Comparative Study of Wastewater Disinfection By-product Formation during Wastewater Disinfection by Peracetic Acid versus Sodium Hypochlorite

by

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ABSTRACT

This study comparatively investigated disinfection by-products (DBPs) formed from wastewater disinfection with peracetic acid (PAA) versus sodium hypochlorite (NaClO). Gas chromatography and mass spectrometry (GC/MS) was used for DBPs analysis. It was found that the changing characteristics of wastewater influenced the detection and reproducibility of DBPs formed, resulting in no identification of any of the DBPs detected.

DBPs formed from oxidation of the 17β-estradiol (E2), clofibric acid, and ketoprofen by PAA and NaClO were also studied. Both PAA and NaClO reduced E2: 13.5% reduction by PAA and two DBPs were detected: 2- or 4-CH_3O-E2 and one unknown compound; 92.3% reduction by NaClO and three DBPs were detected: 2- or 4-chloro-E2, 2- or 4-chloro-E1, and one unknown compound. 26.5% reduction of clofibric acid was found by both PAA and NaClO oxidation, and no DBPs were detected. Neither reduction nor DBPs were found by both PAA and NaClO oxidation of ketoprofen.

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LIST OF ACRONYMS AND NOMENCLATURE

AA	Acetic Acid
AOPs	Advanced Oxidation Processes
AOX	Absorbable Halogenated Organics
APCI	Atmospheric Pressure Chemical Ionization
API	Atmospheric Pressure Ionization
APPI	Atmospheric Pressure Photoionization Ionization
BOD_5	Biochemical Oxygen Demand
CA	Clofibric Acid
CI	Chemical Ionization
COD	Chemical Oxygen Demand
CSOs	Combined Sewer Overflows
DBPs	Disinfection By-products
DOC	Dissolved Organic Carbon
DW	Distilled Water
E1	Estrone
E2	17β-estradiol
E3	Estriol
EC	Enterococci
ECD	Electron Capture Detector
EDCs	Endocrine Disrupting Compounds

EI	Electron Ionization
ESI	Electrospray Ionization
FC	Fecal Coliform
FID	Flamed Ion Detector
FLD	Fluorescence Detector
GC/MS	Gas Chromatography/Mass Spectrometry
HAAs	Haloacetic Acids
HANs	Haloacetonitriles
HKs	Haloketones
HPLC	High Performance Liquid Chromatography
ICP	Inductively Coupled Plasma
K	Ketoprofen
LLE	Liquid-Liquid Extraction
MAC	Maximum Allowable Concentration
MDL	Method Detection Limit
MIP	Molecularly Imprinted Polymer
NDMA	N-nitrosodimethylamine
NH_3-N	Ammonia Nitrogen
NPOC	Non-purgeable Organic Carbon
NSAIDs	Non-steroidal Anti-inflammatory Drugs
PAA	Peracetic Acid
RID	Refractive Index Detector
SE	Secondary Effluents

SIM	Selected Ion Monitoring
SPE	Solid-phase Extraction
SPME	Solid-phase Micro-extraction
SS	Suspended Solids
SUVA	Specific UV_{254} Absorbance
TC	Total coliforms
THMs	Trihalomethanes
TKN-N	Total Kjeldahl Nitrogen
TOC	Total Organic Carbon
TOX	Total Halogenated Organics
U.S.EPA	United States Environmental Protection Agency
UV	Ultraviolet
UV-VIS	Ultraviolet/Visible
WW	Wastewater
WWTP	Wastewater Treatment Plant

Chapter 1

INTRODUCTION

The purpose of this section is to give a general background on wastewater disinfection and issues related to disinfection efficiency, including sodium hypochlorite (NaClO) disinfection, peracetic acid (PAA) disinfection, and endocrine disrupting compounds (EDCs)/ pharmaceuticals in wastewater.

1.1 Wastewater Chlorination

With the rise in population and the greater demand for water resources, human exposure to wastewater discharged into the environment has increased. In order to prevent the transmission of infectious diseases from pathogens (bacteria, viruses, protozoan cysts, and helminthes), and to ensure water is safe for human contact and the environment, disinfection and the corresponding choice of disinfectant have become extremely important in wastewater treatment processes.

At the present time, wastewater effluents are mainly disinfected by chlorine and chlorine-based compounds such as sodium hypochlorite, calcium hypochlorite, and chlorine dioxide. Among these compounds, sodium hypochlorite is commonly applied. Sodium hypochlorite (NaClO), known as bleach, is only available as a solution. It is formed according to the following reaction:

$$Cl_2 + 2\,NaOH \longrightarrow NaCl + NaClO + H_2O \qquad \text{(Reaction1.1)}$$

When sodium hypochlorite is added to water, two reactions take place: hydrolysis and ionization. Hydrolysis is the formation of hypochlorous acid (HClO) as follows:

$$NaClO + H_2O \longrightarrow HClO + NaOH \qquad \text{(Reaction 1.2)}$$

The ionization of hypochlorous acid to hypochlorite ion (OCl^-) can be defined as:

$$HClO \longleftrightarrow H^+ + OCl^- \qquad \text{(Reaction 1.3)}$$

If bromide ions are present, chlorine will also oxidize bromide to form hypobromous acid:

$$HClO + Br^- \longleftrightarrow HBrO + Cl^- \qquad \text{(Reaction 1.4)}$$

Hypobromous acid is an effective biocide and [HBrO]/ [BrO^-] are better oxidants than [HClO]/ [ClO^-] [1]. Since the effluents from most wastewater treatment plants contains significant amounts of nitrogen, usually in the form of ammonia, the active oxidizing agent HClO will react readily with ammonia to form three types of chloramines: monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and nitrogen trichloride (NCl_3). These reactions depend on temperature, contact time, pH, and the ratio of chlorine to ammonia.

Sodium hypochlorite kills pathogens such as bacteria and viruses by breaking the chemical bonds in their molecules as the same as chlorine [2]. The germicidal efficiency of chlorine disinfection depends primarily on the chlorine dosage C_R and contact time t. The required degree of disinfection can be achieved by varying the dose and the contact time for any chlorine disinfection system. The effect of disinfection is determined by pH in water, which has an optimal range in wastewater disinfection between 5.5 and 7.5 [2].

Chlorine has been commonly used for wastewater disinfection because of its low cost, effectiveness against many enteric bacteria, and the great amount of existing knowledge regarding chlorination. However, the use of chlorine has become less popular in recent years due to the harmfulness of DBPs and the toxicity of residual chlorine formed in the disinfection process [1-4]. It has been eliminated in many countries and even banned by the Canadian province of Quebec. It is therefore necessary to search for disinfectant alternatives to chlorine that are effective against microbial contamination of wastewater, and concurrently reduce DBPs.

Ultraviolet (UV) irradiation is one choice due to its effectiveness against chlorine resistant pathogens (*Cryptosporidium parvum* and *Giardia lamblia*) and no formation of DBPs; ozone is also considered as an alternative to chlorine because it is an extremely active oxidant and its biocidal properties are not affected by ammonium ion or pH [2]. However, with respect to wastewater treatment, UV and ozone disinfection can be very energy-intensive. High costs caused by high operational and maintenance needs make them limited worthy for larger wastewater treatment facilities.

Therefore, another disinfectant which is the newest alternative for applications in North America has currently become attracting particular interest, that is peracetic acid (PAA). PAA has been used in Europe, especially in Italy, for wastewater disinfection for many years [5-10], but and it has been recognized as an alternative disinfection technology by the U.S.EPA [2].

1.2 PAA Disinfection

Peracetic acid (PAA, CH_3CO_3H) is the peroxide of acetic acid (AA, CH_3CO_2H), which is a clear, colorless liquid and has a strong pungent acetic acid odor. PAA has an acidic pH of less than 2, so it can be highly corrosive. Exposure to PAA can cause irritation to the skin, eyes and respiratory system. PAA maintains its efficacy for pH values ranging from 5 to 7 [11]. It is freely soluble in water and in polar organic solvents like alcohol, ether, and sulfuric acid [12].

Commercial PAA is only available in a quaternary equilibrium mixture, which is produced from the reaction of AA with hydrogen peroxide (H_2O_2) in the presence of sulfuric acid as a catalyst. Although there are several different concentrations of PAA solution available in the market, their components are the same, containing AA, H_2O_2, PAA, and water. Their equilibrium reaction is as follows:

$$CH_3CO_2H + H_2O_2 \longleftrightarrow CH_3CO_3H + H_2O \qquad \text{(Reaction 1.5)}$$

In the PAA mixture solution, both PAA and H_2O_2 can contribute to the disinfection power; however, PAA has more potential than H_2O_2 [12, 13]. The reason is that it can rapidly react at low concentrations while H_2O_2 requires much higher dosages than PAA for the same level of disinfection [12]. More importantly, researchers found that by varying the ratio of PAA to H_2O_2, it is the PAA, not the H_2O_2 that is responsible for the biocide action [8]. Given to explosiveness, stability, and reactivity properties, PAA solutions between 10% and 15% are used in many industrial applications [12].

PAA has been widely used as a disinfectant and for sterilizing in the food processing and beverage industries because of its powerful antimicrobial action on bacteria, fungi, and viruses at low temperatures along with the absence of toxic residuals [12]. It is also used or studied for various environmental applications including ion exchangers, cooling tower, combined sewer overflows (CSOs), membrane hollow fibers, as well as pathogen reduction in biosolids [12]. Nevertheless, the most important and popular application is for wastewater disinfection.

When PAA is consumed in an aqueous solution, three reactions happened [12]: spontaneous decomposition, hydrolysis, and transition-metal-catalyzed decomposition. However, for wastewater disinfection, pH is usually at range of 5.5 to 8.2, where PAA spontaneous decomposition is the main concern. It leads mainly to the formation of AA, oxygen, H_2O_2 and water [12, 13].

The disinfecting mechanism of PAA is still not entirely known. There are mainly two suggested mechanisms: hydroxyl radicals (·OH) release theory and "active" oxygen release theory. Hydroxyl radicals (·OH) release theory [5, 12] is based on advanced oxidation processes (AOPs). The presence of H_2O_2 within the commercial product of the PAA contributes not only to the formation of new PAA, but also to the formation of new hydroxyl radicals [12]. The oxidizing action and formation of a chain reaction of other free radicals guarantee a rapid degradation of the polluting molecules. "Active" oxygen release theory [6, 12] is illustrated that the oxygen-oxygen bond released from PAA acts on the base of DNA molecules, disrupts cell membranes of the pathogens by blocking the sulfhydryl (–SH) and

sulfur (S–S) bonds within enzymes and proteins, and thus impedes the cellular transport processes. Both of them show that PAA has strong oxidant potential.

PAA, compared to chlorine and chlorine-based compounds, is particularly suitable for wastewater disinfection mainly due to the following advantages. Firstly, studies have shown that PAA disinfection efficiency is not significantly affected by suspended solids concentrations and has a low dependence on pH [7, 12-15]. Secondly, it is so far reported that PAA produces little to no toxic DBPs, and PAA residuals appear to be non-toxic compounds, generally AA and oxygen in receiving waters [12]. This means no quenching requirement needed, which can, in a certain degree, balance the relatively expensive cost of PAA compared to NaClO. Thirdly, PAA displays strong oxidant and disinfectant capabilities. Its oxidation potential is larger than that of chlorine or chlorine dioxide [7, 12, 15]. It has broad spectrum of activity even in the presence of heterogeneous organic matter, so it needs less doses and shorter contact time. [8-10,14-15]. In addition, with the appropriate personal safety apparatus, PAA is easy to handle, ship, storage, and it can retrofit chlorination equipment without the need of expensive capital investment [12].

Although PAA has its own disadvantages such as increasing organic content in the effluent and higher relative cost compared to chlorine, limited research shows that it produces no/little toxic or mutagenic DBPs (aldehydes and halogenated phenols) even when high levels of organic matter are present [6, 10, 12, 16, 17], and its cost can be lowered as the demand for PAA increases. In such a case, PAA may also become cost-competitive with chlorine, and might replace chlorine-based disinfectants in wastewater treatment processes.

1.3 EDCs/Pharmaceuticals in Wastewater

Endocrine disrupting compounds (EDCs) and pharmaceuticals are groups of emerging contaminants that have been detected at trace concentrations in waters around the world. EDCs are comprised of a vast group of chemicals that interfere with hormones functions in animals, causing cancerous tumors, birth defects, and other developmental disorders. Pharmaceuticals found in waters include prescription and nonprescription human and veterinary drugs. According to the current research [18-22], municipal wastewater treatment plants (WWTP) effluents have been identified as a major source of EDCs in surface waters.

Among the extensive suite of various EDCs/Pharmaceuticals found in water, 17β-estradiol (E2), clofibric acid and ketoprofen have been selected and focused on in this research to study PAA oxidation efficiency and DBP formation. The reasons are that these chemicals are frequently measured but not easily removed by either chlorination or ozone oxidation in WWTP [18, 20-21, 23-25], and their high degree of persistence in environment has led to long-lasting effects on various water matrices, aquatic wildlife, farm animals, and even food chain [23-28].

