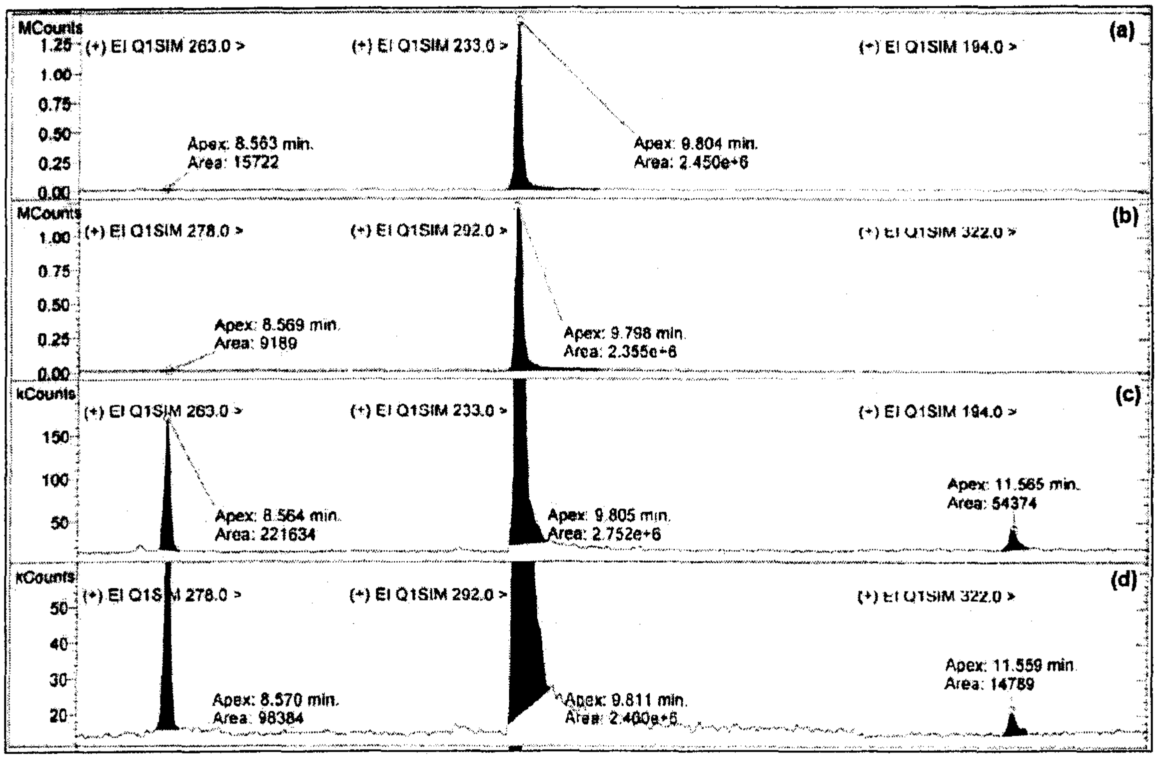


Figure 9-22 A typical SIM spectrum of drug-mixed standard added 10 ppm 2,3-D

The difficulties experienced in this study and those reported by other researchers mentioned previously (in Chapter 7, PhACs Analytical Methods) suggest the inherent challenges of using GC-MS for analyzing PhACs. Although the process of whole research is really hard, lots of valuable results are still obtained and presented in the following sections.

9.2 Freeze Concentration Results

9.2.1 Ibuprofen

The experimental results indicated that ibuprofen was the most stable drug compared to A.S.A and gemfibrozil; therefore, more experimental results were attained.

Based on single-stage freezing and two-stage freezing, the effect of different parameters on the freeze concentration of the drugs was presented in the following sections.

9.2.1.1 Single-stage Freezing of Ibuprofen

1) Effect of Initial Drug Concentration in Feed Water on Impurity Removal on Single-Stage Freezing

In this study, pH and conductivity as two typical parameters were firstly measured for each water sample. Additionally, slight impurities in distilled water as the main solvent for each group of experiment were also taken account into the experimental results. Therefore, the counterparts of the corresponding blanks were tested at the same time of analyzing water samples.

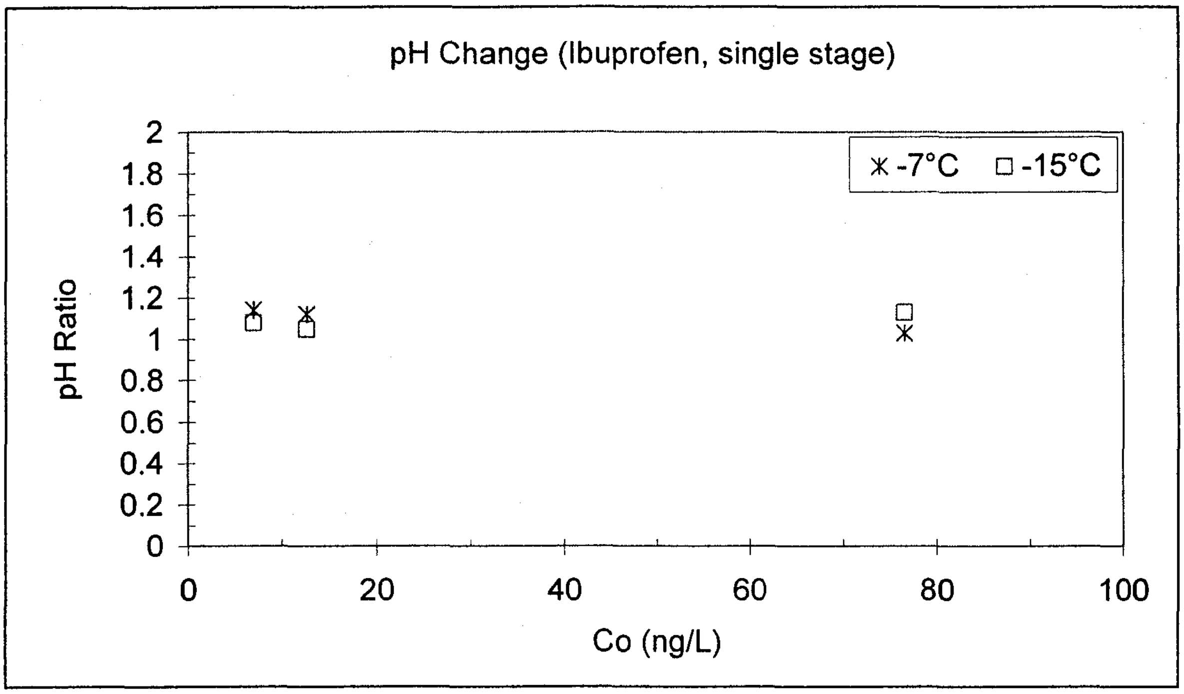


Figure 9-23 pH change in unfrozen liquid after single-stage freeze concentration for different ibuprofen concentrations in feed water

$$\text{pH ratio} = \frac{\text{pH of unfrozen liquid}}{\text{pH of feed water}}$$

Change of pH in the unfrozen liquid samples versus the feed water sample is depicted in Figure 9-23. There is no obvious change in pH values with increasing impurity concentration. This phenomenon may be explained by the factor that the ibuprofen is a weak acidic and has pK_a value of around 5.0 (Carballa et al. 2005). For a given acid, pK_a values will vary depending on solvent other than impurity concentration solution (Internet-20, 2007). In this study, distilled water is the main solvent to prepare synthetic water samples. The distilled water pH was in the range of 5.1 to 6.4 as shown in Figure 9-24, and pH value of feed water was ranged from 5.0 to 6.2. The samples did not show significant difference with increasing ibuprofen concentrations.

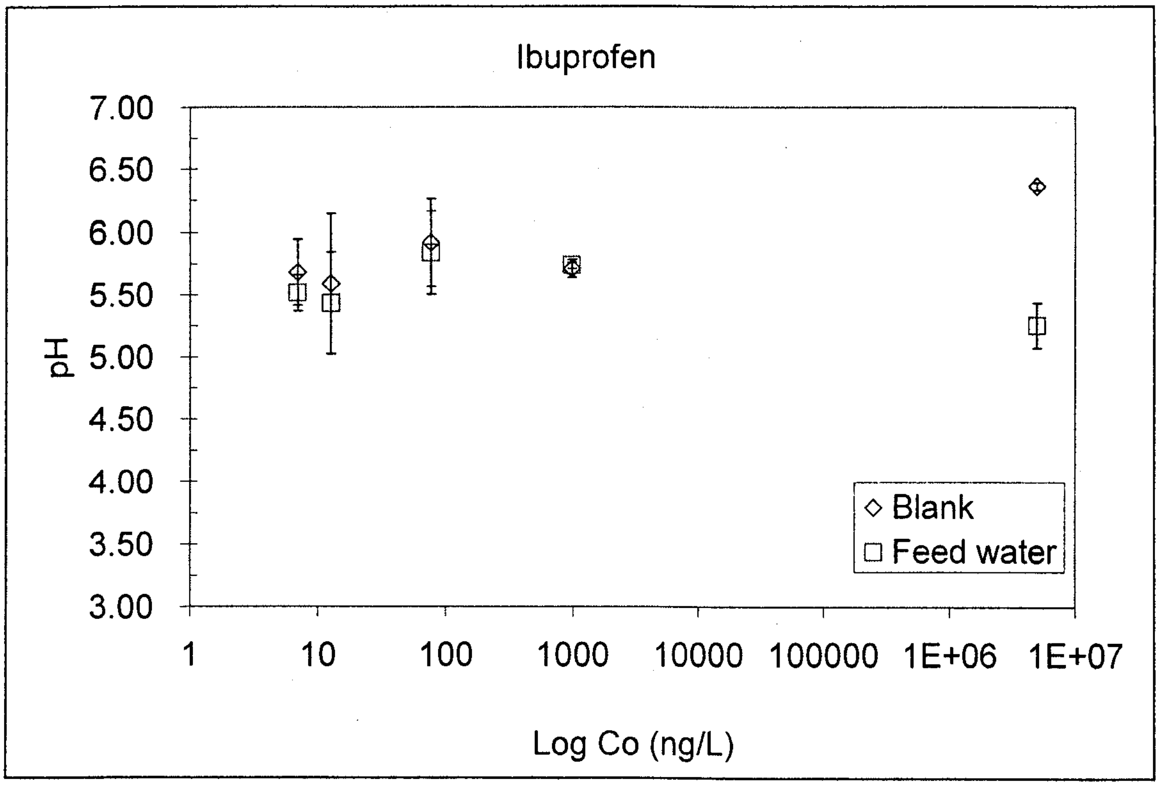


Figure 9-24 Comparison of pH between blank and ibuprofen feed water
Blank is distilled water used to prepare the corresponding feed water.

In contrast to pH, conductivity is a measure of the ability of an aqueous solution to carry an electric current. This ability depends on the presence of ions; on their total concentration, mobility, and valence; and on the temperature of measurement (Clesceri et al. 1998). Consequently, the comparison of the conductivity values of blank and feed water for each group of freeze concentration was done, and the corresponding results are showed in Figure 9-25.