17β-estradiol (E2) is a sex hormone, and is abbreviated E2 as it has 2 hydroxyl groups in its molecular structure (Figure 1.1 [28]), compared to estrone (E1) and estriol (E3). Its estrogenic effect is about 10 times as potent as estrone's and about 80 times as potent as estriol's. 17β-estradiol (E2) is mainly excreted in the urine of humans and animals. It exists in different water matrices, and it is mainly removed through chemical oxidative processes in WWTP. However, incomplete elimination of estrogenic compounds during WWTP results in the presence of these substances at the ng/L level in the treated wastewaters effluents, and

further into the aquatic environment. DBPs are also generated during this process by conventional treatment such as chlorination and ozonation. So far, still no research on reduction of 17β-estradiol (E2) by PAA oxidation. Therefore, 17β-estradiol (E2) has been selected as a representative to study PAA oxidation efficiency and the corresponding DBP formation.

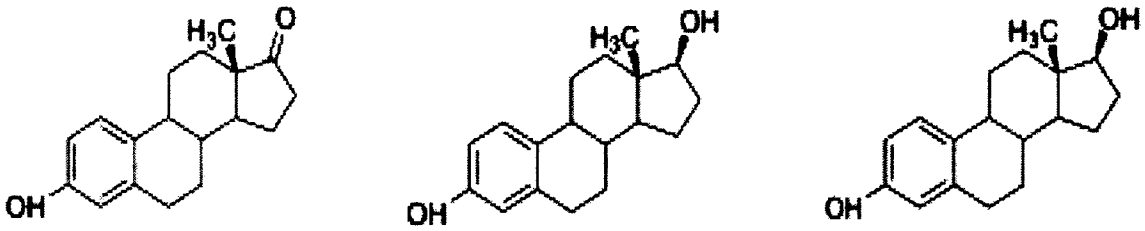
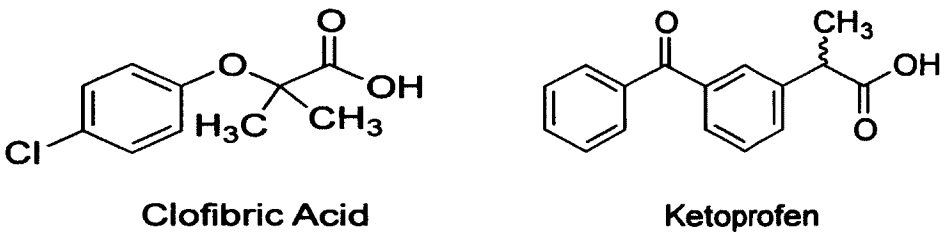


Figure 1.1: Groups of estrogens: (a) E2, (b) E1, (c) E3 [28]

Clofibric acid and ketoprofen (Figure 1.2 [26]) are two of the oldest drugs detected and found to be ubiquitous in the environment have also been studied. Clofibric acid is a metabolite of clofibrate, etofibrate and etofyllinclofibrate which are drugs functioned as blood lipid regulator. Ketoprofen, one of the non-steroidal anti-inflammatory drugs (NSAIDs), is largely used for analgesic, anti-inflammatory and antipyretic properties in various applications [26, 27]. Both of them are also excreted in the urine, enter into WWTP, and incomplete removed by different processes by both wastewater and drinking water systems. Likewise, the oxidation of these two chemicals by PAA has not been studied so far, and these issues are the subjects of recent and ongoing studies, including this project, a more detailed review is presented in the literature review chapter.



Clofibric Acid Ketoprofen

Figure 1.2: Structures of clofibric acid and ketoprofen [26]

1.4 Objectives

The main goal of this research was to study PAA DBP formation during wastewater disinfection and compare them with the corresponding DBPs from NaClO at the same experimental conditions. This included the ability of PAA and NaClO to react with select EDCs and pharmaceuticals. Jar-testing apparatus at laboratory scale was used to simulate disinfection contact chamber conditions. A gas chromatography/mass spectrometry (GC/MS) analyzer was used for DBP identification and quantification. The specific objectives are as follows:

- Compare reduction and potential DBP formation of selected endocrine disrupting compounds (EDCs)/pharmaceuticals, specifically 17β-estradiol (E2), clofibric acid , and ketoprofen from PAA and NaClO oxidation

- Investigate and identify potential genotoxic and mutagenic DBPs formed from wastewater disinfection with PAA or NaClO

- Investigate how different dosages of PAA/NaClO and contact time impact the following parameters in wastewater: pH, specific UV_{254} absorbance (SUVA),

chemical oxygen demand (COD), ammonia nitrogen (NH_3-N), as well as total organic carbon (TOC)

Chapter 2

LITERATURE REVIEW

In order to clearly understand PAA disinfection by-products (DBPs) formation during wastewater treatment, the detailed, current situation will be reviewed in this chapter, which includes 1) PAA disinfection efficiency; 2) wastewater chlorination DBPs; 3) wastewater DBPs formation by PAA; 4) DBPs formed from oxidation of 17β-estradiol, clofibric acid, and ketoprofen by NaClO/PAA; 5) analytical methods for DBPs.

2.1 PAA Disinfection Efficiency

PAA disinfection efficiency for different microorganisms in different secondary effluents is summarized in Table 2.1. The most commonly measured physic-chemical parameters include pH, TOC, COD, ammonia, and UV_{254} absorbance. It is shown that PAA disinfection can effectively reduce bacteria including *Total and Fecal Coliforms*, *E. coli*, *Enterococci*, and *Fecal Streptcocci* at very low dosage (1-10 mg/L) and contact time (< 30 min). However, for virus like *F-RNA coliphages*, *DNA-phage*, *RNA-phage* and *MS-2*, higher dosage and/or contact time is needed to achieve the same reduction level.

Comparative studies of the effects of PAA and NaClO on indicator bacteria and virus in secondary wastewater effluents indicated that PAA could be a viable alternative to chlorine-related compounds in disinfection process [7, 30, 31]. A pilot-plant study [7] found that the disinfectant actions of PAA against *total and fecal coliforms, E. coli, Pseudomonas* and *Salmonella sp.* was similar to that shown by NaClO, but for *fecal streptococci* and *bacteriophagesa anti-E.coli*, PAA had less bactericide activity than NaClO.

Table 2.1: Summary of study on PAA disinfection efficiency in WWTP secondary effluents

Wastewater Characteristics	Dosage (mg/L)	Time (min)	Pathogens	Log Removal	Source
pH: 6.82-7.31 T: 9-19 °C COD: 27-89 mg/L Turbidity: 1.2-4.8 NTU 253.7 nm transmittance: 33-58.1%	2-7	27	Total coliforms (TC), enterococci (EC)	3 log	[8]
			F-RNA coliphages	1 log	
COD: 123-240 mg/L SS: 16-45 mg/L Turbidity: 16-31 NTU Low UV transmittance: 4.6-29.5%	4.5	60	Fecal Coliform (FC), EC	1 log	[14]
	1.5-3	60	MS-2	1 log	
pH: 7.3-8.1 COD: 23-75 mg/L Turbidity: 1.2-2.9 NTU Total solids: 1-30 mg/L Total ammonia: 0.5-1.7 mg/LNH_4 Total P: 0.7-6.1 mg/L	2-8	10-30	TC, FC	2-4 log	[5]
			Faecal Strept.	3-6 log	
			E. coli	3-4 log	
			Pseudomonas sp.	2-3 log	
Turbidity 2-8 NTU	25	10	DNA-phage	5 log	[22]
			RNA-phage	2 log	
pH: 7.4-7.8 COD: 54-140 mg/L TSS: 4-8 mg/L	≤1	10	TC, E. coli	2 log	[9]
pH: average 6.5 BOD_5: average 41 mg/L TSS: average 17.6 mg/L	1.5-2	20	TC, E. coli	5 log	[15]
			EC	2-3 log	
			Total heterotrophic	1-2 log	

Similar study had been conducted by [30] at laboratory scale, which concluded that PAA might be a more efficient disinfectant than NaClO for treatment of waters containing easily oxidizable organic matter. It showed that low PAA doses (1.5-3 mg/L) were needed to achieve 2-3 log *E. faecalis*, *E. coli* and *S. enteritidis* reductions compared to 12-18 mg/L NaClO for 1-2 log reduction at 10 min contact time. By using higher PAA doses (7-15 mg/L) *MS2-coliphage* could be inactivated.

Although very few studies assessed protozoa removal efficiency by PAA disinfection, it is known that significantly higher dosage and contact time are needed. That is why the idea of using PAA disinfection alone or in combination with a short UV exposure is proposed and processed in some wastewater treatment plants [5, 30, 32].

All of these results mentioned above indicate that PAA could represent a good alternative to chlorine for microbial reduction. However, an ideal disinfectant should not only guarantee the maximum efficiency in pathogenic microorganism removal, it also needs to make sure little/no toxic and undesirable byproducts generated during disinfection process. To better understand how PAA DBPs are formed and how different it is from chlorine DBPs formation, the current situation about DBPs formation by chlorine and PAA disinfection is illustrated in the following two sections.

2.2 Chlorine Disinfection By-products

To date, there are a variety of harmful or toxic DBPs found during water and wastewater chlorination. The DBPs occurring most frequently and with the highest concentration by chlorine disinfection are trihalomethanes (THMs: chloroform, bromodichloromethane,

dibromochloromethane, and bromoform) and haloacetic acids (HAAs: monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid) [2]. Another DBP that has recently surfaced in disinfected wastewater effluents is N-nitrosodimethylamine (NDMA), a member of a class of compounds known as nitrosamines, which are among the most powerful carcinogens known [2, 33-34]. The level of NDMA formed as a result of sewage disinfection with chlorine was found to reach 100 ng /L in treated wastewaters by [34]. There are also other emerging DBPs such as haloacetonitriles (HANs), haloketones (HKs), chlorophenols, aldehydes and other minor DBPs [35].

Although occur at trace concentrations (usually < 1 μg/ L), most of these DBPs may have acute or chronic toxic effects on both aquatic organisms and humans [35-36]. The effects of these DBPs on aquatic organisms include alteration of normal physiological processes, induction of genetic mutations, cancer, and defects in offspring, reproductive impairment of sexually mature individuals, and even death [3-4]. These effects can drastically alter the population size and may results in serious upsets in the food web.

Possible hazards to humans associated with these DBPs may contain the following aspects: cancer, reproductive effects, developmental effects, and the organ-specific toxicities. THMs and HAAs can lead to bladder, liver, kidney, and colon cancers. The influences of them on human reproductive function are related to low birth weight, spontaneous abortion, small for gestational age, neural tube defects, and sperm abnormalities. For the developmental effects, teratogenesis, the process by which congenital malformations are produced in an embryo or fetus, by HAAs is the main concern. The general toxicity is mainly

referred to organ-specific toxicities, which include hemolytic anemia such as methemoglobinemia, liver toxicity, kidney toxicity, and neurotoxicity. [3, 4, 36]

Chlorination DBPs are formed due to a series of complex reactions between free chlorine (HClO and ClO^-) and a group of organic compounds in water and wastewater [1]: including oxidation (chlorine is reduced to chloride ion), addition (chlorine is added to unsaturated double bonds), and substitution (C-chlorinated compounds or N-chlorinated compounds are formed). Among these reactions, substitution is the key way to form chlorinated DBPs.

Formation of these DBPs is affected by the following operational parameters [1-2, 38-40]: initial mixing, contact time, pH, temperature, free chlorine and bromine concentration, as well as presence of bromide and organic/inorganic precursor. Controlling these parameters can help decrease hazardous DBPs formation during wastewater chlorination, but it also associated with a lot of difficulties.

Initial mixing can affect the formation of THMs because of the competing reactions between chlorine + ammonia and chlorine + organic substances. However, increasing flow rate might reduce chlorine disinfection efficiency. Increasing contact time has also been noted to increase DBPs formation. However, various hydraulic properties often make control of contact time a challenge [41]. Temperature and pH are another two essential parameters during chlorine disinfection. An increase in pH and temperature can generally increase DBPs formation. However, it has been shown that HAAs formation decrease as pH increases [39, 41]. Therefore, the determination of the optimum pH range for disinfection is often necessary during water and wastewater treatment processes.

Cl_2/N ratio, the presence of bromide, as well as the type and concentration of the organic/inorganic precursor play the most important role in chlorination DBPs formation. Free chlorine can oxidize bromide to bromine and the bromine ion can combine with precursors to form THMs [40]. Decreasing chlorine dose and Cl_2/N mass ratios will lead to a decrease in reaction extent and thus cause a decrease of THMs and HAAs formation [38], but it is difficult to achieve an optimum Cl_2/N ratio.

Organic acids were found to be the dominant precursors of DBPs with or without the presence of ammonia, which indicated that the triple bonds of carbon-carbon and carbon-oxygen as well as C–O structures contributed to the formation of DBPs significantly [38]. Therefore, to reduce DBPs formation potential, removal of these substances by using enhanced coagulation and membrane filtration prior to disinfection or dechlorination after disinfection can be an effective approach. However, coagulation and filtration can only partially remove precursors because of their low molecular weights [41]. Chemical dechlorination by adding sulfite is not only difficult to reach near zero levels of residual chlorine, but it also lower pH of the effluent and produce toxic compounds (such as sulfate) formation [42]. Besides, these enhanced treatments may increase operating and maintenance costs significantly.