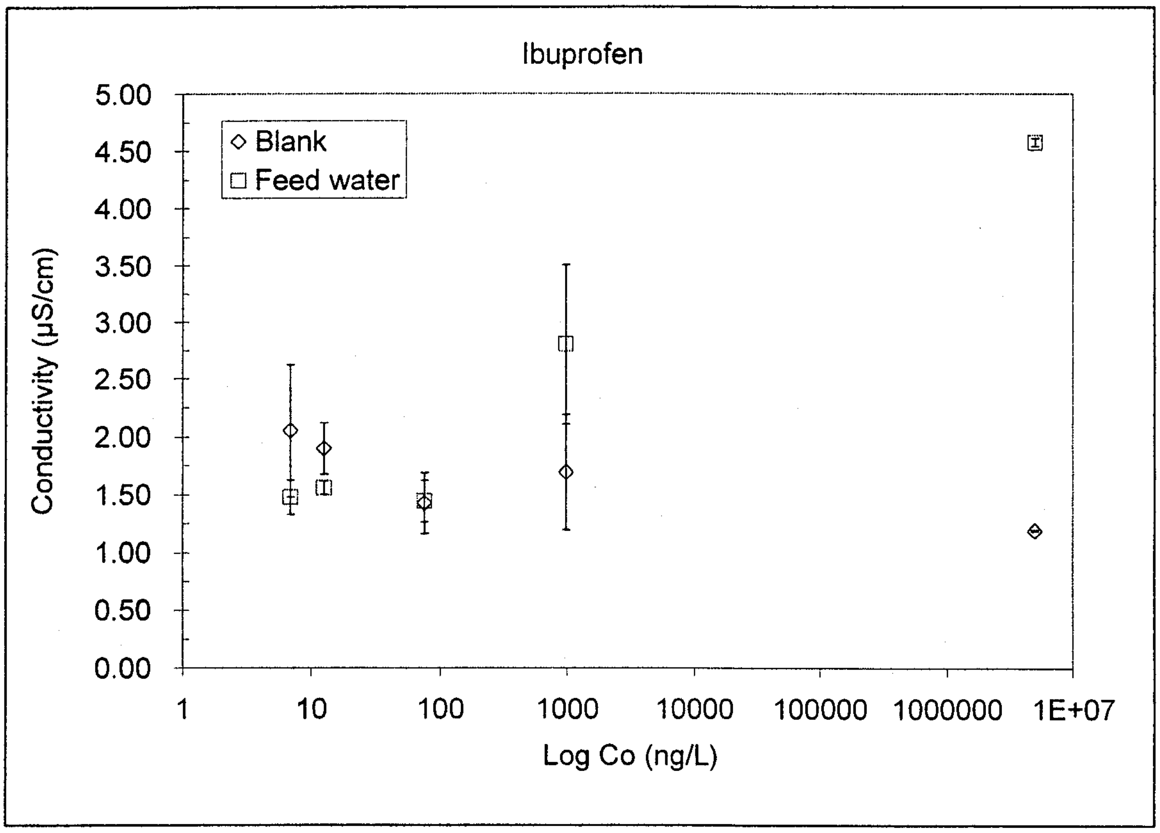


Figure 9-25 Comparison of conductivity between blank and ibuprofen feed water
Blank is distilled water used to prepare the corresponding feed water.

According to Figure 9-25, the conductivity of the distilled water used to repair the synthetic water samples varied. When the concentration of the drug was low, there was a slight decrease of the sample conductivity. The small decrease of the sample conductivity might be caused by bounding of the some impurities in the distilled water with ibuprofen molecules. With further increase of the drug concentration, the sample conductivity increased accordingly. As a result, conductivity of a sample is an important impurity concentration indictor. Consequently, the conductivity ratios with corresponding concentration ratios are also taken account of the presentation of the experimental results as follows.

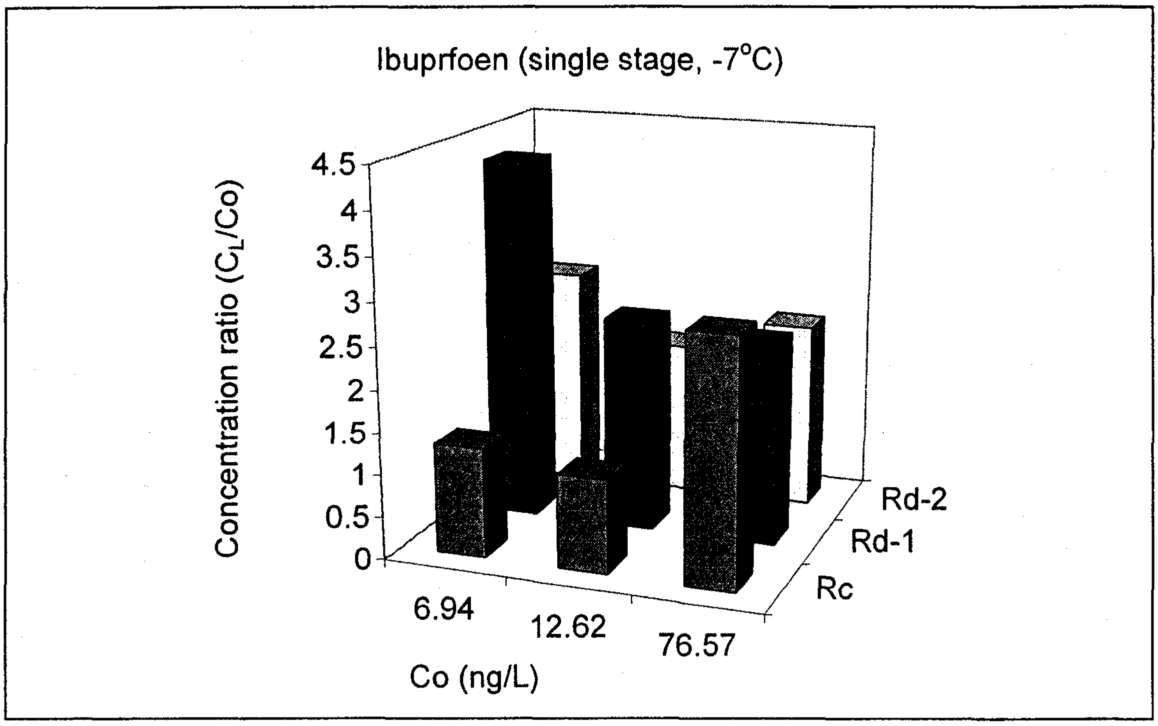


Figure 9-26 Concentration ratio and conductivity ratio against ibuprofen concentrations in feed water (-7°C).

$$\mathrm{Rc} = \text{Concentration ratio} = \frac{\text{Drug concentration in unfrozen liquid } (C_L)}{\text{Drug concentration in feed water (Co)}}$$

$$\mathrm{Rd} - 1 = \text{Conductivity ratio} - 1 = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of feed water}}$$

$$\mathrm{Rd} - 2 = \text{Conductivity ratio} - 2 = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of blank (distilled water)}}$$

Figure 9-26 and Figure 9-27 show that the ibuprofen concentration ratio in the liquid phase to the feed water at -7°C and -15°C, respectively. At the warmer temperature (-7°C), the recovery of ibuprofen from feed water with high initial concentration is greater than that with lower initial concentration in feed water. However, results from -15°C freezing experiments (Figure 9-27) present that lower recovery efficiency was obtained from the more concentrated feed water.

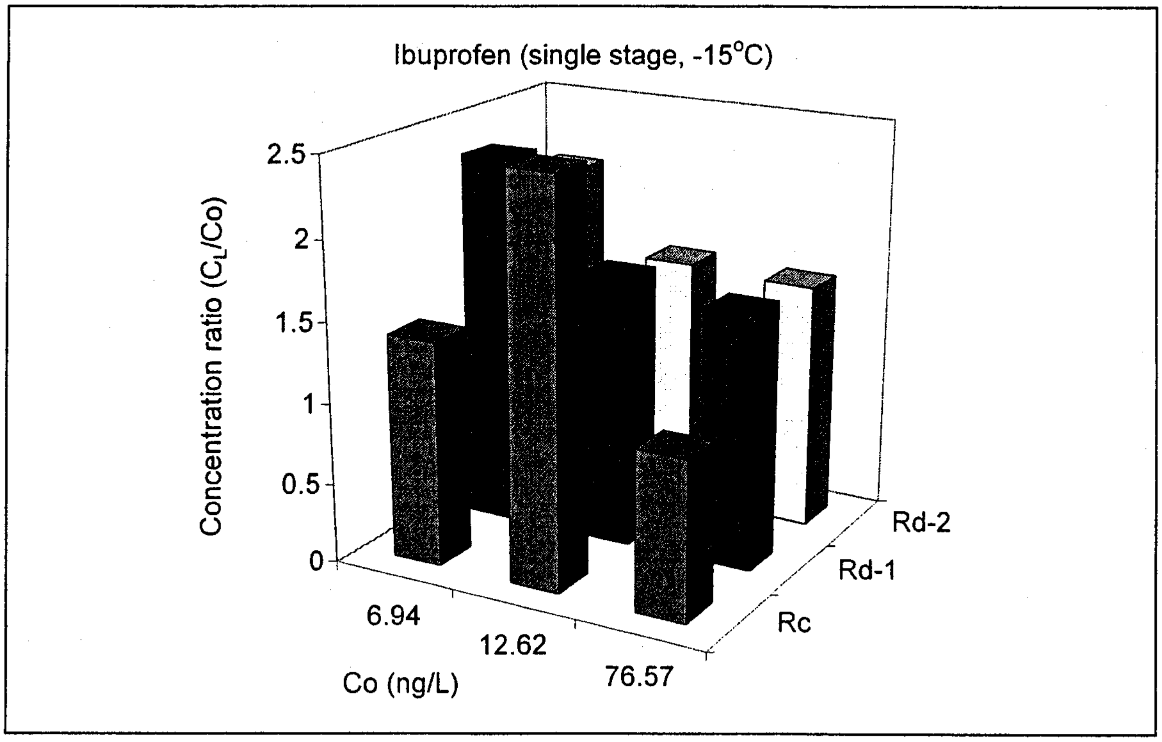


Figure 9-27 C_L/ C_O after single-stage freezing against ibuprofen concentrations in feed water (-15°C).

$$\text{Rc} = \text{Concentration ratio} = \frac{\text{Drug concentration in unfrozen liquid } (C_L)}{\text{Drug concentration in feed water (Co)}}$$

$$\text{Rd - 1} = \text{Conductivity ratio - 1} = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of feed water}}$$

$$\text{Rd - 2} = \text{Conductivity ratio - 2} = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of blank (distilled water)}}$$

Results of -15°C freezing experiments seems to agree with findings of other researches, i.e., as feed water impurity concentration increases, the separation efficiency would decrease as the chances to capture of impurities within ice structure is enhanced when there are more impurities in the solutions. The possible reasons that might cause the discrepancy at -7°C are sample preparation and analytical errors.

Additionally, Figure 9-26 and Figure 9-27 also illustrate that at low concentration solutions, the conductivity ratios are significantly higher than the concentration ratios, with an example of experiments at warmer temperature (-7°C), for the group of samples of 6.94±3.10 ng/L ibuprofen in feed water, C_L/C_O of 1st-stage unfrozen liquid to feed water is only about 1.3, but the conductivity of unfrozen liquid is around 2.2 folds of that of the feed water. The significant differences between conductivity ratios and concentration ratios thus prove that the operation errors during sample preparation processing and the low precision of GC-MS at low concentration. For feed water with initial ibuprofen high drug concentration (76.57±16.90 ng/L), the C_L/C_O value was greater than the corresponding conductivity ratio. The possible explanation is that the sample preparation errors leaded to GC-MS responded the no enough high concentrations accordingly to actual concentration in the feed water with really low initial drug concentration; but after freezing cycle, the impurity content in the unfrozen liquid was much higher, which was perhaps ranged into the good response range of the GC-MS, and the sampe preparation errors can be neglected compared to the enough high concentrations. As a result, the calculated C_L/C_O value became higher than the conductivity ratios. Contamination of the distilled water during sample preparation for the low drug concentration samples could be another reason that causes low conductivity ratios at high impurity concentration. In general, for analyzing the low concentration sample, although conductivity may not directly reflect the actual drug concentration like GC-MS, it seems more consistent in measuring the strength of the ionic species in the water samples.

Comparing the conductivity ratios between unfrozen liquid and feed water in Figure 9-26, the freeze concentration at -7°C was not affected by the initial impurity concentration in feed water. For the experiments at -15°C, the conductivity ratios between unfrozen liquid and feed water in Figure 9-27 show that the dilute solution is easily concentrated by freeze concentration as mentioned previously. The differences between experimental results at the two freezing temperatures are produced by two primary factors including freezing temperature and solvent used to prepare the corresponding water samples.

According to Rd-2, the conductivity ratios in Figure 9-26 and Figure 9-27, the experimental results are consistent with findings of other researches such as Yang et al. (1990) and Liu et al. (1999), i.e., when impurity concentration was low, most of the impurities could be separated by freeze concentration.

2) Effect of Freezing Temperature on Ibuprofen single-stage Freeze Concentration

Figure 9-28 compares the impurity concentration ratios of the water samples to the feed water and to the blanks at temperature of -7°C and -15°C freeze concentration. As shown in the figure, there were little difference in pH ratios differences between the two freezing temperatures while some differences were observed for the drug concentration and conductivity.

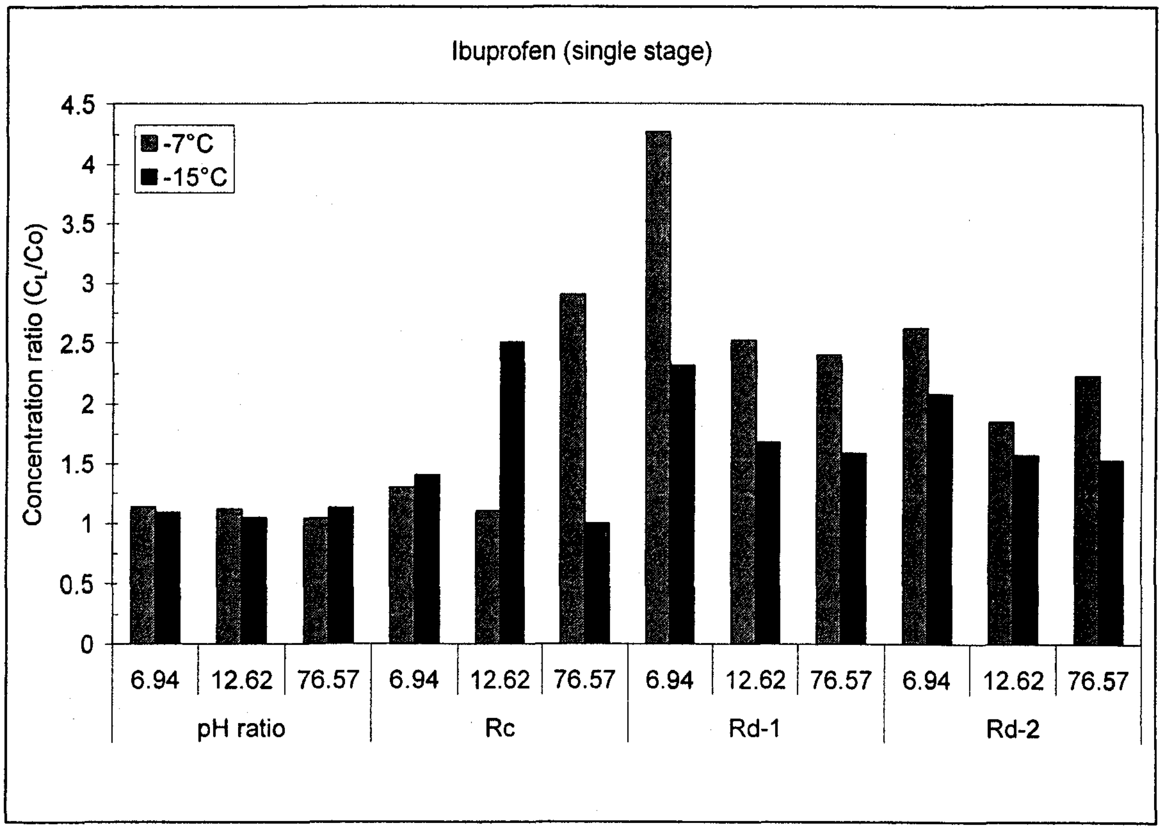


Figure 9-28 Effect of freezing temperature on single-stage freeze concentration of ibuprofen solution

$$\text{pH ratio} = \frac{\text{pH of unfrozen liquid}}{\text{pH of feed water}}$$

$$\text{Rc} = \text{Concentration ratio} = \frac{\text{Drug concentration in unfrozen liquid } (C_L)}{\text{Drug concentration in feed water (Co)}}$$

$$\text{Rd - 1} = \text{Conductivity ratio - 1} = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of feed water}}$$

$$\text{Rd - 2} = \text{Conductivity ratio - 2} = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of blank (distilled water)}}$$

As for drug concentration ratios, the results were mixed as mentioned earlier. At high initial feed water impurity concentration (~77 ng/L), freezing at the warmer temperature had higher concentration efficiency than that at -15°C. Combining concentration ratios and conductivity ratios, freeze concentration at -7°C is more effective than that at -15°C. That is because a lower freezing temperature produces a slower moving freezing front which allows impurities to be dissipated away, not trapped in the ice structure. Similar results were reported by other researchers, for example, Liu et al. (1997, 1998), Martel et al. (2002), and Gao et al. (2004), and others.

9.2.1.2 Second-Stage Freeze Concentration of Ibuprofen

1) Effect of Initial Drug Concentration in Feed Water on Impurity Removal on Second-Stage Freezing

High impurity separation efficiency can be achieved by using two stages or multi-stage freeze concentration as demonstrated in Baker (1967) and other researchers. In this experimental study, two stage freeze concentration was used for the unfrozen liquid from 1st stage freezing to find out the capacity of the freeze concentration of impurity and volume reduction.

Figure 9-29 show the results obtained the results obtained from testing at -7°C, pH changes are still small. Concentration and conductivity of unfrozen liquid to the feed water (unfrozen liquid collected from 1st-stage freeze concentration) followed the similar trends as the feed water concentration to the 1st-stage freezing. It is obviously that the lower original impurity concentration attained higher concentration factor.

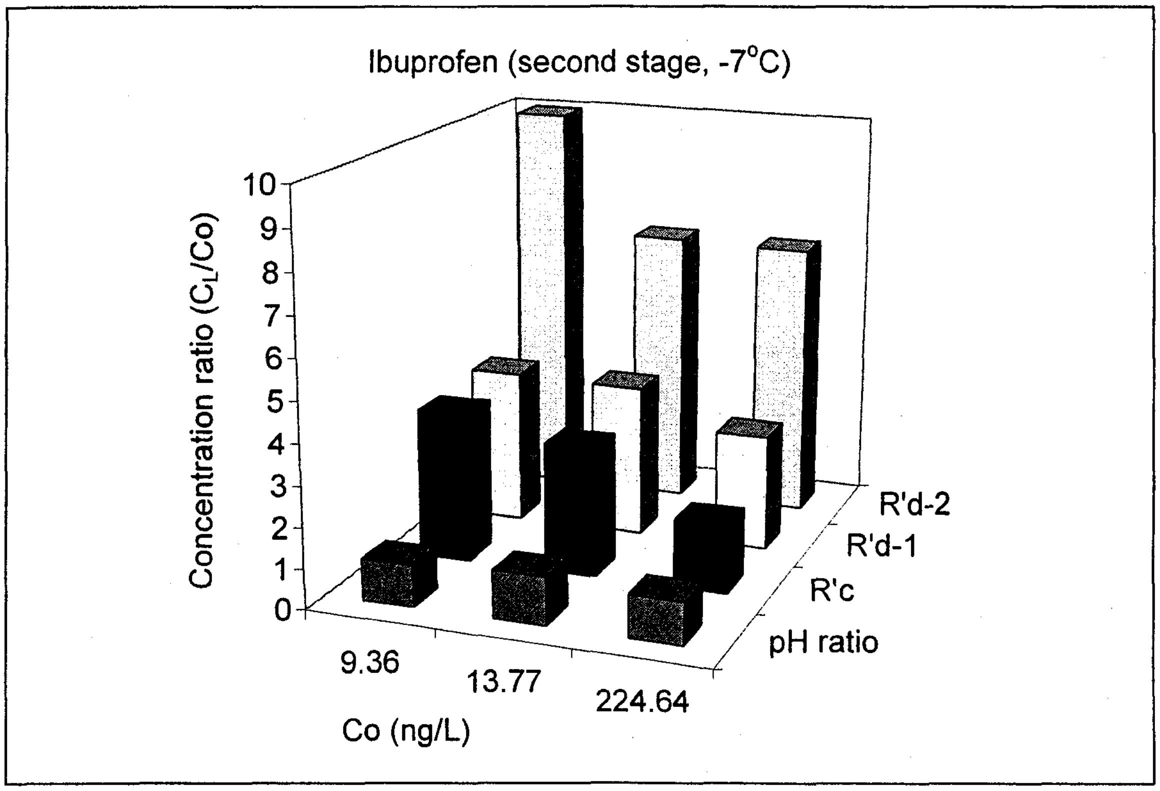


Figure 9-29 Effect of feed water concentration on ibuprofen 2nd-stage freeze concentration (-7°C).

$$\text{pH ratio} = \frac{\text{pH of unfrozen liquid}}{\text{pH of feed water to second stage freezing}}$$

$$\text{R'c} = \text{Concentration ratio} = \frac{\text{Drug concentration in unfrozen liquid } (C_L)}{\text{Drug concentration in feed water to second stage freezing (C'o)}}$$

$$\text{R'd - 1} = \text{Conductivity ratio - 1} = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of feed water to second stage freezing}}$$

$$\text{R'd - 2} = \text{Conductivity ratio - 2} = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of blank (distilled water)}}$$

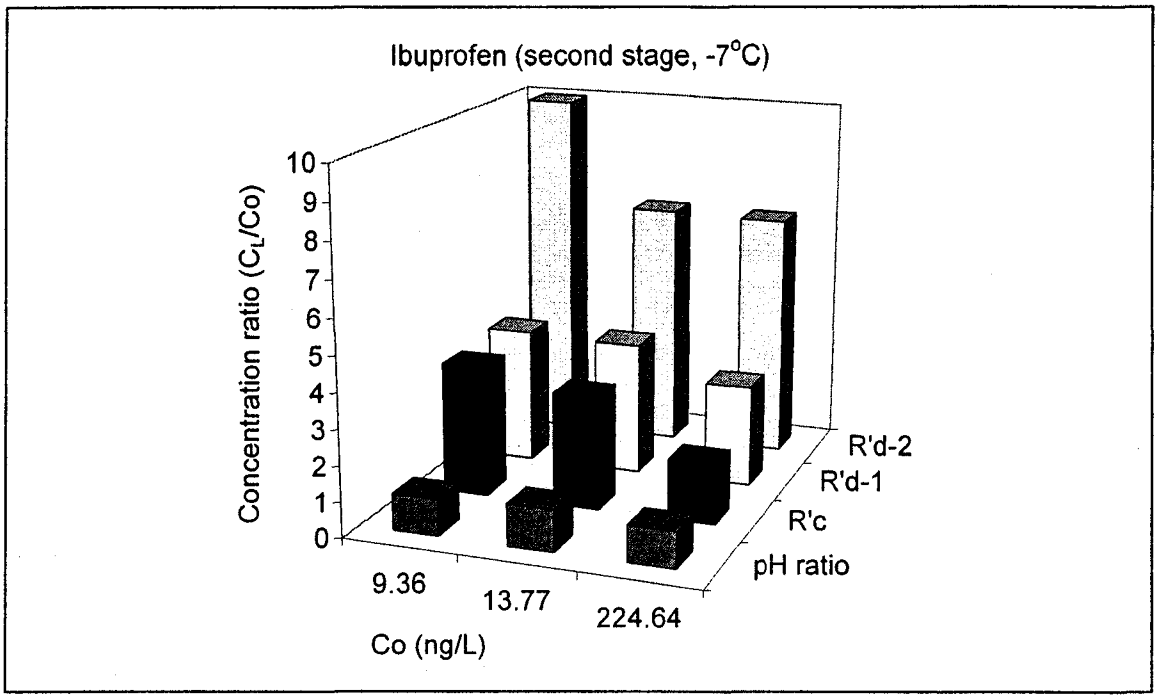


Figure 9-30 Effect of feed water concentration on ibuprofen 2nd-stage freeze concentration (-15°C).

$$\text{pH ratio} = \frac{\text{pH of unfrozen liquid}}{\text{pH of feed water to second stage freezing}}$$