The challenges mentioned above make searching an effective, economic and safe alternative disinfectant necessary and urgent. PAA as such is a promising candidate, and has been introduced into wastewater treatment plants to replace chlorine.

2.3 PAA Disinfection By-products

As illustrated in Section 1.2, PAA goes through three reactions in an aqueous solution, but the pH range of wastewater (6 to 8.2, shown from Table 2.1) results mainly in PAA spontaneous decomposition of PAA to acetic acid and oxygen [12]. From this point, PAA may not produce harmful products. Carboxylic acids, formed through the oxidation of natural organic matter in the water by PAA, were reported to be the predominant DBPs and they are not recognized as being mutagenic [12, 43]. A pilot study has been undertaken to assess genotoxicity of DBPs resulting from wastewater disinfection with PAA/NaClO by using in vitro bacterial reversion assays and plant genotoxicity tests, which concluded that moderate doses of PAA (2 and 4 mg/L) do not lead to the formation of significant amount of genotoxic DBPs [10]. In addition, not surprisingly, no chlorinated DBPs were observed in PAA treated waters [12, 43], even at high TOC content [6]. This is one of the most important advantages of PAA over other typically used chemical disinfectants.

Although PAA is considered to form little to no toxic or mutagenic DBPs, the possibility that it could form DBPs cannot be completely ignored due to its strong oxidizing power. There are two groups of DBPs reported so far: aldehydes and brominated by-products [6, 12, 16, 17]. It was shown that less than 30 μg/L of aldehydes were formed when PAA reacted with organic compounds such as amino acids, phenols, and other aromatics in treated wastewater [44], quoted by [6, 12, 16]. At both pilot and laboratory level, [16] assessed and detected some formation of aldehydes, but their concentration was well below Italian maximum allowable concentration (MAC, 1 mg/L for wastewater discharge into surface water and 0.5 mg/L for wastewater reuse, compared to 0.8 μg/L formaldehyde set by Ontario

Provincial Water Quality Objectives [45]) in the experimental conditions investigated and was considered to be negligible. Two aldehydes, nonanal and decanal, were confirmed by [6], which pointed out the former presented in a slightly higher percentage of abundance than the latter.

Aldehydes formation is directly proportional to PAA dosage, although it varies with PAA/COD ratio [16]. It was demonstrated that aldehydes formation follows a two-step "formation and destruction" mechanism [16]: PAA (CH_3COOOH) react with organic precursor present in the wastewater effluents first to form acetic acid (CH_3COOH), aldehydes (HCHO), and water; aldehydes is then further oxidised by excessive PAA to form carboxylic acids, or ultimately to carbon dioxide. This mechanism explains why the detected concentrations of aldehydes in previous studies are negligible.

Another category of reported PAA DBPs is halogen-related organics, mainly referred to bromo- phenols. Generally, high concentrations of PAA, organic precursors and bromide are needed for the formation of halogenated by-products [6, 12, 16, 17]. The phenolic substrates, which are responsible for slow reacting THM precursors, were found to be organic precursors for halogen-containing organics [16]. They can react with free radicals PAA generated to form brominated phenols [6, 12, 16].

It was found that PAA was unable to oxidize chloride to hypochlorous acid (HClO). The current research results shows that the addition of PAA to an experimental water enriched with chlorides does not increase the concentration of absorbable halogenated organics (AOX) nor of total halogenated organics (TOX) [44], quoted by [6, 12, 16]. However, the electrochemistry of PAA shows that it has the power to oxidize bromide to hypobromous

acid, subsequently forming brominated organics [6, 12, 16, 17]. In order to understand the mechanism and kinetics of potential formation of halogenated phenols in the presence of PAA, a laboratory set of model reactions in bi-distilled water was investigated by [16] at 30, 5 mg/L PAA dosage and 50, 120 min contact time. It was shown that both PAA and H_2O_2 could oxidize bromides (HBr) to Br_2 in the acidic conditions (pH 3.8-4.2), according to

$$2HBr + CH_3COOOH \longrightarrow Br_2 + CH_3COOH + H_2 \qquad \text{(Reaction 2.1)}$$

$$2HBr + H_2O_2 \longrightarrow Br_2 + 2H_2O \qquad \text{(Reaction 2.2)}$$

The Br_2 formed by both PAA and H_2O_2 then proceeded following a well-known equilibrium:

$$Br_2 + H_2O \longrightarrow HBrO + HBr \qquad \text{(Reaction 2.3)}$$

$$HBrO \longrightarrow H^+ + BrO^- \qquad \text{(Reaction 2.4)}$$

$$Br_2 + Br^- \longrightarrow Br_3 \qquad \text{(Reaction 2.5)}$$

Br^- formation from this process will react with phenols to form brominated phenols. What needs to be noticed is that formation of brominated phenols following PAA addition only achieved under unrealistic conditions (pH = 3.8-4.2 and very large excess of reagents), which is not possible in actual wastewater because of the existence of ammonia [16, 17].

In addition, the low DBPs production observed may be due to the low precursor concentration, correlated to the overall good quality of the effluents used for these tests. The insufficient amount of information on PAA DBPs formation has therefore led to the need to further perform research on DBPs formation during wastewater disinfection with PAA.

2.4 DBPs Formation from the Target EDCs/Pharmaceuticals by NaClO/PAA

As mentioned before, although at the ng/L level in treated wastewater, EDCs/Pharmaceuticals, especially 17β-estradiol (E2), clofibric acid and ketoprofen, persistently exist in various matrices with potential long-lasting estrogenic effects in aquatic organisms [18-29]. Different methods have been investigated to remove these contaminants, including diverse physical methods (such as activated carbon adsorption, reverse-osmosis, ultrafiltration and nanofiltration) and chemical methods (such as disinfection and oxidation) [18-25]. However, given to high-energy consumption (post-treatment of materials and waste generate) of the available physical methods, chemical oxidation processes have been considered to be more cost-effective processes in wastewater treatment plant [18, 25, 46].

Currently, research on removal efficiency of EDCs/pharmaceuticals in water or wastewater has been mainly conducted by chlorine or ozone disinfection [21, 23, 46-49]. There is still no study on PAA removal efficiency of these pollutants, not to mention investigation on DBPs formation from PAA disinfection. Therefore, this section primarily focuses on the impact of chlorine disinfection on the removal of EDCs/pharmaceuticals.

Chlorine disinfection, as a typical disinfection method of wastewater effluent, has been proven to efficiently remove a significant number of EDCs/pharmaceuticals via oxidation, substitution, and addition reactions, as mentioned in Section 1.3. At the concentration of 10 mg/L chlorine and time of 60 min, more than 85% of the most pharmaceutical (except ketoprofen and clofibric acid) would be transformed [18, 46].

There are three main parameters influencing chlorine oxidation efficiency of EDCs/Pharmaceuticals: dosage and contact time, pH, as well as types and structure of EDCs/Pharmaceuticals [23, 25, 46-47]. It was found that chlorination of estrogens was time dependent and higher efficiencies had been obtained with longer contact times and higher dosage. Doses of chlorine between 1 and 4 mg/L could provide good removal efficiencies for estrogens [25]. Estrogenic activity of 17β-estradiol (E2) decreased with the increase of chlorination time. For instance one study that examined estrogen in well water found that an initial concentration of 100 ng/L of 17β-estradiol (E2) could be reduced to below the method limit of detection (30 ng/L) using a chlorine dosage of 2 mg/L and 30 min while a lower dosage and contact time of 0.5 mg/L and 10 min were insufficient. [25] However, after 120 min at dosage of 1.46 mg/L, chlorination yielded no further significant decrease in the activity of the solution [47]. It was also illustrated by [46] that free chlorine compared to combined-chlorine could react much more rapidly with these chemicals.

The effect of pH on EDCs/pharmaceuticals removal efficiency during wastewater chlorination is also significant. It was found that these chemicals react faster with free chlorine at pH = 5 and 7 than pH = 9 because pH shifts the equilibrium distribution of hypochlorous acid and hypochlorite ion [23, 25, 46].

Another very important factor responsible for removal of EDCs/Pharmaceuticals by chlorine is structure of these chemicals. Generally, those easily removed chemicals are more reactive compounds, which have aromatic rings with functional groups like hydroxyl, and amine in their structures [46]. These groups contain electron rich bonds that can substitute in organic molecules to increase reactivity with chlorine. This explains the reason why

17β-estradiol (E2), as one of the most important phenolic steroids, can be readily oxidized by chlorine, but clofibric acid and ketoprofen, with carboxylic groups, show very weak reactivity with free chlorine. Transformation of aromatic ether- and amine-containing pharmaceuticals, including one of our target compounds (ketoprofen), during chlorine disinfection has been studied by [46]. It was found that ketoprofen reacted too slowly in the pH range of natural waters to be significantly transformed during water treatment processes even after a reaction time of 5 days. And no loss of pharmaceuticals or free chlorine was observed in any of the controls [46].

17β-estradiol (E2) is rapidly oxidized to estrone (E1) with hypochlorous acid [23]. Seven chlorinated DBPs from removing E2 were identified by [47] under the following operational conditions: 1.46 mg/L NaClO, 50 μg/L E2, pH 7.5, and T = 25 °C. The seven chlorinated derivatives are: 2-chloro-E1, 2-chloro-E2, 4-chloro-E1, 4-chloro-E2, 2,4-dichloro-E1, 2,4-dichloro-E2, and 4-[2-(2,6-dichloro- 3-hydroxyphenyl) ethyl]-7R-methyloctahydroinden-5-one. Among these DBPs, dichloro-E1 was identified as the most abundant DBP, followed by monochloro-E1 and dichloro-E2 [47, 48]. Another four unknowns (A-D) were also found. The molecular structures, molecular weight, oxidation pathways of these derivatives are proposed by [47].

The estrogenic activities in the effluent of a WWTP with chlorine disinfection have been investigated by [49], which found that the chlorinated wastewater samples had on average 56% less estrogenic activity than the not chlorinated ones. However, despite the fact that chlorination resulted in a relatively low level of estrogenicity, DBPs formed during wastewater chlorination may induce some estrogenic activity in treated wastewater. The

estrogenicity of chlorinated E2 derivatives were assessed by yeast two-hybrid assays based on a human estrogen receptor and a co-activator [23, 25, 46, 47]. It was found that 4-chloro-E2 produced strong estrogenic activity similar to that of E1, while 2,4-dichloro-E2 elicited an estrogenic activity approximately 40% weaker than that of 4-chloro-E2, but stronger than that of dichloro-E1. This illustrated that 2 and 4 positions tend to decrease the estrogenic activity of chlorinated estrogens. The order of EC10 values (the concentration of the test solution producing a chemiluminescent intensity 10 times that of the blank control) of chlorinated derivatives was: 4-chloro-E2 < E2 < 2-chloro-E2 < 2,4-dichloro-E2 [25, 57]. Three brominated derivatives such as 2-bromo-E2, 4-bromo-E2, 2,4-dibromo-E2 were also found by [48], which concluded that these brominated E2 showed slightly weaker activity than the corresponding chlorinated derivative.

So far, the long-term effects of 17β-estradiol, clofibric acid and ketoprofen into the aquatic environment are still not entirely known. Removal of 17β-estradiol by chlorination is partially efficient but chlorinated or brominated E2/E1 might be more toxic and induce higher estrogenic effects than E2 itself. PAA as a promising alternative to chlorine has not been ever studied on its oxidation efficiency and DBP formation from oxdizing these EDCs/Pharmaceuticals. Therefore, an investigation on reduction and DBPs formation of 17β-estradiol, clofibric acid and ketoprofen by PAA oxidation is urgently needed.

2.5 Methods for DBPs Analysis

Many different methods can be used to determine concentrations of contaminants and DBPs in wastewater. Since the concentrations of DBPs formed during wastewater chlorination, PAA disinfection, as well as oxidation of 17β-estradiol (E2), clofibric acid and ketoprofen

are very low (sub-μg/L), this review focused on methodologies applicable for trace analysis. The measurement of organic substances in water and wastewater commonly consists of an extraction of the chemicals from water, concentration of the extract, chromatographic separation, detection and quantification. This section provides general information on the above processes based on review of specific methodologies and compounds provided in Table 2.2.