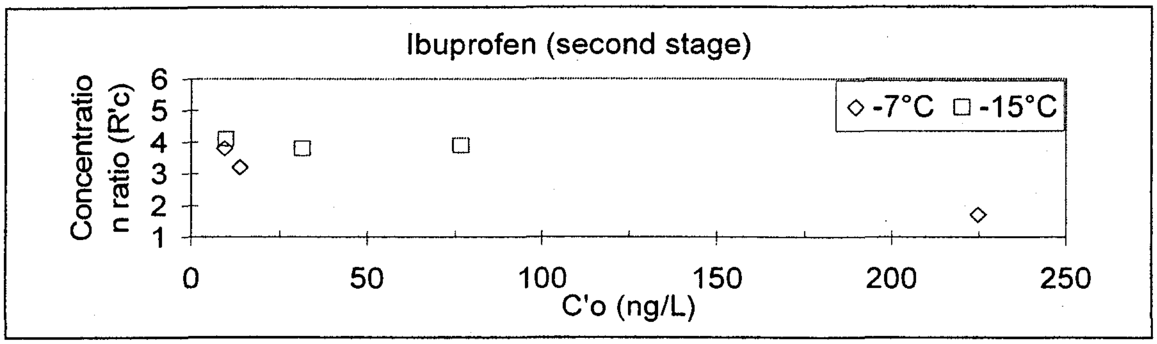
$$\text{R'c} = \text{Concentration ratio} = \frac{\text{Drug concentration in unfrozen liquid } (C_L)}{\text{Drug concentration in feed water to second stage freezing } (C'o)}$$

$$\text{R'd - 1} = \text{Conductivity ratio - 1} = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of feed water to second stage freezing}}$$

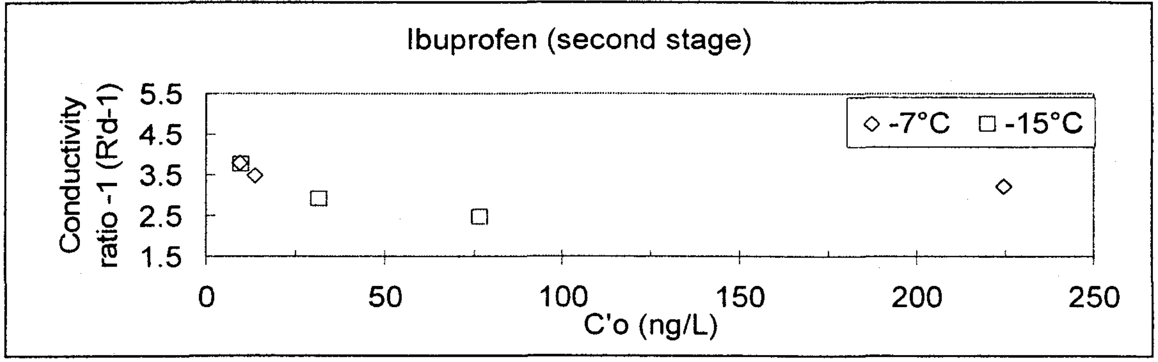
$$\text{R'd - 2} = \text{Conductivity ratio - 2} = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of blank (distilled water)}}$$

Figure 9-30 shows the results from -15°C freeze concentration tests. The results were close to those of -7°C. The trends of impurity concentration ratios also suggested that dilute solution was more effectively separated.

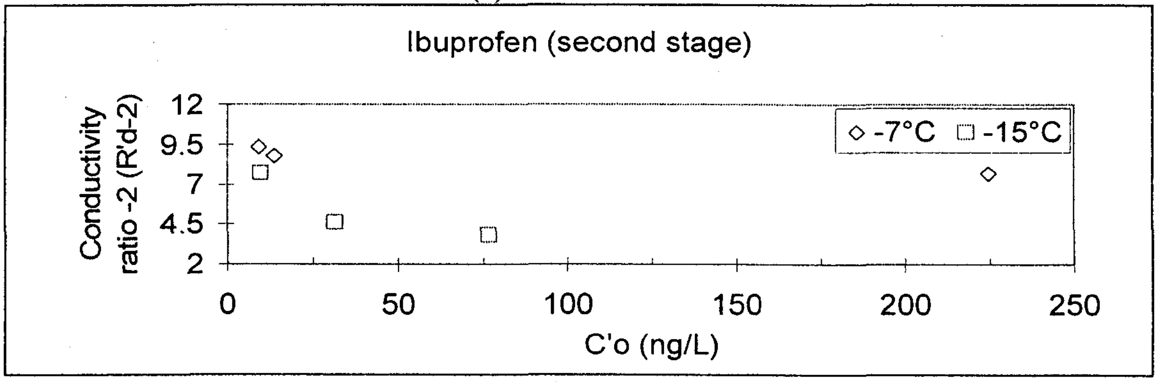
2) Freezing Temperature Effect on Second-Stage Freeze Concentration



(a) R'c vs. C'o



(b) R'd-1 vs. C'o



(c) R'd-2 vs. C'o

Figure 9-31 Effect of freezing temperature on ibuprofen two-stage freeze concentration

$$R'c = \text{Concentration ratio} = \frac{\text{Drug concentration in unfrozen liquid } (C_L)}{\text{Drug concentration in feed water to second stage freezing } (C'o)}$$

$$R'd-1 = \text{Conductivity ratio} - 1 = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of feed water to second stage freezing}}$$

$$R'd-2 = \text{Conductivity ratio} - 2 = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of blank (distilled water)}}$$

Figure 9-31 shows the comparison of experimental results at two freezing rates. Higher drug concentration ratios of unfrozen liquid obtained during the 2nd-stage freezing to the feed water of 2nd stage freezing (unfrozen liquid from 1st stage freezing) were observed for the freezing at the warmer temperature (-7°C).

9.2.2 Gemfibrozil

9.2.2.1 Effect of Initial Drug Concentration in Feed Water on Impurity Removal on Single-stage Freeze Concentration

Similar to ibuprofen, gemfibrozil has a pK_a value of 4.7 which means its chemical characteristic such as a weak acid closed to ibuprofen. Therefore, pH of gemfibrozil solution does not clearly vary with gemfibrozil concentration, even when the concentration of gemfibrozil reaches of 5 mg/L (Figure 9-32).

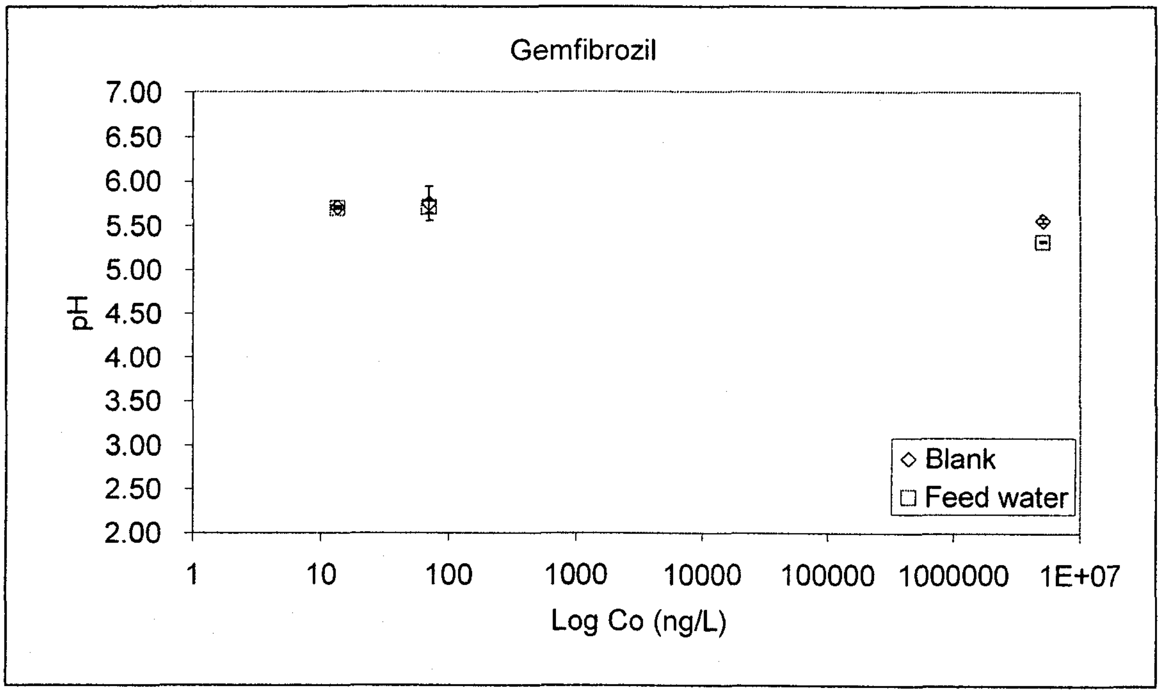


Figure 9-32 Comparison of pH between distilled water and gemfibrozil feed water Blank is distilled water used to prepare the corresponding feed water.

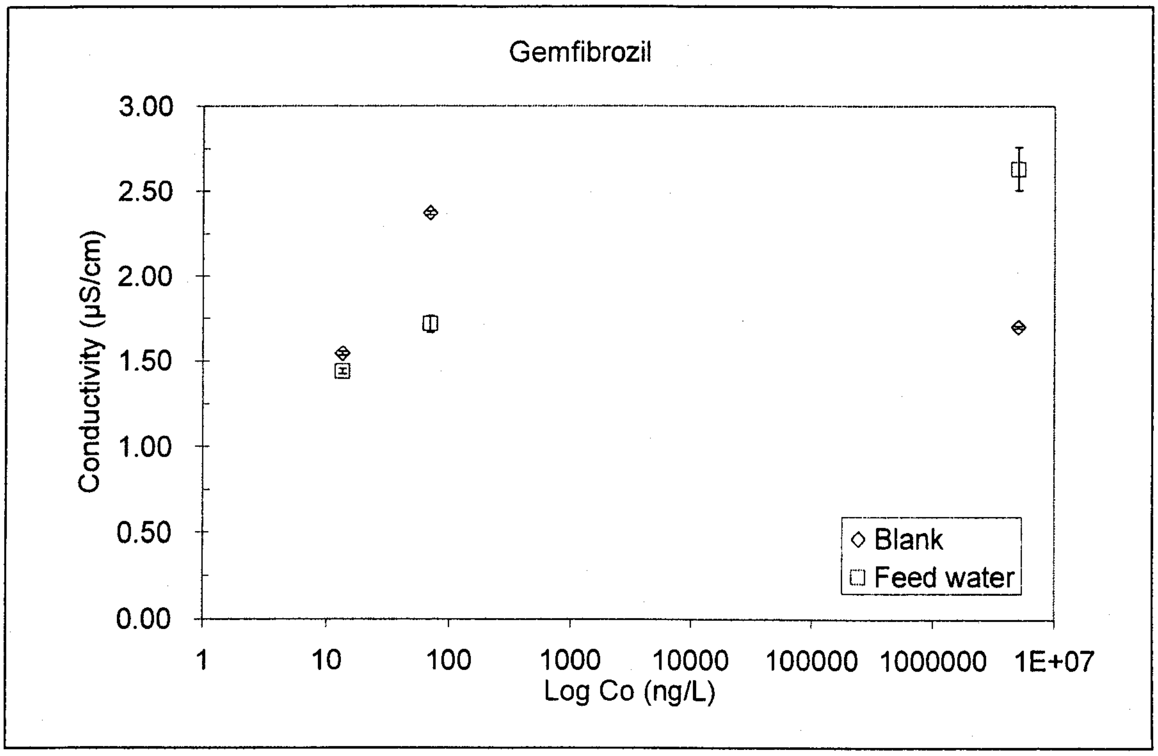


Figure 9-33 Comparison of conductivity between distilled water and gemfibrozil feed water

Blank is distilled water used to prepare the corresponding feed water.

Comparison of conductivity of blank and the gemfibrozil feed water suggests the behavior of gemfibrozil in distilled water is more like that of ibuprofen. Also, the fluctuation of conductivity of the distilled water used to prepare the synthetic water samples was observed. Again, the decrease of sample conductivity occurred after addition of the drug. The sample conductivity did increase with increase in drug concentration (Figure 9-33), The corresponding conductivity ratios of the feed water to blank samples are presented in Figure 9-34.