Table 2.2: Case studies on the extraction and analysis methods for the target compounds

Target Compounds	Extraction	Chromatography/ Toxicity	Detection Limit	Source
17β-estradiol (E2)	LLE	GC/MS-EI-SIM	0.13-0.37 ng/L	[23]
	SPE	FAB-MS, H-NMR Spectra, HPLC	---	[48]
	---	GC-FID	50 μg/L	[58]
	SPE	LC/MS, HPLC; Yeast two-hybrid Assays	---	[47]
	SPE	E-screen Assay	---	[49]
	SPE	GC/MS-MS-EI	---	[60]
	SPE-MIP	HPLC	---	[61]
Clofibric acid / Ketoprofen	SPE	GC/MS-SIM	7 μg/L for clofibric acid 230 μg/L for ketoprofen	[26]
	SPE	GC/MS	3-114 ng/L	[19,29]
	SPE	HPLC	---	[46]

Target Compounds	Extraction	Chromatography/ Toxicity	Detection Limit	Source
	SPE	GC/MS-MS	1 ng/L	[62]
	LLE, SPE	GC/MS-EI	50.6 ng/L	[50]
THMs/HAAs	HS-SPME	GC-ECD	100 μg/L	[49]
	LLE	GC-ECD	---	[38-40]
	SPE	GC-ECD	---	[35]
	LLE	GC/MS	0.2 μg/L	[36]
	LLE	HP-GC-ECD	0.06-0.5 μg/L	[37]
	LLE	GC-ICP-MS	0.01 μg/L	[52]
	LLE	GC/MS-SIM	0.04-0.83 μg/L	[51]
NDMA	SPE	GC/MS	5 ng/L	[33]
	LLE, SPE/SPME	HPLC, GC/MS	LLE+GC/MS: 200 μg/L SPE/SPME+GC/MS: 20-39 ng/L HPLC: 50 μg/L	[34]
Aldehydes/ Halogenated phenols	SPE	GC/MS	0.5 μg/L	[16,43]
	SPE	GC/EI-MS	0.1 μg/L	[59]
	SPME, LLE	GC/MS	0.1-2 μg/L	[6]

(LLE: liquid-liquid extraction; GC/MS-EI-SIM: gas chromatography/mass spectrometer using electron impact in selected ion monitoring; HPLC: high performance liquid chromatography; GC-FID: gas chromatography-flamed ion detector; SPE: solid phase extraction; LC/MS: liquid chromatography/mass spectrometer; MIP: molecularly imprinted polymer; HS-SPME: headspace-solid phase micro-extraction; GC-ECD: gas chromatography-electron capture detector; GC-ICP-MS: gas chromatography-Inductively coupled plasma-mass spectrometer)

2.5.1 Extraction/Concentration-Sample Enrichment

Wastewater DBPs and the target three EDCs/Pharmaceuticals have been found to induce toxic effect on aquatic organisms at sub-μg/L, which requires that detection limit of analytical techniques for these chemicals has to be achieved in ng/L. However, the majority analytical instruments are not able to directly detect compounds at these levels without an extraction step. Conventional extraction techniques such as liquid-liquid extraction (LLE), solid-phase extraction (SPE) and solid-phase micro-extraction (SPME) have been used to extract the compounds mentioned above, shown in Table 2.2.

Liquid-liquid extraction (LLE) in water and wastewater treatment generally uses solubility differences of different substances to selectively draw the target analytes from water into the organic layer. It is commonly performed at laboratory scale after a chemical reaction using a separate funnel. Common LLE solvent pairs are water-dichloromethane [6, 23, 43, 48, 50], water-ether [38-40, 51], water-pentane [52], and water-hexane [38]. Repeated extractions are usually needed to ensure higher extraction efficiency. Because no solvent is completely insoluble in water, one additional step is usually carried out before evaporating the organic solvent: drying over anhydrous sodium sulfate or other drying agent. The advantages of LLE are simplicity, high extraction efficiency and sensitivity by using an adequate solvent. The major drawbacks are the higher demand for solvent volume and time, labor intensity, as well as the emulation formation [53-55].

Solid-phase extraction (SPE) removes the target analytes from a water sample by distribution and retention on a solid phase (adsorbents), followed by the evaporation with a

stream of nitrogen or air [54, 56]. There are three kinds of adsorbents packed into a cartridge or column: carbon-based, silica-based, and polymer-based sorbents [53].

Among these adsorbents, Octadecyl (C18) cartridges are the most widely used material for the extraction of chlorinated hydrocarbons, phenols and chlorophenols, as well as pharmaceuticals [16, 35, 43, 47, 50]. It provides great extraction efficiency for the analytes with low polarities from water samples [53]. Compared to LLE, SPE requires less solvent, more quickly, and provides more highly concentrated extracts. However, SPE also has disadvantages such as low extraction efficiency for very polar analytes, high requirement for uniformity of packing, and dependence on water matrix [54].

Compared to SPE, solid-phase micro-extraction (SPME) is a more modern, solvent-free extraction technique, which is based on the equilibrium of the analytes between the sample and a fused silica fiber coated externally with a solid phase. SPME combines sampling, analytes separation, concentration, and sample introduction within one step. It is a more rapid and sensitive technique for both polar and non-polar analytes in water samples, although low storage stability of the samples may be involved. [53-56]

2.5.2 Methods Based on Gas Chromatography

Gas chromatography (GC) has been used for organic compounds analysis in water and wastewater for many years. The principal of GC [57] is as follows: A 1-2 μL liquid sample collected by an auto-sampler is injected into the GC and vaporized at a high temperature (normally 250 °C); a carrier gas (usually helium, a mobile phase) then carry the vaporized sample into a column (packed or capillary tube, a stationary phase); because different

compounds in a sample have different physical and chemical properties, their interaction with column will be different, which causes these compounds to exit the column at different time (retention time). During GC analysis, the compounds are separated without decomposition.

In most GC systems, a detector is used to monitor the compounds as they leave the column. The most widely used detectors include flame-ionization detector (FID), electron capture detector (ECD) and mass spectrometry (quadrupole MS), seen from Table 2.2. Each detector has its own sensitivity for a certain groups of compounds. FID is said to be sensitive for non-aromatic compounds, while ECD is highly specific for detection of halogenated compounds [62-63]. Compared to FID and ECD, MS is more popular and highly preferred because of its great sensitivity and excellent selectivity.

MS separates compounds from GC based on their mass to charge ratio (m/z) after vaporization and ionization processes by electron ionization (EI) or chemical ionization (CI). It could achieve qualitative identification of compounds rapidly by comparison of the experimental mass spectra with those in a library of spectra of known compounds. Two scan modes could be used in MS: Full-scan mode and selected ion monitoring (SIM) mode. Full-scan MS monitors a wide range of fragments (generally 50-400 m/z). From full-scan spectrum, an unknown compound could be predicted. In SIM mode, only one or a few target masses are monitored, thus the sensitivity of the quantification is significantly increased.

To achieve a much finer degree of identification, GC and MS are usually used together (GC/MS) to reduce the possibility of error occurred in GC or MS alone. It is extremely unlikely that two different molecules will behave in the same way in a GC/MS analysis.

Based on GC/MS, GC/MS-MS (triple quadrupole) as an emerging technique is used to quantify the extremely low levels of target compounds in a complex sample [64]. Except for mass spectrum obtained by the first MS, a scanning mode from the second MS in GC/MS-MS could build an ion spectrum (fragment ions of a precursor ion), resulting in lower background noise and higher signal to noise ratios [56, 64-65]. The detection and quantification limit is thus significantly improved.

Although the GC-based techniques, especially GC/MS, have many advantages for water samples analysis, the complexity of operation, low sensitivity for nonvolatile and thermally fragile compounds, costly maintenance for GC instrument have led to the increased popularity of another group of analytical methods, that is liquid chromatography (LC)-based techniques [56, 64].

2.5.3 Methods Based on Liquid Chromatography

Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid. The most popular used LC for the qualitative and quantitative analysis of a wide range of analytes is high performance liquid chromatography (HPLC). HPLC works by interaction of a liquid (mobile phase) at high pressure by a pump with the stationary phase in a small size column. Combination of water with organic solvents such as acetonitrile and methanol is commonly used for mobile phase in HPLC. [64-65]

There are four dominant detectors used in LC analysis: ultraviolet/visible (UV-VIS) spectroscopic detectors, electrical conductivity detector (ECD), fluorescence detector (FLD), and refractive index detector (RID) [56, 62-63]. Among these detectors, UV detection is

generally used due to its ease of use and cost-efficiency. However, compared to GC/MS, the sensitivity and selectivity of these LC systems are lower and cannot effectively detect trace concentrations in water samples [56]. Therefore, LC combined with a MS detector or a tandem MS-MS detector is developed for identifying compounds that are difficult to analyze by GC/MS.

The working mechanisms of LC/MS and LC/MS-MS are similar to that of GC/MS and GC/MS-MS, except the ion sources used for MS. The common ionization technique used by LC/MS and LC/MS-MS is atmospheric pressure ionization (API), which includes electrospray ionization (ESI, for large molecules), atmospheric pressure chemical ionization (APCI, for a wide range of polar and nonpolar molecules), and atmospheric pressure photoionization (APPI, similar to APCI, more sensitive). Although LC/MS and LC/MS-MS results in an easier detection of compounds, high sensitivity and selectivity, it is too expensive and very difficult to operate. Besides, matrix suppression associated with LC/MS analysis is still a problem so far. [56, 64] Therefore, GC/MS is still a predominant technique for water analysis.

Chapter 3

MATERIALS AND METHODS

This chapter aims to provide detailed information of materials and methods used in this project, which were illustrated according to different experimental runs and operating procedures.

3.1 Experiment Runs

In order to study the formation of DBPs during wastewater disinfection with PAA, it is important to understand changes to the wastewater characteristics during PAA disinfection, which may have significant influence on the quantity and spectrum of DBP formation. Therefore, the project was started with a series of preliminary tests studying the impact of different dosages of PAA/NaClO and contact time on parameters such as pH, specific UV_{254} absorbance, TOC, COD, NH_3-N in wastewater. After that, reactions of selected EDCs/Pharmaceuticals (17β-estradiol, clofibric acid and ketoprofen) with PAA/NaClO were conducted to evaluate PAA oxidation efficiency and DBPs formation, followed by investigation of wastewater DBPs formed during PAA/NaClO disinfection. The acronym PAA/NaClO refers to testing with PAA or NaClO respectively and is simplified in this form for sake of convenience.

3.1.1 Wastewater Characteristics

Un-disinfected secondary effluents (SE) from Robert O. Pickard Environmental Center Wastewater Treatment Plant (ROPEC WWTP, Ottawa, ON, Canada) were collected and

transported to Environmental Engineering Laboratory (stored at 4 °C refrigerator) at Carleton University. The specific wastewater treatment processes before disinfection consist of preliminary screening, grit removal, primary clarification, and secondary suspended growth biological treatment including fine pore aeration and secondary clarifiers. The main physico-chemical characteristics of SE wastewater recorded by ROPEC in January 2012 are described in Table 3.1. (The last three parameters-pH, TOC, and UV absorbance were measured in the Environmental Engineering lab)

Table 3.1: Main characteristics of SE wastewater before disinfection in January 2012

Parameters	Unit	Sample #	Minimum	Maximum	Average
$cBOD_5$	ppm	9	5	8	6
COD	ppm	16	39	49	44
TSS	ppm	16	6	18	11
TP	ppm	15	0.18	0.62	0.4
TKN-N	ppm	15	17	23.8	20.4
NH_3-N	ppm	14	16.5	21.6	19.11
Alkalinity	ppm	11	144	175	159
SO_4	ppm	4	61	76	67
pH (25 °C)	---	30	6.42	6.58	6.49
TOC	ppm	20	12.64	13.53	13.25
UV_{254} absorbance	cm^{-1}	22	0.2278	0.2463	0.2343

(Sample #: number of samples; $cBOD_5$: biochemical oxygen demand, 5-day; COD: chemical oxygen demand; TSS: total suspended solids; TP: total phosphorus; TKN-N: total kjeldahl nitrogen; NH_3-N: ammonia; UV absorbance: ultraviolet absorbance at 254 nm and optical path of 1 cm for DBPs formation potential analysis)-data provided by Robert O. Pickard Plant

3.1.2 Preliminary Tests

The preliminary experiments were to investigate how the main physico-chemical parameters in wastewater were affected by different dosages of PAA/NaClO and contact time. A jar testing apparatus at bench scale processed all the runs in 1000 mL beakers. Parameters including pH, UV_{254} absorbance, TOC, COD, and NH_3-N in wastewater were evaluated at the dosages of 0, 1, 2, 4, 6, 8, 10 ppm and contact time of 0, 10, 15, 30, 60 min for both PAA and NaClO disinfection. The same sets of experiments were also conducted in distilled water as comparison. Experimental runs were summarized in Table 3.2. The equipment and methods used for analyzing these parameters were as follows.

pH and UV_{254} absorbance Analysis

pH of samples was measured by a pH meter (Oaklon, pH/mV/°C). The accuracy of the pH meter was first verified by using pH Check Buffers in the range of 4-10. After rinsing with deionized water, the pH electrode was placed into the bottle containing pH 4.00 buffer and then calibrated when the reading was stable. The same process was conducted for pH 7.00 and 10.00 calibrations. Once the pH meter was calibrated, samples were measured.

A Varian 100 Bio UV-Visible spectrophotometer connected with a personal computer was used for the measurement of UV absorbance of samples at 254 nm. The UV spectrophotometer was turned on and warmed up for 20 min before analysis. During the waiting period, the wavelength of the spectrophotometer was set to 254 nm. Samples were measured after zeroing of deionized water to avoid interference.

Table 3.2: Summary of preliminary experiments conducted

Preliminary Tests	Parameters	Regents/ Equipment	Contact time (min)
Wastewater (WW)/distilled water (DW)	pH	A pH meter	60
	UV_{254} absorbance	UV spectrophotometer	10
			60
	TOC (mg C /L)	TOC-V_{CPN} Analyser	0
			10
			15
			30
			60
	COD (mg O_2 /L)	Hach COD vials + DRB200 Reactor + Hach DR 2800 spectrophotometer	0
			10
			15
			30
			60
	NH_3-N (mg N /L)	Hach NH_3-N vials + Salicylate + Cyanurate regents powder pillows + Hach spectrophotometer	0
			10
			15
			30
			60

(All the runs were conducted at PAA/NaClO dosage of 0, 1, 2, 4, 6, 8, 10 ppm)

TOC Analysis

A TOC-V_{CPN} analyzer (Shimadzu Corporation) together with a Shimadzu ASI-V auto sampler, Harris gas (compressed air and oxygen) cylinders, and a computer, was used to quantify the total organic carbon in the samples.

Non-purgeable Organic Carbon (NPOC) method was used to determine TOC by purging the acidified sample (pH = 1.5) with purified air to remove inorganic carbon. Before starting measurement, the instrument and sample table were prepared first. The carrier gas (air) cylinder was turned on, and the pressure was set to between 3-6 bars. A series of operating parameters were checked after turning on the power to TOC analyzer, including carrier gas pressure (200 kPa), flow rate (150 mL/min), water levels (deionized water) in the humidifier, cooler drain container, dilution bottles, wash bottles, and an acidification bottle (hydrogen chloride, HCl).

Once all the conditions went well, the computer was turned on. TOC-Control-V software was opened to edit the sample tables according to the procedures provided in TOC-V_{CPN} manual. Five point calibration curves (0-20 ppm NPOC) were produced by the use of potassium hydrogen phthalate solutions. When samples (duplicate samples) were ready, TOC vials filled with samples (up to 1 cm from the bottom of vial trap) were placed in the vial rack. The position of each vial corresponded with settings in the sample table. One blank sample with deionized water was placed after every five working samples to avoid contamination. 2-3 determinations were conducted for each sample, which resulted in an approximate 30 min running time.

COD Analysis

A Hach Method 8000 based on the reagents/apparatus including Hach COD digestion reagent vials, DRB200 Reactor, and HACH DR 2800 spectrophotometer determined COD values. The DRB200 Reactor was turned on; the temperature and heating time were set to 150 °C and 2-hour, respectively. Appropriate range (ultralow range of 0-40 mg/L for distilled water, low range of 3-150 mg/L for wastewater) of reagent vials were set in a tube rank and marked. Once samples were ready, caps of these regent vials were removed; 2 mL of collected samples were added to the corresponding sample vials by using a volumetric pipette. The blank sample was prepared by adding 2 mL of deionized water to a blank vial. Capping tightly, these vials were inverted gently 10 to 15 times to thoroughly mix the regents and samples. After 2-hr digestion period by DRB200 reactor, vials started to cool. When the vials cooled down to 120 °C, they were removed to the tube rank for rapid cooling to room temperature. A Hach DR 2800 spectrophotometer was then used to measure COD values. A proper range of COD program was selected: 431 COD ULR for ultra-low range test and 430 COD LR for low range tests. After swiping the outside of the vials with a paper towel, the blank sample was inserted to the cell holder and zeroed, followed by the reading of sample COD values.

Ammonia Analysis

NH_3-N was measured by Hach Method 10031-Salicylate Method based on High Range (0-50 mg/L NH_3-N) Test 'N Tube AmVer™ Nitrogen Ammonia Reagent and Hach DR 2800 spectrophotometer. Similar to COD, NH_3-N regent vials were set in a tube rank and marked first. Hach DR 2800 spectrophotometer was turned on and method 343 N, Ammonia HR TNT was selected. 0.1 mL of both samples and reagent blank (deionized water) was added to

the corresponding vials. After adding one Ammonia Salicylate Reagent Powder Pillow and one Cyanurate Powder Pillow, the vials were caped tightly and shake thoroughly to dissolve the powder. Waiting for a 20-minute reaction period, the samples were measured after zeroing the blank.

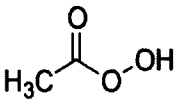
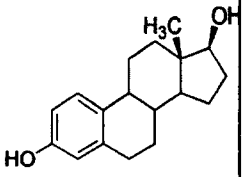
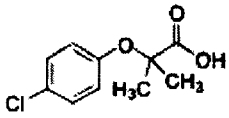
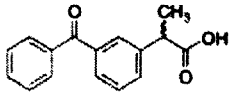
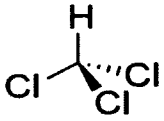
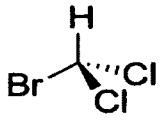
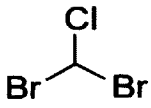
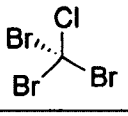
3.1.3 Oxidation Tests of Selected EDCs/Pharmaceuticals

EDCs/Pharmaceuticals including 17β-estradiol (E2), clofibric acid, and ketoprofen were chosen to study PAA oxidation efficiency and possible DBPs formation. These three chemicals as well as methanol (≥ 99.8%) as a solvent were purchased from Sigma-Aldrich and Caledon Laboratory Chemicals, respectively. The characteristics of these chemicals were summarized in Table 3.3. A Varian CP 3800 Gas Chromatography-Flame Ionization Detector (FID)/Varian 1200 Quadrupole Mass Spectrometry (GC-FID/MS) analyzer was used for samples analysis.

The same operating processes were used for these three chemicals. The stock standard solutions were first prepared in methanol to a concentration of 100 mg/L: 10 mg of 17β-estradiol (E2)/clofibric acid/ ketoprofen in a 100 mL of volumetric flask was measured on an analytical balance; the flask was then filled with methanol to the marked line and covered by a screw cap; by inverting and turning the flask about 30 times, the solution was thoroughly mixed and ready to use. Working standard solutions were prepared from the stock solutions.

Two kinds of calibration curves at the concentration range of 1, 2, 4, 6, and 10 ppm were constructed by GC/MS: direct analysis and processed standards.

Table 3.3: Characteristics of the target chemicals

Chemicals	Formula	MW (g/mol)	Density (g/cm^3)	Melting Point (°C)	Boiling Point (°C)	Structure
Peracetic Acid (15%)	$C_2H_4O_3$	76.05	1.13 @ 20°C	-49	109	
Sodium Hypochlorite (10.3%)	NaClO	74.44	1.166 @ 20°C	-25	40	$Na^+ (ClO)^-$
Sodium Thiosulfate	$Na_2S_2O_3$	158.11	1.667	48.3	100	
17β-estradiol	$C_{18}H_{24}O_2$	272.38	---	176-180	---	
Clofibric Acid	$C_{10}H_{11}ClO_3$	214.65	---	120-122	---	
Ketoprofen	$C_{16}H_{14}O_3$	254.28	---	---	---	
Chloroform	$CHCl_3$	119.38	1.489 @ 20°C	-63.5	61.2	
Bromodichloromethane	$CHBrCl_2$	163.83	1.98 @ 25°C	-55	87	
Dibromochlormethane	$CHBr_2Cl$	208.28	2.451 @ 25°C	-22	119-120	
Bromoform	$CHBr_3$	252.73	2.89 @ 25°C	5-8	146-150	

Calibration Procedure One: Direct Analysis. Diluted standard solutions were directly dissolved in methanol and analyzed by GC/MS. The target concentrations were achieved by serial dilutions in methanol with 10 mL volumetric flasks and graduated cylinders. The transferred volumes corresponding to the target concentrations in 10 mL volumetric flasks were: 1 mL from 100 mL standard stock solution to 10 ppm solution, 6 mL from 10 ppm to 6 ppm solution, 6.7 mL from 6 ppm to 4 ppm, 5 mL from 4 ppm to 2 ppm, and 5 mL from 2 ppm to 1 ppm. Disposable pipettes were used to transfer these solutions during the serial dilution process.

Calibration Procedure Two: Processed Standards. The other calibration curve was built based on a series of sample treatment processes (jar testing, acidification, extraction, evaporation, and methanol derivative, seen in Section 3.2) for 17β-estradiol (E2)/clofibric acid/ketoprofen mixed in distilled water. For 1, 2, 4, 6, and 10 ppm applied in 1000 mL distilled water, the corresponding volumes needed from 100 ppm stock solution were: 10, 20, 30, 60, and 100 mL. By jar testing mixing for 60 min, 250 mL of these samples as calibration samples were collected, treated, and finally analyzed by GC/MS.

Similar runs were also done for (distilled water only), (10 ppm PAA/NaClO + distilled water), and (1 ppm 17β-estradiol (E2)/clofibric acid/ketoprofen+10 ppm PAA/NaClO + distilled water). Through comparisons of the various mass spectrums obtained from these runs: before and after treatment with the two disinfectants, new peaks found in the disinfected mass spectrum were considered to be DBPs or degradation products, which were finally identified and quantified by an appropriate GC/MS method, seen in Section 4.2 and Section 4.3. The sampling operations at each programmed experimental conditions were

repeated three times, and for each time three injections were conducted to determine the reproducibility of the obtained results. UV_{254} absorbances for both untreated and PAA/NaClO-treated samples were also measured to analyze DBPs formation potential.

3.1.4 Wastewater DBPs Formation by PAA/NaClO

THMs, as the most commonly detected DBPs in wastewater chlorination were first targeted in this project to evaluate toxicity in PAA/NaClO treated wastewater. Standards solutions including chloroform (Caledon Laboratory Chemicals), bromodichloromethane, dibromochloromethane, and bromoform (Sigma-Aldrich, Canada) were purchased. Pentane (Anhydrous $\geq$ 99%, Sigma-Aldrich) was used as a solvent for these chemicals. The main properties of THMs are illustrated in Table 3.3.

Two different concentrations of standard stock solutions were prepared: 10 g/L and 50 mg/L. For each THM, an analytical balance was used to measure 100 mg of a standard solution in a 10 mL volumetric flask. The 10 mL flask was then filled with pentane to the marked point, inverted and turned to achieve mixing. In this way, a 10 g/L stock solution was produced. Based on 10 g/L solution, 1 g/L and 50 mg/L solutions were made by serial dilutions: 1 mL of 10g/L solution and 0.5 mL of 1 g/L solution were successively transported by two volumetric pipettes to two 10 mL volumetric flasks.

50 mg/L standard stock solutions were mainly used to develop a method by GC/FID. The detailed information about method development for four THMs was described in Section 4.1. 10 g/L standard stock solutions were used for THMs calibration by GC/MS. There were two calibration curves constructed according to two different mass spectra modes: full-scan

and selected ion monitoring (SIM), which are described in Section 3.3.2. The target concentration range of 1, 2, 4, 6, and 10 ppm THMs for both calibration methods were achieved by serial dilutions.

The process was similar as the first calibration method of selected EDCs/Pharmaceuticals. Five 10 mL of volumetric flask and graduated cylinders were prepared and marked from #1 to #5, representing 10 ppm to 1 ppm in order. 1 mL of 10 g/L solution measured by #1 graduated cylinder was added to #1 volumetric flask, filled with pentane, forming 10 ppm working solution. Similarly, 6 mL, 6.7 mL, 5 mL, and 5 mL of 10 ppm, 6 ppm, 4 ppm, and 2 ppm solutions were separately added to #2, #3, #4, and #5 volumetric flasks to form 6 ppm, 4 ppm, 2 ppm, and 1 ppm working solutions. These working solutions were analyzed by GC/MS for further confirmation and quantification (calibration).

After the calibration curves were produced, un-disinfected and disinfected wastewaters samples were analyzed by both GC/FID and GC/MS. Dosage of 10 ppm PAA/NaClO and a contact time of 60 min were chosen to pioneer the experiment to investigate potential DBPs formation. Triplicate samples were studied and UV_{254} absorbance was monitored.

3.2 Sample Preparation Procedures

General sample preparation procedures were illustrated in this section, shown as Figure 3.1, including stock solution preparation and volumes calculation, cleanup for glassware, sample preparation by jar testing, quenching and acidification, liquid-liquid extraction, rotary evaporator drying, and solvent derivative for GC/MS analysis. Processes after extraction

including evaporation, derivation, and GC/MS analysis were conducted in the lab of Dr. Jeffrey Manthorpe in the Chemistry Department, Carleton University, Canada.

3.2.1 Stock Solution Preparation and Calculation of Volumes Needed

All the experimental trials conducted used PAA/NaClO as disinfectants and sodium thiosulfate ($Na_2S_2O_3$) as quenching agent. PAA commercial stock solution (15% w/w PAA, 10 % w/w H_2O_2, 33% w/w acetic acid and 42% water, FMC corporation-peroxygens division, Philadelphia, PA) and NaClO stock solution (10.3% w/w, LAVO "12", Montreal, QC) were diluted (1: 500) first by deionized water in 500 mL or 1000 mL volumetric flasks, respectively. A 1 L stock solution of 500 ppm anhydrous sodium thiosulfate ($\geq$ 98% $Na_2S_2O_3$, Sigma-Aldrich, Canada) was prepared.