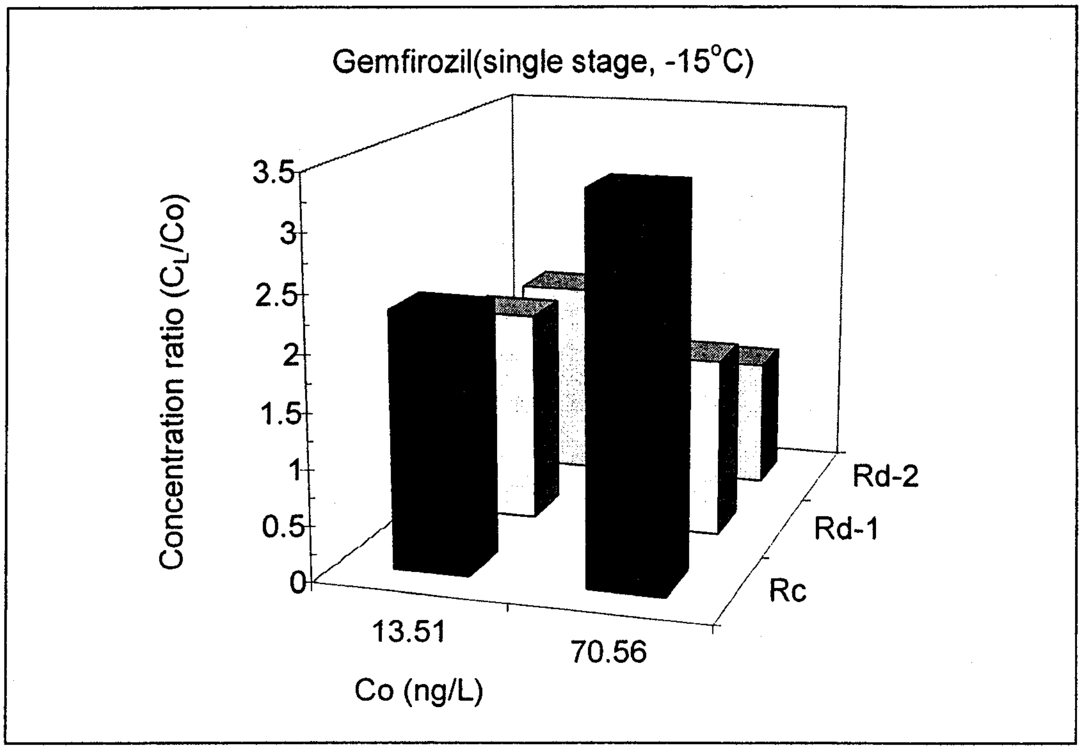


Figure 9-34 Effect of gemfibrozil concentrations in feed water on 1st stage freeze concentration (-15°C).

$$\text{Rc} = \text{Concentration ratio} = \frac{\text{Drug concentration in unfrozen liquid } (C_L)}{\text{Drug concentration in feed water (Co)}}$$

$$\text{Rd - 1} = \text{Conductivity ratio - 1} = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of feed water}}$$

$$\text{Rd - 2} = \text{Conductivity ratio - 2} = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of blank (distilled water)}}$$

According to Figure 9-34, the conductivity changes of unfrozen liquid based on distilled water indicate that the dilute solution has achieved effective concentration. However, drug concentration ratios were in contradiction with the results of conductivity ratios. Considering the operation errors in low drug concentration sample preparation processing, these errors significantly influence the concentration values of the original samples and unfrozen liquid after 1st stage freeze concentration at initial concentration of about 14 ng/L but the influences from operation errors became very low for those feed water samples of around 71 ng/L, therefore, higher drug concentration ratios were obtained.

9.2.2.2 Effect of Initial Drug Concentration in Feed Water on Impurity Removal on Second-Stage Freeze Concentration

Figure 9-35 shows the changes in concentration and conductivity of gemfibrozil solution after 2nd stage freeze concentration. The concentration ratios of the the second stage freezing was slightly lower than those of the first stage freezing. Increase in impurity concentration might be the reason.

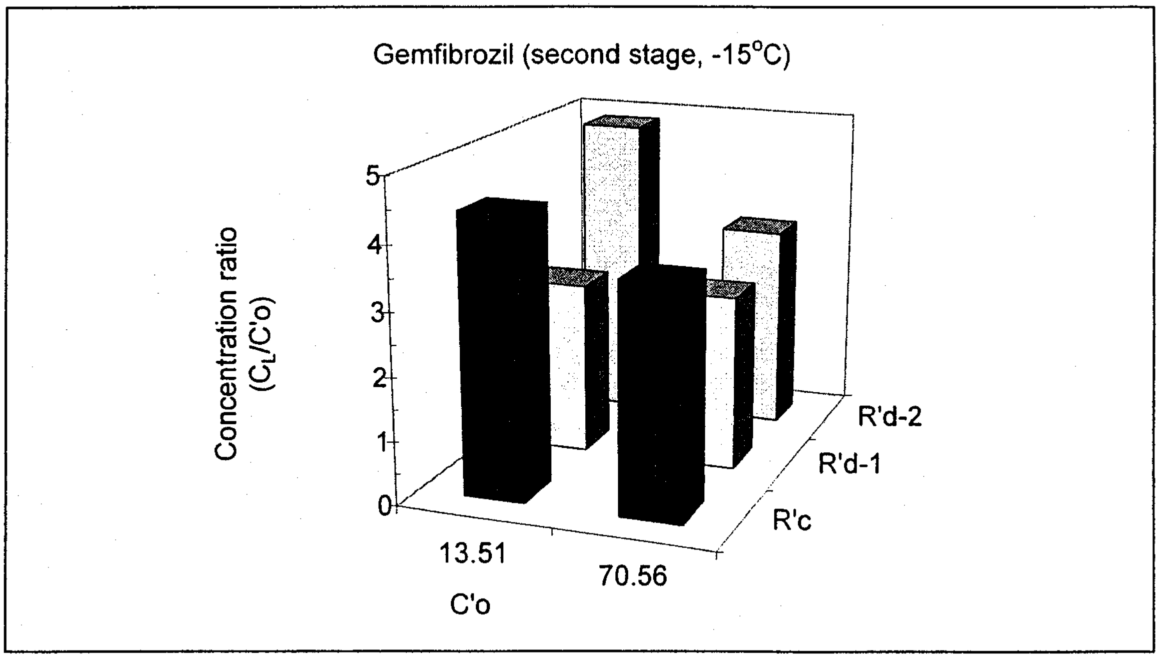


Figure 9-35 Effect of feed water concentration on gemfibrozil 2nd stage freeze concentration (-15°C).

$$R'c = \text{Concentration ratio} = \frac{\text{Drug concentration in unfrozen liquid } (C_L)}{\text{Drug concentration in feed water to second stage freezing } (C'o)}$$

$$R'd-1 = \text{Conductivity ratio}-1 = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of feed water to second stage freezing}}$$

$$R'd-2 = \text{Conductivity ratio}-2 = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of blank (distilled water)}}$$

9.2.3 Comparison of Ibuprofen and Gemfibrozil

9.2.3.1 Single Stage Freeze Concentration

Figure 9-36 shows the comparative results of the two drugs passed through single stage freezing. Generally, no obvious differences are apparent in pH ratio and conductivity ratios based feed water and blank. The concentration ratio of gemfibrozil was slightly higher than that of ibuprofen when the feed water had higher initial drug concentration.

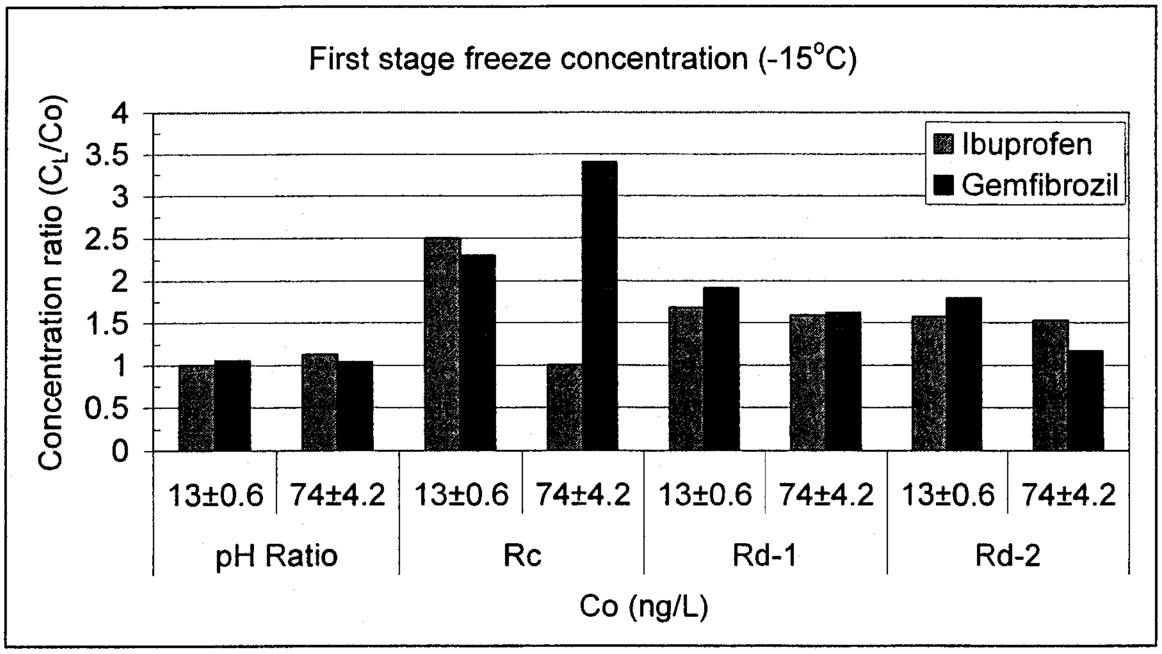


Figure 9-36 Comparison of different drugs in single-stage freezing (-15°C).

$$\text{pH ratio} = \frac{\text{pH of unfrozen liquid}}{\text{pH of feed water}}$$

$$\text{Rc} = \text{Concentration ratio} = \frac{\text{Drug concentration in unfrozen liquid } (C_L)}{\text{Drug concentration in feed water (Co)}}$$

$$\text{Rd - 1} = \text{Conductivity ratio - 1} = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of feed water}}$$

$$\text{Rd - 2} = \text{Conductivity ratio - 2} = \frac{\text{Conductivity of unfrozen liquid}}{\text{Conductivity of blank (distilled water)}}$$

9.2.3.2 Two Stages Freeze Concentration

The concentration ratios of the two drugs (for the freezing at -15°C) are plotted in Figure 9-37 for comparison.

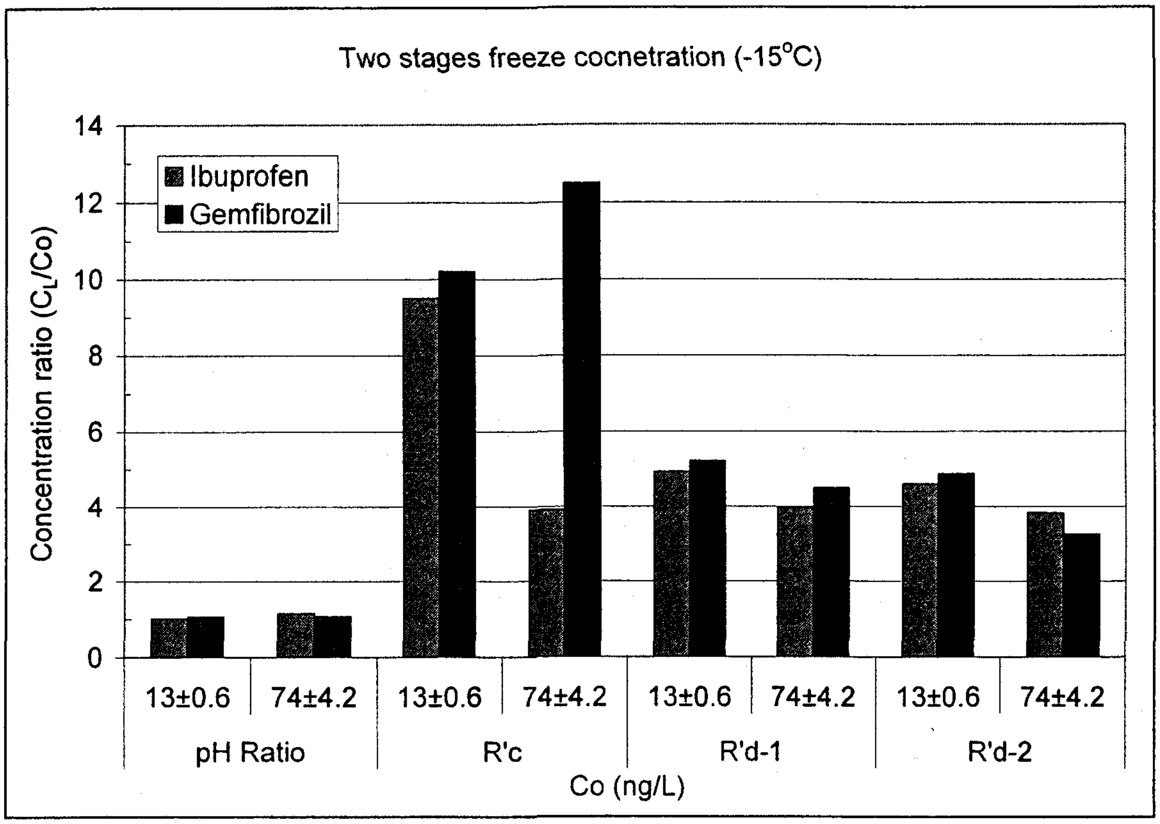


Figure 9-37 Comparison of different drugs in two stages freezing (-15°C).