For the target PAA/NaClO dosages (1, 2, 4, 6, 8, 10 ppm) in 1 L distilled water/ wastewater, their corresponding volumes of diluted PAA and NaClO stock solutions were calculated before conducting the experiments. The calculation procedures for 1 ppm PAA/NaClO are shown in Table 3.4. Different volumes applied for different dosages are summarized in Table 3.5. In case the concentrations of diluted PAA and NaClO decreases as time goes, PAA and NaClO solutions were diluted just before each run.

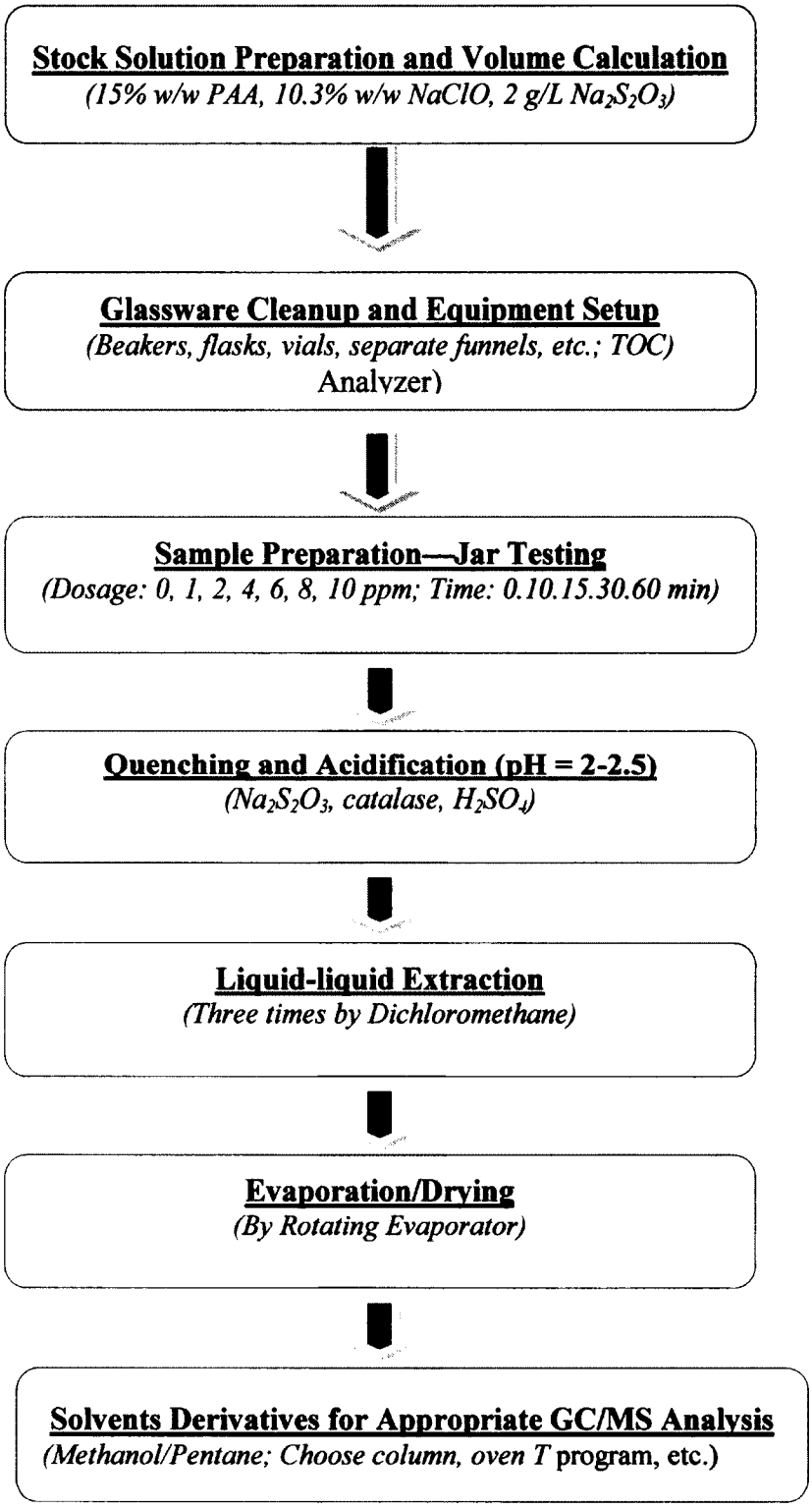


Figure 3.1: Sample preparation procedures

Table 3.4: Volumes needed for 1 ppm PAA/NaClO in 1 L water samples

Parameters		15% (w/w) PAA	10.3% (w/w) NaClO
Specific Gravity (SG)		1.13	1.166
Density		$1.13 * 10^6$ ppm	$1.166 * 10^6$ ppm
Concentration	In stock solution	$15\% * 1.13 * 10^6$ ppm = 169500 ppm	$10.3\% * 1.166 * 10^6$ ppm = 120098 ppm
	In diluted solution (1: 500)	169500 ppm / 500 = 339 ppm	120098 ppm / 500 = 240.196 ppm
Volume of diluted solution for 1 ppm applied dosage in 1 L distilled water/wastewater		1 ppm * 1000 mL / 339 ppm = 2.95 mL	1 ppm * 1000 mL / 240.196 ppm = 4.16 mL

Table 3.5: Volumes added for different dosages of PAA/NaClO in 1 L water samples

Dosage (ppm)	PAA (mL)	NaClO (mL)
1	2.95	4.16
2	5.9	8.32
4	11.8	16.64
6	17.7	24.96
8	23.6	33.28
10	29.5	41.6

For 2 g/L $Na_2S_2O_3$, the theoretical concentration needed for 1 ppm PAA in 1 L distilled water/wastewater was calculated according to the following reaction equation:

$$CH_3COOOH + Na_2S_2O_3 \rightarrow CH_3COOH + Na_2SO_4 + 1/8\ S_8 \qquad \text{(Reaction 3.1)}$$

MW (g/mol): 76 158

Concentration (ppm): 1 158 / 76 = 2.08

Volume of $Na_2S_2O_3$ added (mL): 2.08 ppm * 1000 mL / 2000 ppm = 1.04 mL

The corresponding volumes of $Na_2S_2O_3$ added for the target dosages were illustrated in Table 3.6.

Table 3.6: Theoretical calculated volumes of 2 g/L $Na_2S_2O_3$ for quenching different dosages of PAA/NaClO

PAA/NaClO Dosage (ppm)	Volume of $Na_2S_2O_3$ Calculated (mL)	
	For 1000 mL samples	For 100 mL samples
1	1.04	0.1
2	2.08	0.2
4	4.16	0.4
6	8.32	0.8
8	12.48	1.2
10	16.64	1.6

3.2.2 Glassware Cleanup and Equipment Setup

To avoid any interference during operation processes, all the beakers, flasks, graduated cylinders, vials, and separatory funnels were cleaned before and after experiments in the following order:

Tap water was first used to remove bigger residuals in the glassware. To loosen and remove most contaminants, glassware was soaked with soapy water and cleaned thoroughly by scrubbing with a brush. Distilled water was then used to rinse glassware at least three times to make sure no ions remained in the glassware, followed by a final rinse by acetone (Caledon Laboratory Chemicals). The cleaned glassware was eventually suspended upside down for air-drying on the shelf.

3.2.3 Jar Testing

A jar testing apparatus at laboratory scale was used in this project to simulate disinfection contact chamber conditions in WWTP. Generally, 4 beakers (2 for two different dosage of PAA and 2 for NaClO at the same dosages) at a time filled with 1000 mL distilled water or wastewater samples were used to conduct reactions. The freshly prepared PAA/NaClO working solutions were dosed according to volumes determined in Table 3.5 before turning on the stirrers. During this process, graduated cylinders and volumetric pipettes were used for volume measurement and transfer. In order to quickly and thoroughly mix the disinfectants and samples, the stirrers were operated at a high RPM of 120 for the first 5 min and reduced to 60 RPM to simulate the actual mixing speed in WWTP. After a target time, a certain amount of samples were collected: 100 mL for parameters measurement and 250 mL for DBPs analysis.

3.2.4 Quenching and Sample Acidification

To evaluate effects of different dosages (0, 1, 2, 4, 6, 8, 10 ppm) and contact time (0, 10, 15, 30, 60 min) on parameters change and DBPs formation in disinfected samples, PAA/NaClO

residuals need to be quenched first. 1 L of 500 ppm sodium thiosulfate ($Na_2S_2O_3$) was used to quench both PAA and NaClO according to the volumes calculate in Table 3.6. For PAA-disinfected samples, PAA was quenched after H_2O_2 decomposition by 0.1 mL catalase (bovine liver C30-aqueous suspension, 10,000-40,000 units/mg protein by Sigma-Aldrich). $Na_2S_2O_3$ was allowed to react for 10 min with PAA/NaClO and stirred either at RPM of 100 by jar testing or by a stirring rod. The *N, N* diethyl-p-phenylene-diamine (DPD) colorimetric method was used to check the residual concentrations after initial quenching.

Hach Method 10070, high range (0.1-10 mg/L as Cl_2) of total chlorine DPD method was used to measure residual concentrations in the samples, which was based on DPD total chlorine reagent powder pillows, 10 mL/1 cm sample cell and Hach DR 2800 spectrophotometer. First, the spectrophotometer was turned on and the 88 Chlorine F&T HR program was selected. A sample cell filled with 5 mL of the sample was inserted into the cell holder, zeroed, and then removed. One DPD total chlorine powder pillow for 25 mL samples was successively added to this cell, shaking around 20 seconds to dissolve reagent. Waiting for 3 minute longer, the cell was inserted into the cell holder to measure the chlorine concentration.

If residuals were still found in the quenched samples, their corresponding quenching volumes were adjusted and checked again until no residual was detected. The final actual volumes of 2 g/L $Na_2S_2O_3$ used for quenching PAA/NaClO in distilled water/wastewater are shown in Table 3.7. The detailed adjustment processes are shown in Appendix A.

Acidification, as a following step, was achieved by adding 95-98% sulfuric acid (H_2SO_4, Anathema Canada Inc., Montreal, QC) to achieve a pH between 2 and 2.5. Sulfuric acid as a strong acid was chosen because it could react with the extra sodium thiosulfate after quenching by the following reactions:

$$H_2SO_4 + Na_2S_2O_3 \longrightarrow Na_2SO_4 + S + SO_2 + H_2O \qquad \text{(Reaction 3.2)}$$

Table 3.7: Actual volumes of 2 g/L $Na_2S_2O_3$ used for quenching PAA/NaClO in 100 mL distilled water/wastewater

Dosage (ppm)	Theoretical value (mL)	Actual values for DW (mL)		Actual values for WW (mL)	
		PAA	NaClO	PAA	NaClO
1	0.1	1.2	0.1	1.2	1.2
2	0.2	2.4	0.2	2.4	2.4
4	0.4	4.8	0.4	4.8	4.8
6	0.8	7.2	0.8	7.2	7.2
8	1.2	9.6	1.2	9.6	9.6
10	1.6	12.0	1.6	12.0	12.0

3.2.5 Liquid-liquid Extraction

An acidified wastewater sample (250 mL in 500 mL flask) was then transported to a fume hood workstation for liquid-liquid extraction process. A 500 mL stopcock-closed separatory funnel was supported in a ring stand, and dichloromethane (≥ 99%, Caledon Laboratory

Chemicals) was prepared as an extract solvent. Because the density of dichloromethane (1.33 g/cm^3) is higher than water, the lower layer after separating was collected.

The specific procedures were described as follows: A 250 mL sample was poured into the separatory funnel and 125 mL dichloromethane was added. After placing a stopper, the funnel was removed out of the ring, rocked once gently, and inverted to release excess pressure by opening the stopcock. After that, the funnel was shaked vigorously for about 1 min to sufficiently mix the two solvents. During this process, pressure was released at intervals of a few seconds. After thoroughly mixing, the funnel was put back in the ring and kept undisturbed until the layers were clearly separated. While waiting, the stopper was removed, and a 500 mL Erlenmeyer flask was placed under the funnel. Once two layers were separated, the stopcock was open allowing the lower layer to drain into the flask. To the point that the upper liquid barely reached the stopcock, the stopcock was closed.

This process was repeated three times to achieve high extraction efficiency. The extracted solvents were combined and a total volume of 375 mL was collected. The extracted samples were then carefully transported to the chemistry lab for further treatment and analysis.

3.2.6 Evaporation/Drying

0.50 g of sodium sulfite (Anhydrous, ACS Grade, Bioshop, Canada) was added to remove any possible water present in the extracted samples. Medium frits connected with adapters were used to filter these samples to 1 L round bottom flasks.

A Heidolph Laborota 4011 digital Evaporator was used for evaporation and drying processes. Water supply, Heidolph Rotavac Valve Control pump, Heidolph Vac Control Automatic, and the evaporator were turned on. The 1 L round bottom flask filled with extracted samples was then clipped to the evaporator by using a connector and a Keck clip. Their height was adjusted to keep at least 1/3 volume of flask under the water bath (40 °C). Vacuum for dichloromethane was set to 600-700 mbar and speed was adjusted to 115-120 rpm. The evaporation process for dichloromethane with a 375 mL sample was approximately 30 min long. When no liquid found in the flask, the vac control was turned off and the speed was returned to 0. The waste bottle was emptied. The dried round bottom flask was removed and transported to workstation for ethyl acetate (≥ 99.5%, Caledon Laboratory Chemicals, Canada) further derivative. 15 mL of ethyl acetate was added to the round bottom flask, shake for 5 min, and transferred by a disposable pipette to a 50 mL pear-shaped flask. An appropriate bump trap instead of a connector was then clipped with the pear-shaped flask to start drying process. For ethyl acetate, vacuum was adjusted to 120-150 mbar and running time last at least 1 hour long.

3.2.7 Solvent Derivatives

The completely dried samples were then dissolved in the appropriate solvents preparing for GC/MS analysis. In this project, two solvents were used: methanol for deriving the oxidation products of selected EDCs/Pharmaceuticals by PAA/NaClO; pentane for DBPs formed from wastewater disinfection with PAA/NaClO.

3.3 GC/MS Analysis

A Varian GC-FID/MS analyzer was used to identify and quantify the possible DBPs in these samples. GC-FID was first used to determine an appropriate method for identifying a certain group of compound. Once the method was decided, MS detector was turned on, replacing FID for further qualitative and quantitative analysis.

3.3.1 GC Method Development

GC method development is the first and the most important step during GC/MS analysis. Professional knowledge and significant amounts of practice and experience are needed to build a proper method for a specific group of compounds. This section aimed to describe the ideas and approach to developing a GC method involved in this project. The specific methods development processes for different target compounds were illustrated in Chapter 4.

Method Development, conducted by GC-FID, is a process of determining optimized GC conditions for detecting a target compound. From equipment setting, column selection, to temperature and flow rate adjustments, appropriate GC operating conditions are very important to achieving good performance and reliable results. Specifically, these conditions include column stationary phase, column dimensions, column oven temperature, carrier gas selection, gas flow rate, and injection technique. The impacts of these conditions on GC performance and general application guide are summarized in Table 3.8 [57, 66].

Table 3.8: Main parameters for method development during GC/MS analysis [57, 66]

GC Conditions	Parameters	Impacts	Application Notes
Stationary Phase	(Thickness, Uniformity, Chemical Nature)	Retention time, Separation efficiency	1) Similar polarity as the solute 2) Use least polar stationary phase
Column Dimensions	**Length**	Shorter L: Adv.: shorter retention time, lower pressure Dis-adv.: lower efficiency	1) Shorter: for samples containing small number of compounds 2) Longer (≥ 60m): for extremely complex samples 3) Commonly used: 20-30 m
	Diameter	Smaller D: Adv.: higher efficiency Dis-adv.: higher pressure, lower capacity, longer retention time	1) 0.18-0.25 mm: for higher column efficiency needed 2) 0.32 mm: for larger injection volumes (>2 μL)
	Film Thickness	Thinner Film: Adv.: shorter retention time Dis-adv.: lower capacities	1) Thin film: high boiling solute 2) Thick film: very volatile solute
Column Oven T	Isothermal	Poor separation and resolution for complex samples	Only used for solutes with similar retention time
	T program	Adv.: better separation efficiency for complex samples, shorter retention time Dis-adv.: longer GC oven	No shortcuts- -**Trial and error**: Setting Initial T and hold time, adjusting ramp rate, determining final T and

GC Conditions	Parameters	Impacts	Application Notes
		cooling time, more difficult	time
Carrier Gas And Makeup Gas	Gas Selection	Resolution: Hydrogen > Helium > nitrogen Retention time: Hydrogen < Helium < nitrogen (very long)	Depends on detector, sample, and availability, priorities are: 1) **Helium (He)**: carrier gas 2) **Hydrogen (H_2), Nitrogen (N_2), Air**: makeup gas
	Flow Rate	<u>Lower v</u>: Adv.: increase resolution Dis-adv.: longer retention time	
Injector	T	**250°C** (default)	Default settings are sufficient, but 1) 150-200°C: volatile samples 2) 275-300°C: high boiling samples
	Volume (μL)	**1 μL** (default)	
	Split Ratio	Separation efficiency, accuracy, re-productivity	1) Splitless: for trace level analysis 2) Split ratio: at least 1/10, 1/50

(Bold: parameters mainly concerned in this project)

Seeking an accurate and ideal method for a target chemical always begin with the selection of a column. Among the various GC conditions, four parameters play significant roles in selecting a proper column: stationary phase (film coated onto the inner wall of a column), column diameter, column length, and film thickness. These parameters have

greatest influence on both GC performance (retention time, sample capacity, separation efficiency) and resolution of results, illustrated in Table 3.8. It seems that longer, smaller diameter, thicker columns can achieve higher separation efficiencies, but at the same time retention time and pressure are also significantly increased. Therefore, selecting the right column cannot only rely on these application guides, but it also needs a series of trial and error processes.

Another challenge is the application of column oven temperature. Because isothermal temperature condition is only suitable for a limited number of analyses, a temperature program is usually required to achieve higher separation efficiency [66]. Developing a temperature program includes setting the initial temperature and hold time, adjusting the different ramp rates for different middle temperatures, as well as determining final temperature and hold time. This information might be found from the application notes provided by supplier, but in most cases, adjustment and trial and error are involved. The uncertainty in selecting a column and oven temperature program ensures that method development is an extremely challengeable and time-consuming task during GC-FID/MS analysis.

In this project, to save time and reduce trial and error, an initial GC column for a target substance was selected based on the suggestion by GC manufacturers and suppliers, advice of experienced GC users, and availability. Searching for previous analysis information for the target group of compounds was conducted in this project to obtain the initial temperature program and split ratio for a similar column used. The maximum allowable operating temperature (isothermal and programmed) could be found from the column certificate

provided by the supplier. Injector temperature, inject volume, detector temperature, and carrier gas were defaulted to 250 °C, 1 μL, 300 °C, and helium, respectively.

Once all GC conditions were initially determined, a FID method could be built. The first trial FID tests include a solvent blank run and a higher concentration (such as 20 g/L) standard solution of a compound (compound + solvent blank) run. Comparison of two FID results, whether the target compound was identified or not, as well as the resolution of peaks could be shown. When no compound peaks were found, different temperature programs were attempted. When there were compound peaks but the resolution of peaks was not satisfactory, carrier gas flow rate and injector split ratio were adjusted. The worst cases happened in this project, after the initial column was selected numerous attempts at different temperature programs still could not separate some target compounds or result in satisfactory peak resolution, eventually a different column was used. After a series of trial and error processes, an appropriate method for a certain group of compounds was eventually decided and saved in the FID mode. Retention time for a target compound could also be found.

3.3.2 MS Analysis and Data Representation

After the target compound was identified by a method finally developed by GC-FID, MS was then turned on to conduct further analysis.

Because MS is very sensitive for contaminants, the performance of MS was checked before analysis. Air/water level, including N_2, N_2/O_2, N_2/H_2O was conducted first for leak checking. If there was leak reported, transfer line/ column nut, GC injector septum, valve block, and clip in the column were checked. Once no significant leak was detected, various

electronics diagnostics were run for troubleshooting, followed by an auto-tune conducted for hardware checks, baseline calibration, and resolution check. When all the three operation went well, a MS method could be built.

GC conditions in the MS methods were set to the same conditions as that in FID method. The mass range was set depending on two different analysis methods: Full-scan MS, and Selected ion monitoring (SIM). In this project, the mass range for Full-scan MS analysis was set as 75 to 400 m/z, while for SIM analysis, the mass range was set according to the mass of a target compound. Full-scan MS was first used to detect all the peaks within a spectrum, a plot of intensity versus mass-to-charge ratio. It provided more information than SIM, such as the mass fragments and isotopic composition for different peaks, which could help confirm a known compound and also identify an unknown compound in a sample. If only a small quantity of a target compound could be detected in Full-scan mode, the sensitivity of MS for this compound needed to be improved. SIM could definitely accomplish it because only the target compound was analyzed. SIM was mainly aimed at the known compounds.

The mass spectra obtained from MS analysis need to be further interpreted. Interpretation of mass spectra was achieved by combination of various techniques, knowledge and experience. A library of mass spectra was first used to compare the experimental mass spectrum against the spectra in the library. If library could not identify a peak, manual interpretation followed. Manual interpretation was based on the mass spectrum analysis. In a mass spectrum, the tallest peak is assigned 100% value, and the other peaks are assigned different proportionate values. A parent peak, which resulted from losing one electron from the molecule, could indicate the total mass of an unknown compound. The

value of this peak could be used to fit with a chemical formula that contained the elements from different reactants. This formula was then checked by a large amount of fragment peaks in the spectrum. Identification of these peaks in the spectrum helped in structure elucidation of an unknown compound. The detailed identification processes for different peaks found in different samples were illustrated in Chapter 4.

Chapter 4

METHOD DEVELOPMENT: RESULTS AND DISCUSSION

As explained in Chapter 3, method development is a very complicated, time-consuming, and challenging task during GC analysis. A series of trial and error processes was needed to develop a proper method for analyzing a specific compound. Based on the guidelines and concepts explained in Section 3.3, methods for analyzing the target compounds including four THMs (chloroform, bromodichloromethane, dibromochloromethane, and bromoform), 17β-estradiol (E2), clofibric acid, and ketoprofen were all developed.

4.1 Method Development for THMs

Method development processes for THMs included six stages: stages 1-3 preliminary peak identification by GC/FID, stage 4 -5 peak confirmation by MS, and stage 6 summary of the method that was finally used for THMs.

4.1.1 Stage 1-Varian CP3800 GC + J&W DB-1 column, Environmental Engineering Lab

At the beginning of this project, the available equipment and column were Varian CP3800 GC in Environmental Engineering Lab, and Agilent J&W DB-1 column 15 m * 0.32 mm * 0.25 μm, respectively. Since the auto-sampler in the GC was broken, sampling and injection were conducted manually using a 10-μL syringe. The column oven temperature program was decided based on the GC operating manuals and methods described by [82]. In case of the

DB-1, 15 m * 0.32 mm * 0.25 μm column, an initial temperature of 40 °C that was held 2 min was used to improve the resolution of earlier eluting peaks [82]. A mild ramp rate of 10 °C /min was chosen to reach the final temperature of 250 °C, as suggested by the manual. The final hold time was set to 10 min. THMs calibration mix (200 mg/L each component in methanol, ≤ 99.92 % methanol, and ≤0.02% THMs) purchased from Sigma-Aldrich, Canada was used to identify and calibrate peaks. The specific GC conditions used in stage 1 are summarized in Table 4.1.

After GC conditions were set in FID method and activated, 1 μL of triplicate standard samples (200 mg/L THMs in methanol) was separately injected into the GC. Results are shown in Figure 4.1.

As shown in Figure 4.1b, six peaks were found: one large peak (methanol) at 0.5 min and five small peaks (1-5) at 15 min, 23.3 min, 24.5 min, 26.5 min, and 29.2 min, respectively. The small peaks could not be shown in Figure 4.1a, because the intensities of these peaks were too small compared to the first peak (methanol). The identification of the four THMs from these five peaks was almost impossible for GC/FID. The reason was that the standard of THMs was a mixture of four THMs that could not be monitored separately by FID, and the FID was unable to provide component and structure information for structure determination. Therefore, separate standards and better equipment were needed.