$$\text{pH ratio} = \frac{\text{pH of unfrozen liquid from second stage freezing}}{\text{pH of feed water to first stage freezing}}$$

$$\text{R'c} = \text{Concentration ratio} = \frac{\text{Drug concentration in unfrozen liquid from second stage freezing } (C_L)}{\text{Drug concentration in feed water to first stage freezing (Co)}}$$

$$\text{R'd-1} = \text{Conductivity ratio-1} = \frac{\text{Conductivity of unfrozen liquid from second stage freezing}}{\text{Conductivity of feed water to first stage freezing}}$$

$$\text{R'd-2} = \text{Conductivity ratio-2} = \frac{\text{Conductivity of unfrozen liquid from second stage freezing}}{\text{Conductivity of blank (distilled water)}}$$

In general, the results are close; there is no significant difference between the removal efficiency of ibuprofen and gemfibrozil. The only discrepancy was the drug concentration ratio of gemfibrozil at initial feed water concentration of 74±4.2 ng/L. The experimental error (s) related to chemical analysis might have been the cause in addition to the difference in freeze separation/concentration efficiency.

9.2.4 Association of Drug Concentration with Total Organic Carbon (TOC)

For water/wastewater treatment plants, it is impractical, if not possible, to identify and to monitor of all of PhACs in their effluents. For organic contaminants, the effluent quality is practically measured by gloss parameters such as BOD, COD and TOC. Effluent quality control in terms of the organic contaminants is also accomplished by controlling the concentrations of TOC, BOD, or COD. Therefore, it is important to find out the association of PhACs concentrations with the gross organic parameters as no such studies have been conducted.

In this study, the TOC concentrations of five drugs (A.S.A, ibuprofen, gemfibrozil, metoprolol and sulfamethoxazole) were measured and freeze concentration efficiency of ibuprofen and gemfibrozil and mixed drugs in one stage and two stage processes was also investigated at -7 °C. Ice samples from both 1st-stage and 2nd-stage freezing were also collected in addition to the unfrozen liquid and the feed water samples (see the schematic illustration of freeze concentration tests in Figure 9-38).

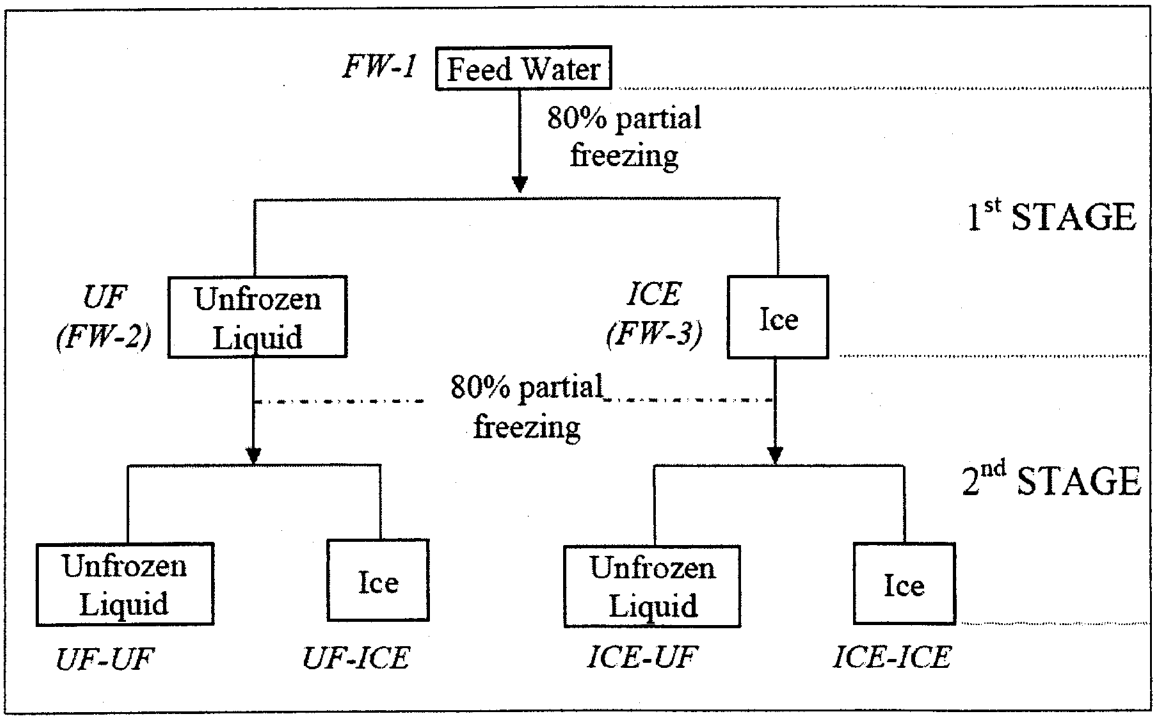


Figure 9-38 Schematic of two-step cascade freeze concentration of high concentration drug solution

FW = Feed water to first stage freeze concentration;
UF = FW-2 = Unfrozen liquid from first stage freeze concentration;
ICE = FW-3 = Ice formed in first stage freezing (melted ice is collected as feed water of melted ice refreezing);
UF-UF = Unfrozen liquid from refreezing of unfrozen liquid from first stage freeze concentration;
UF-ICE= Ice from refreezing of unfrozen liquid from first stage freeze concentration;
ICE-UF= Unfrozen liquid from refreezing of melted ice obtained in first stage freezing;
ICE-ICE=Ice from refreezing of melted ice obtained in first stage freezing.

9.2.4.1 Drug Concentration versus TOC

TOC concentration of the samples was determined by subtracting the TOC in the blank and the small volume of solvent. The plot of TOC versus selected drug concentrations in the water samples was shown in Figure 9-39.

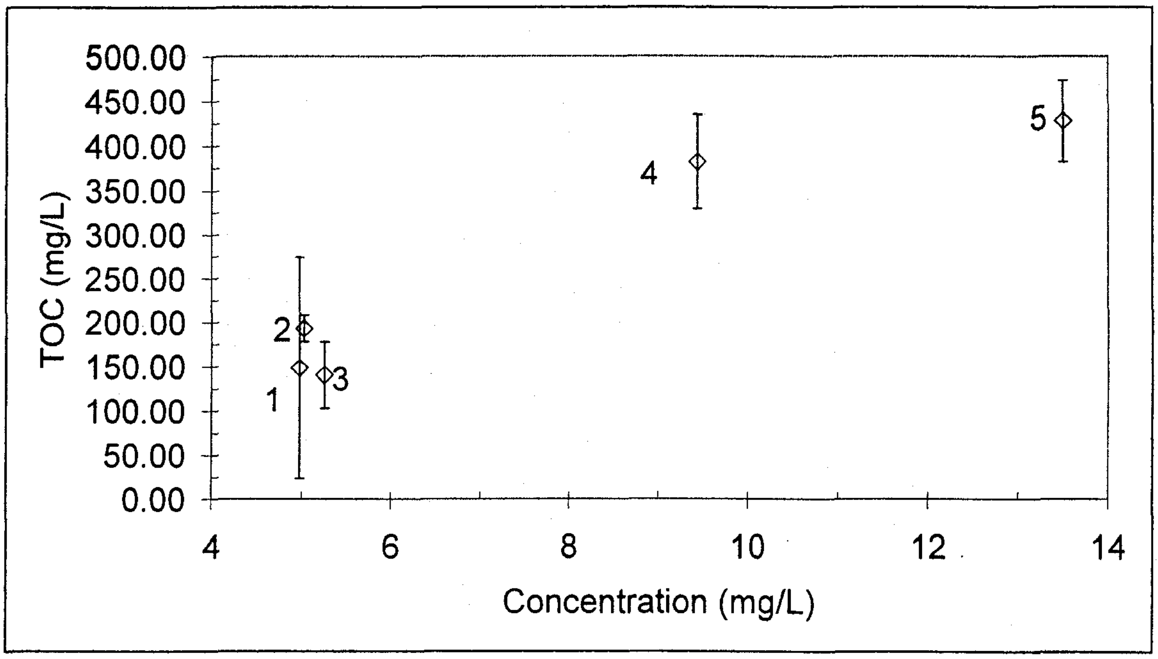


Figure 9-39 TOC vs. drug concentration
Point #1: Single drug solution (IBP of 4.98 mg/L);
Point #2: Single drug solution (GEM of 5.03 mg/L);
Point #3: Five drugs mixed solution (1.05±0.03 mg/L for each drug of A.S.A., IBP, GEM, MET and SUL)
Point #4: Five drugs mixed solution (1.47±0.03 mg/L for each acidic drug of A.S.A., IBP and GEM; 2.52±0.01 mg/L of MET and SUL, respectively)
Point #5: Five drugs mixed solution (1.49±0.03mg/L for each acidic drug of A.S.A., IBP and GEM; 4.52±0.01 mg/L of MET and SUL, respectively)

According to Figure 9-39, TOC concentration generally increased with increasing drug concentrations, and the increase trend presents like logarithmic curve. For example, when total drug concentration increased from around 5 mg/L to about 9.5 mg/L (the section from points #1,2,3 to point #4 in Figure 9-39), the corresponding TOC increased about 200 units. However, there is not significantly difference between Point#4 and 5 with total drug concentration of about 9.5 mg/L and 13.5 mg/L, respectively. Additionally, there are probably some equipment errors, especially for high drug concentration samples. Because the accurate range of TOC analyzer is limited, sometime the TOC values of high drug concentration samples maybe out off the range. In this study, TOC concentrations of the water samples were surprisingly high, and could not be explained by experimental errors. The TOC analysis was carried out on different days and blank samples were measured each time with the samples, and duplicate samples were also taken. The TOC of distilled water sample (3.24 mg/L) and distilled water plus solvent (TOC changed with the solvent volume) were reasonable and confirmed that no contamination of samples or malfunction of TOC analyzer occurred. The TOC analyzer was calibrated each time before each sample analysis. It seems that the drugs (which are not 100% pure) and the additives have high TOC concentrations. In fact, effluents from wastewater treatment plants may contain hundreds or even thousands of different drugs, it is impossible to control the concentration of every drug in wastewater during practical water industry. Based on the finds of this study, overall, the residue drugs may contribute to significantly increase of TOC concentration. TOC concentration is thus critical for drug residue control.

9.2.4.2 TOC of Frozen Ibuprofen, Gemfibrozil and Mixed Drugs

Different concentration ratios of unfrozen liquid or ice from different freezing cycle to the corresponding feed water are shown in Figure 9-40. For example, the first field in Figure 9-40 is the C/Co ratios of unfrozen liquid and ice to the feed water. With freezing about 84% (volume) of the feed water whose initial ibuprofen concentration was 4.98 mg/L, the ice obtained from the first freezing contained only *ca.* 21% of the drug concentration in the feed water and the unfrozen liquid (UF) drug concentration was 1.64±0.42 times that of in the feed water. Refreezing of the unfrozen liquid and the ice obtained from the 1st stage freeze concentration helped of to achieve further concentration of the drug or purification of the ice was achieved. During the second stage freezing, 77±1% volume of the feed water (i.e., the unfrozen liquid from the first stage freezing) was frozen. As indicated in the Figure 9-40, the concentration ratios for the unfrozen liquid and ice obtained from the second stage freezing were slightly lower compared to those obtained from the first stage freezing. UF-UF and UF-ICE were 1.08±0.01 and 0.54±0.06, respectively. Freezing of the melted ice obtained from the first stage, resulted in higher impurity concentration ratios, the liquid (ICE-UF) contained 3.90±0.09 times of

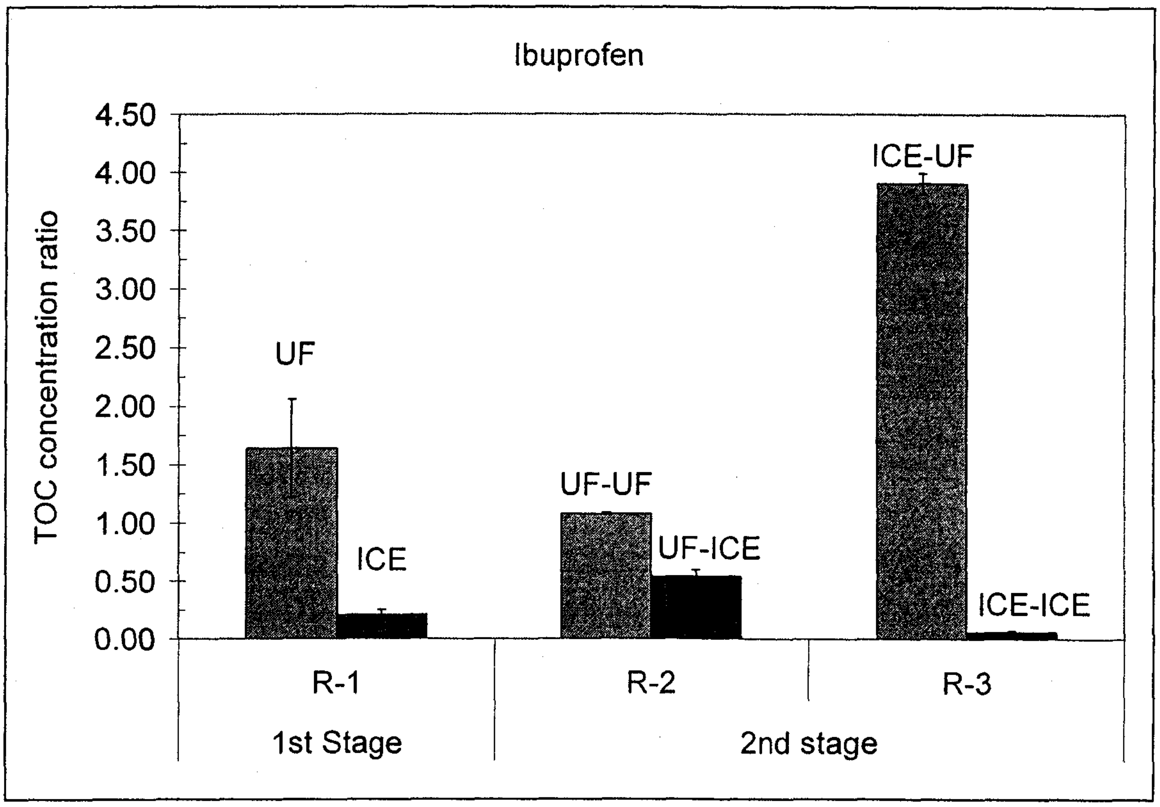


Figure 9-40 Freeze concentration results of 4.98 mg/L ibuprofen single solution

$$R-1 = \text{TOC concentration ratio}-1 = \frac{TOC_{UF} \text{ or } TOC_{ICE}}{TOC_{FW-1}} \quad (TOC_{FW-1} = 4.98 \text{ mg/L})$$

$$R-2 = \text{TOC concentration ratio}-2 = \frac{TOC_{UF-UF} \text{ or } TOC_{UF-ICE}}{TOC_{FW-2}} \quad (TOC_{FW-2} = TOC_{UF})$$

$$R-3 = \text{TOC concentration ratio}-3 = \frac{TOC_{ICE-UF} \text{ or } TOC_{ICE-ICE}}{TOC_{FW-3}} \quad (TOC_{FW-3} = TOC_{ICE})$$

drug concentration and the ice (ICE-ICE) only 6±1% of compared to the feed water (i.e. melted ice). Overall, in terms of mass of TOC causing materials, the removal efficiency was around 84% for the *ca.* 80% reduction of the total feed water after one stage freeze concentration.

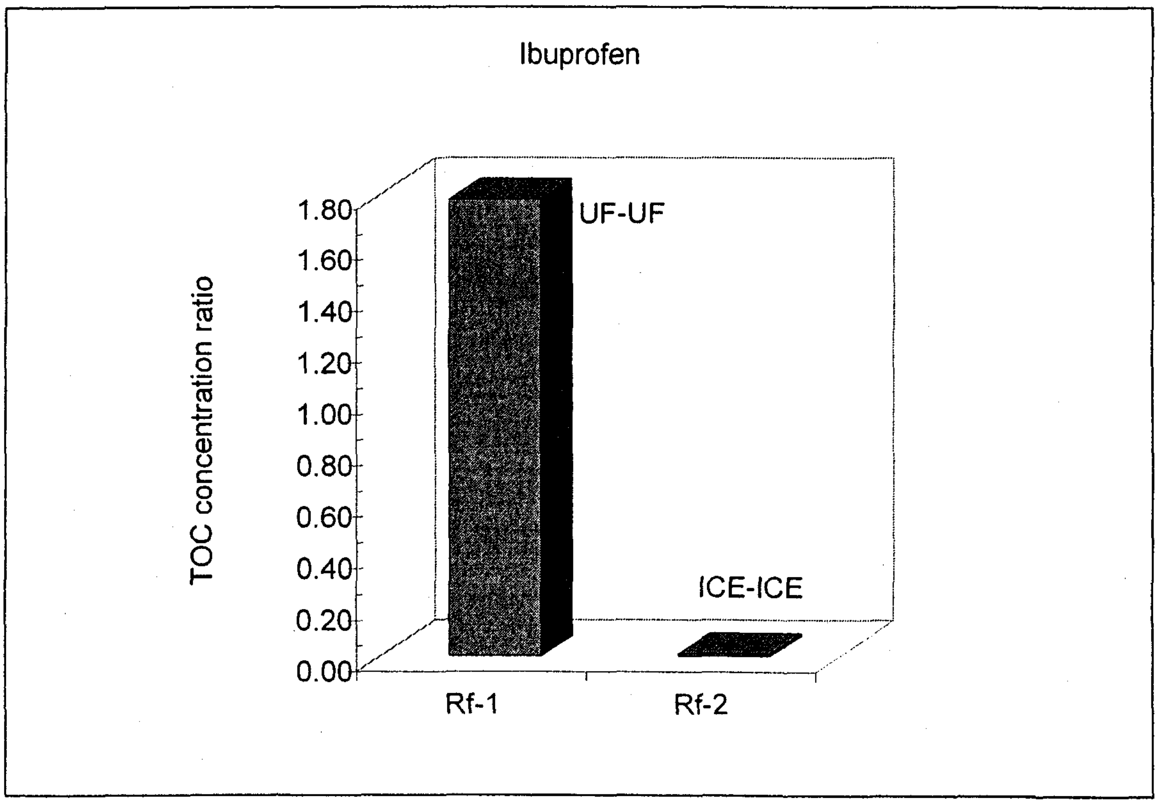


Figure 9-41 Final products of two stages freeze concentration of 4.98 mg/L ibuprofen single solution

$TOC_{FW\text{-}I} = 4.98$ mg/L

$$Rf-1 = \text{final product TOC concentration} - 1 = \frac{TOC_{UF\text{-}UF}}{TOC_{FW\text{-}I}}$$

$$Rf-2 = \text{final product TOC concentration} - 2 = \frac{TOC_{ICE\text{-}ICE}}{TOC_{FW\text{-}I}}$$

Figure 9-41 presents two final products during two stages freeze concentration of about 5mg/L ibuprofen single solution. After the second stage freezing, around 64% of the total feed water (volume) reached close to 99% reduction of TOC concentration and approximately 99% for *ca* 64% of the total feed water after two stages freeze concentration.

Table 9-3 Concentration ratios of ibuprofen at each freezing stage

Freezing stage [1]	Components [2]	TOC ratio [3]	Volume Reduction [4]	Removal efficiency [5]
1st stage	$\frac{UF}{FW-1}$	1.59	84%	84.1%
	$\frac{ICE}{FW-1}$	0.21		
2nd stage	$\frac{UF-UF}{FW-2}$	1.08	77%	61.6%
	$\frac{UF-ICE}{FW-2}$	0.54		
	$\frac{UF-UF}{FW-1}$	1.73	13%	90.2%
	$\frac{UF-ICE}{FW-1}$	0.86		
	$\frac{UF-UF}{FW-2}$	3.90	84%	95.5%
	$\frac{UF-ICE}{FW-2}$	0.06		
	$\frac{UF-UF}{FW-1}$	0.81	65%	99.2%
	$\frac{UF-ICE}{FW-1}$	0.01		

The detailed removal efficiency for each stage is listed in Table 9-3. Column 1 figures out which freezing stage the water samples are collected from. The second column explains the comparative objectives which the corresponding TOC concentration ratios are presented in Column 3. Additionally, volume reduction and removal efficiency are listed the last two columns, respectively.

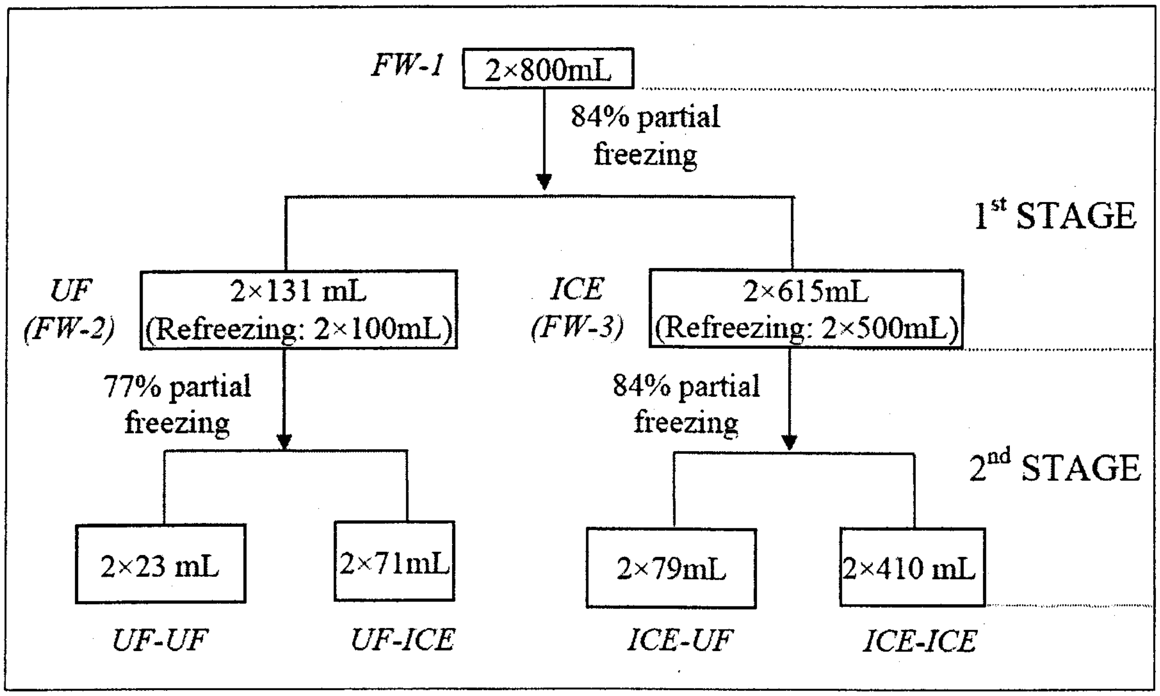


Figure 9-42 Actual volume changes during ibuprofen freeze concentration experiment

FW = Feed water to first stage freeze concentration;
UF = FW-2 = Unfrozen liquid from first stage freeze concentration;
ICE = FW-3 = Ice formed in first stage freezing (melted ice is collected as feed water of melted ice refreezing);
UF-UF = Unfrozen liquid from refreezing of unfrozen liquid from first stage freeze concentration;
UF-ICE= Ice from refreezing of unfrozen liquid from first stage freeze concentration;
ICE-UF= Unfrozen liquid from refreezing of melted ice obtained in first stage freezing;
ICE-ICE=Ice from refreezing of melted ice obtained in first stage freezing.

Figure 9-42 illustrates the actual volumes of various samples obtained or used for the first and the second stage freezing of ibuprofen, which have been mentioned many times in previously sections. From this figure, 80±5% freezing degree is kept for each freezing stage. Reference to Table 9-3, after two stages freeze concentration, about 960 mL high quantity melted ice (UF-ICE and ICE-ICE) may be obtained from 1600 mL feed water contained, which means at least around 60% wastewater reuse. If remaining high drug concentration liquids are further treated, much water will be recovered.

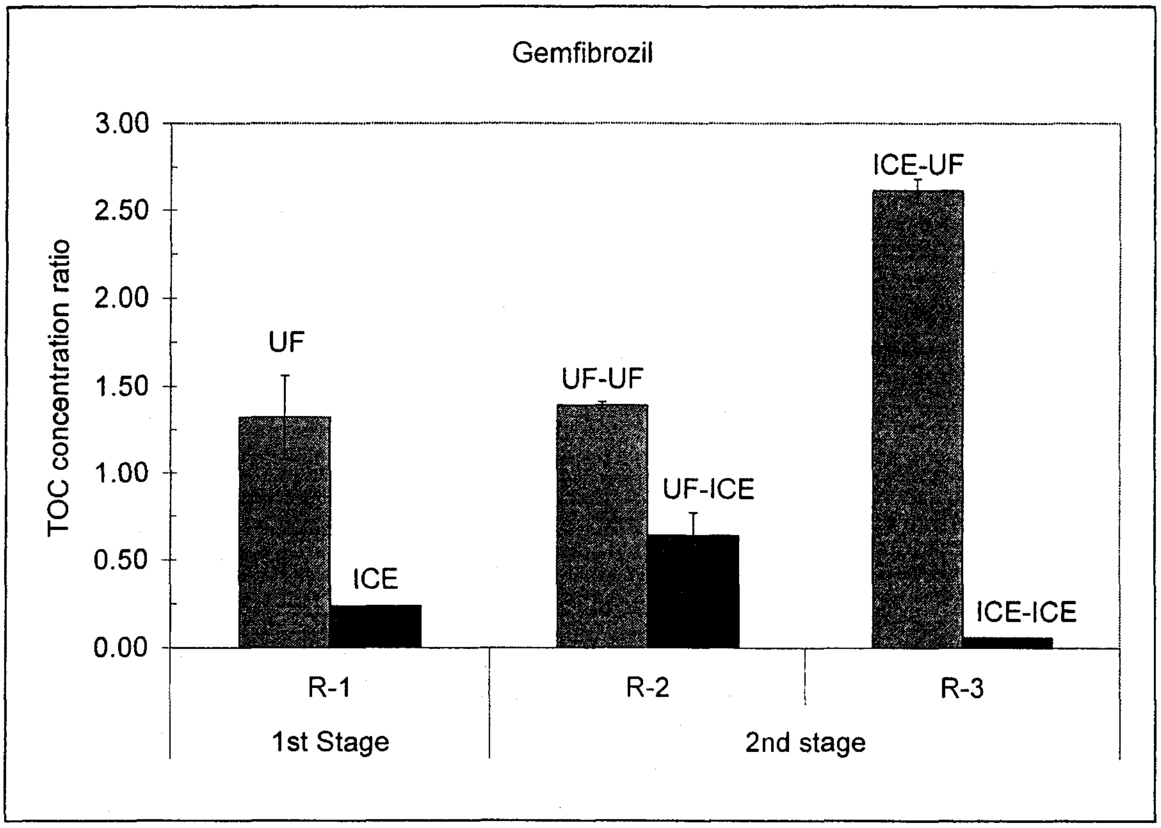


Figure 9-43 Freeze concentration results of 5.03 mg/L gemfibrozil single solution

$$R-1 = \text{TOC concentration ratio} - 1 = \frac{TOC_{UF} \text{ or } TOC_{ICE}}{TOC_{FW-1}} \; (TOC_{FW-1} = 5.03 \text{ mg/L})$$

$$R-2 = \text{TOC concentration ratio} - 2 = \frac{TOC_{UF-UF} \text{ or } TOC_{UF-ICE}}{TOC_{FW-2}} \; (TOC_{FW-2} = TOC_{UF})$$

$$R-3 = \text{TOC concentration ratio} - 3 = \frac{TOC_{ICE-UF} \text{ or } TOC_{ICE-ICE}}{TOC_{FW-3}} \; (TOC_{FW-3} = TOC_{ICE})$$

Results of freezing of gemfibrozil were similar to those of ibuprofen, as shown in Figure 9-43. A TOC concentration ratio of 1.3 was obtained for the unfrozen liquid after freezing about 78 % of the total volume of the feed water which had a 5.03 mg/L of gemfibrozil, and the concentration ratio for the ice of the first stage freezing was 0.12, i.e., the ice contained 12% of the TOC as compared to the feed water. The ice made from the gemfibrozil solution was purer than that of the ibuprofen. Refreezing of the melted ice formed in the first stage freezing further reduced gemfibrozil TOC concentration in the ice, i.e., the final product which contained only 1% of the initial TOC concentration in the feed water (Figure 9-44).

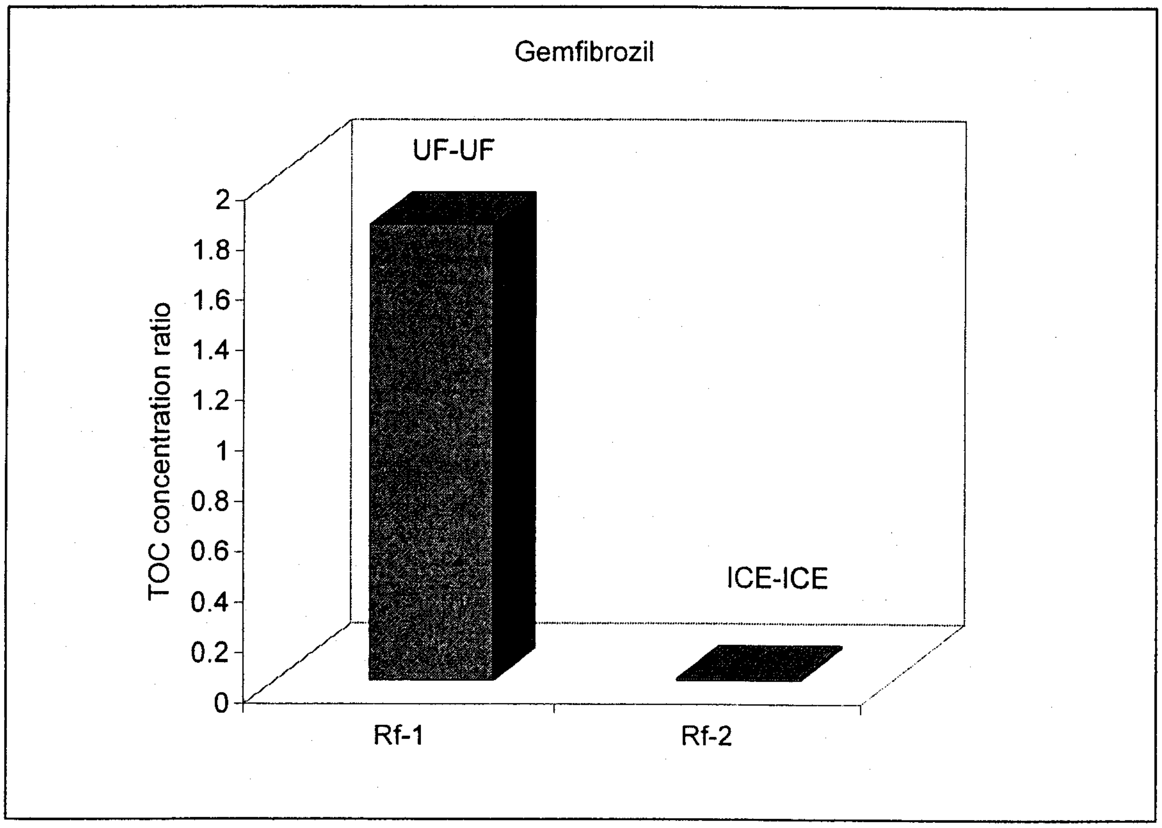


Figure 9-44 Final products of two stage freeze concentration of 5.03 mg/L gemfibrozil single solution

$TOC_{FW\text{-}I} = 5.03$ mg/L

$$Rf\text{ - }1 = \text{final product TOC concentration - }1 = \frac{TOC_{UF\text{-}UF}}{TOC_{FW\text{-}I}}$$

$$Rf\text{ - }2 = \text{final product TOC concentration - }2 = \frac{TOC_{ICE\text{-}ICE}}{TOC_{FW\text{-}I}}$$

Table 9-4 Removal efficiency of freeze concentration to gemfibrozil at each freezing stage

Freezing stage [1]	Components [2]	TOC ratio [3]	Volume Reduction [4]	Removal efficiency [5]
1st stage	$\frac{\text{UF}}{\text{FW-1}}$	1.32	78%	91.9%
	$\frac{\text{ICE}}{\text{FW-1}}$	0.12		
2nd stage	$\frac{\text{UF-UF}}{\text{FW-2}}$	1.39	80%	53.0%
	$\frac{\text{UF-ICE}}{\text{FW-2}}$	0.63		
	$\frac{\text{UF-UF}}{\text{FW-1}}$	1.73	18%	90.2%
	$\frac{\text{UF-ICE}}{\text{FW-1}}$	0.86		
	$\frac{\text{UF-UF}}{\text{FW-2}}$	3.90	80%	95.4%
	$\frac{\text{UF-ICE}}{\text{FW-2}}$	0.06		
	$\frac{\text{UF-UF}}{\text{FW-1}}$	0.81	53%	99.5%
	$\frac{\text{UF-ICE}}{\text{FW-1}}$	0.01		

Table 9-4 listed concentration ratios of all gemfibrozil samples and corresponding drug removal efficiency to each stage freeze concentration. Column 1 figures out which freezing stage the water samples are collected from. The second column explains the comparative objectives which the corresponding TOC concentration ratios are presented in Column 3. Additionally, volume reduction and removal efficiency are listed the last two columns, respectively.

Chapter 10 SUMMARY AND CONCLUSIONS

This study was carried out to get a better understanding of the occurrence of pharmaceutically active compounds (PhACs) in the aquatic environment and water systems, and to evaluate the potential of freeze concentration technique for the removal of PhACs from water systems. The following is a summary and the conclusions drawn from the study:

- Occurrence of residual drugs in municipal wastewater treatment plant effluent (WWTP) and the aquatic environment is the reality; various drugs were detected in municipal wastewater treatment plant effluents, surface, ground water, and in some cases, treated drinking water in difference countries. According to the published literatures the concentration of the drug residues in WWTP effluents and the environment are expected to increase with increased consumption of the drugs, if effective treatment processes are not employed.
- The analytical methodology for the determination of drug residues in complex environmental matrices is still evolving and it may takes years before the universally accepted methods are developed. The GC-MS method used by many researchers requires complex sample preparation, which could influence the accuracy of the analysis.
- Freezing of ibuprofen and gemfibrozil in one stage freeze concentration, about 84% and 92% removal efficiency is for TOC, about 81% and 83% removal efficiency for the two drugs based on the GC-MS analysis. High TOC

concentrations were observed in the water samples prepared with individual or mixed drugs. The removal efficiency of TOC for ibuprofen was around 84% in the first stage freezing and approximately 99% in the second stage freezing, for gemfibrozil circa 92% and 99.5%, respectively.

In general, freeze concentration was effective and not sensitive to the nature of the impurities in the feed water.

Recommendations for Future Research

- Future research is needed to investigate the removal efficiency of PhACs in municipal wastewater treatment plant effluents by the progressive freeze concentration process.
- More types of drugs should be selected to observe their behaviors during the freeze concentration
- Investigations of large scale experiments should be carried out to evaluate the potential of the progressive freeze concentration processes.
- Analytical method researches must be kept to do till simple and high effective standard method(s) occurrence.

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