In addition to the peak identification problem, excessive baseline noise (the problem of signal to noise ratio) was found in Figure 4.1b. Since the signal intensity in FID is related to how well a compound burns, it was probably that THMs did not burn well. The other

possible reason might be the contamination of injector, column, detector and/or septum degradation [82]. By consulting Dr. Onita D. Basu and technician Marie Tudoret Chow in the

Table 4.1: GC conditions for THMs in stage 1

Conditions	Parameters	For THMs
Equipment	GC	Varian CP3800 GC + Manual Sampling/Injection
Default Settings	Carrier Gas	Helium
	H_2, Air, N_2 Flow	30, 300, 29 mL/min
	Injector Temperature	250 °C
	Detector Temperature	300 °C
	Inject Volume	1 µL
Standards	THMs Calibration Mix	200 mg/L each component in Methanol
Column	Stationery Phase	Agilent J&W Scientific DB-1
	Dimensions	15 m * 0.32 mm * 0.25 µm Length * Diameter * Film Thickness
	Maximum Allowable Oven Temperature Programmed	325 °C
	Oven Temperature Program	40 °C hold 2 min; 10 °C /min to 250 °C, hold 10 min; Total 33 min
Injection	Split Ratio	Splitless

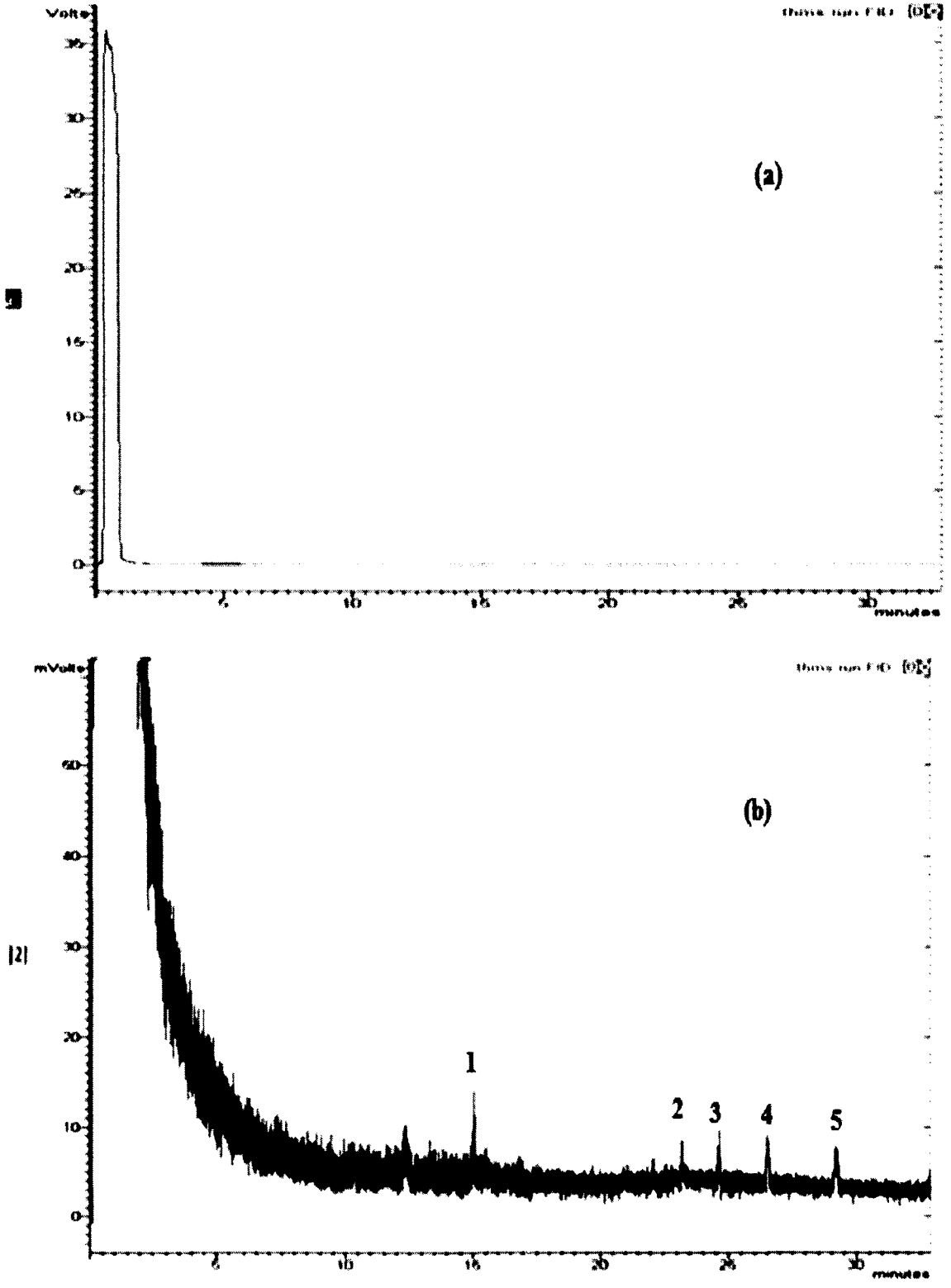


Figure 4.1: Stage 1- FID results for 200 mg/L THMs in methanol: (a) original scale (35 Volts), (b) smaller scale (60 mVolts)

Environmental Engineering Lab, it was found that the GC equipment and column applied in this stage were too old to be further used for sample analysis. The column had been installed in this GC since 1999 and the equipment has not been used for at least seven years. It was considered likely that past contaminants remained in the injector, column, and detector, which could cause a series of problems, even the direct failure of analysis.

Other drawbacks of this GC include the absence of an auto-sampler and an old computer connected to the system. Manual sampling and injection could not only increase the inaccuracy of retention time obtained, but also cause the sample contamination. The computer connected with GC was old and lack of USB interface for data acquisition. Therefore, better equipment was needed.

4.1.2 Stage 2- Varian CP 3800 GC/1200 MS + VF-5ms Column, Chemistry Lab

With the assistance of Dr Jeffrey Manthorpe in the Chemistry department, superior equipment (Varian CP3800 GC/1200 MS with an auto-sampler) and column (VF-5ms, 30 m * 0.25 mm * 0.25 μm) were employed. Since this equipment has the same GC (Varian CP3800) as that used in stage 1, the same default settings in Figure 4.1 were continually used. Separate THMs standards (no solvents) and a split ratio of 1:10 were applied according to the improvement methods proposed in stage 1. Pentane was chosen as a derivative solvent for THMs standards [67]. A mixture of ether and hexane was used as washing solvents before and after each run.

Determination of oven temperature program in this stage was based on the boiling point (a measure of compound volatility). The order of boiling points of the four THMs is:

chloroform ($CHCl_3$, 61.2 °C) < brodichloromethane ($CHBrCl_2$, 87 °C) < dibromochloromethane ($CHBr_2Cl$, 119-120 °C) < bromoform ($CHBr_3$, 146-150 °C). To make sure that the four THMs could be identified, the initial temperature has to be lower than the lowest boiling point (61.2 °C) and the final temperature has to ensure that all materials have eluted from the column. It was noticed that boiling points of $CHCl_3$ and $CHBrCl_2$ differ by only 15.8 °C, which might make separating these two compounds more difficult. Therefore, a relatively low ramp rate of 2 °C /min was used to increase the resolution. The corresponding temperature program suggested by Dr. Jeffrey Manthorpe was shown in Table 4.2, where the adjusted GC conditions were summarized.

THMs standards were run based on the boiling point. Generally, the compounds elute in order of their increasing boiling point [82], which means the order of retention time for the four THMs is: $CHBr_3$ > $CHBr_2Cl$ > $CHBrCl_2$ > $CHCl_3$. Therefore, to ensure all the four THMs could be detected, $CHBr_3$ were conducted first to check whether it could elute within the total running time determined by the oven temperature program. The specific operation order was as follows (Triplicate injections for each run were conducted to check reproducibility of results):

1. Blank-pentane;
2. $CHBr_3$ + pentane;
3. $CHBr_2Cl$ + $CHBr_3$ + pentane;
4. $CHBrCl_2$ + $CHBr_2Cl$ + $CHBr_3$ + pentane;
5. $CHCl_3$ + $CHBrCl_2$ + $CHBr_2Cl$ + $CHBr_3$ + pentane.

Table 4.2: Adjusted GC conditions for THMs in stage 2

Conditions	Parameters	For THMs
Equipment	GC/MS	Varian CP3800 GC/1200 MS + Auto-sampler
Standards	Four THMs	Chloroform, bromodichloromethane, dibromochloromethane, bromoform
Column	Stationery Phase	Factor Four ™ Capillary Column VF-5ms
	Dimensions	30 m * 0.25 mm * 0.25 μm Length * Diameter * Film Thickness
	Maximum Allowable Oven Temperature Programmed	350 °C
	Oven Temperature Program	40 °C hold 15 min; 2 °C /min to 80 °C, hold 5 min; 60 °C /min to 250 °C, hold 3 min; Total 45.83 min
Solvent	Derivative/Washing Solvent	Pentane/(Ether + Hexane)
Injection	Split Ratio	1:10

Through comparison of the FID results obtained from these runs, the four THMs could be easily detected. 20 g/L stock solution of each standard was prepared and analyzed by GC/FID according to the order mentioned above. The FID results for the first three runs are shown in Figure 4.2.

Comparison of these three chromatograms in Figure 4.2, peaks appeared at 1.2 min in Figure 4.2b and at 0.9 min in Figuere 4.2c were probably $CHBr_3$ and $CHBr_2Cl$, respectively. Although these peaks were preliminarily identified, poor separation and peak resolution were found. Pentane was found to elute at the beginning, around t = 0.45 min, followed by the other two peaks ($CHBr_2Cl$ and $CHBr_3$, Figure 4.2 c). The locations of these two peaks were very close to the pentane peak. In this way, the four THMs peaks would elute within 2 min and no additional peaks were located in the remaining 43 min of the program. For this reason, only the first three runs were conducted. To increase the resolution of these peaks, it was thought that decreasing the flow rate might help, but these peaks would elute in the same way (gathered together). Therefore, it was also considered that the oven temperature should be adjusted or perhaps an entirely new column should be tested.

In addition to the problems mentioned above, extra small peaks were detected at the end of the first chromatogram (blank). The location of these peaks indicated that not the sample contamination but injector or column contamination happened. To avoid these contaminations, at least runs of each solvent blank sample need to be run before sample runs. After attempting to adjust the temperature programs and no good results were found, Dr. Onita D. Basu thought a column more specific for identification of volatile compounds like THMs was needed.

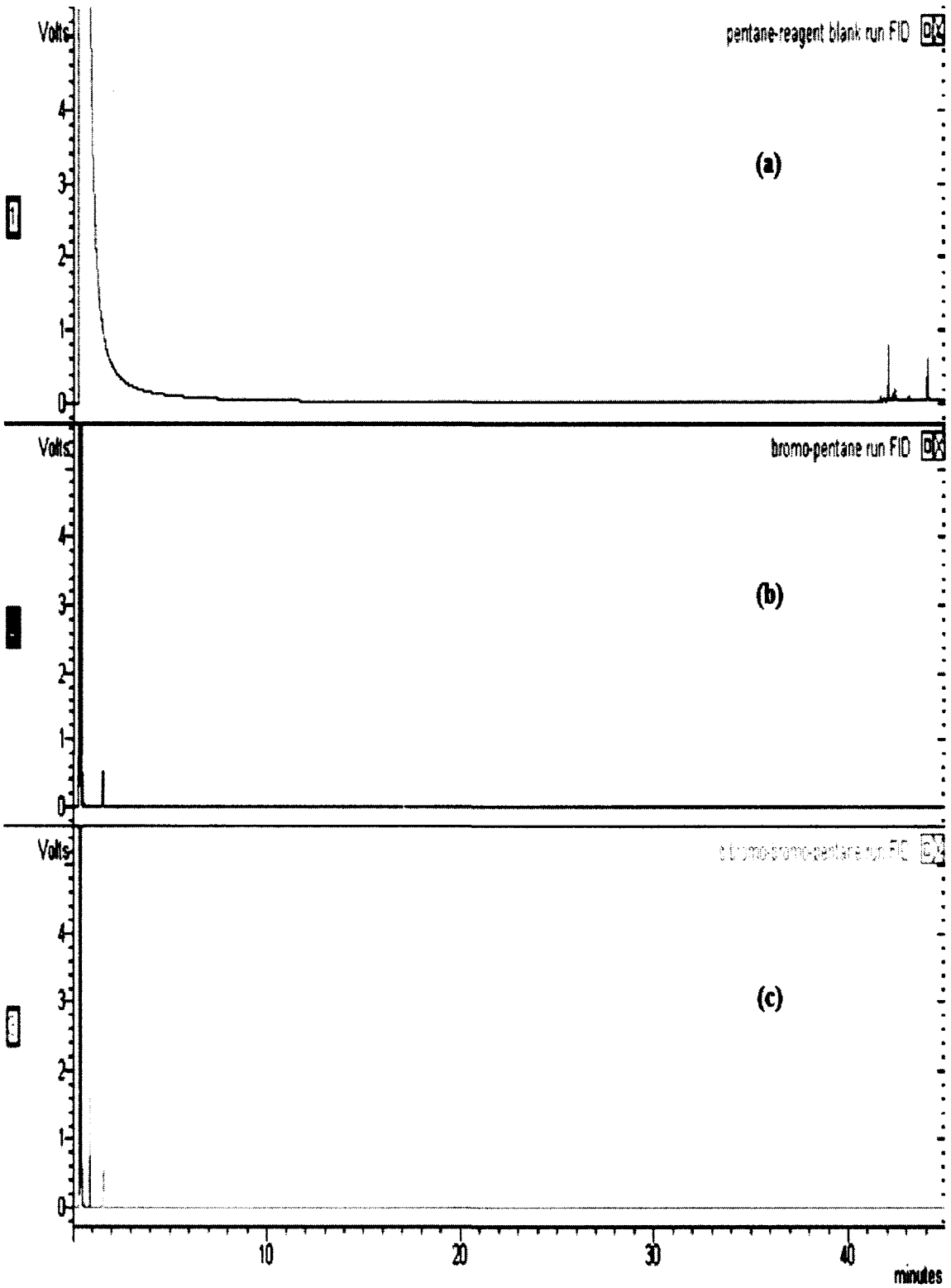


Figure 4.2: Stage 2 - FID results for (a) blank (pentane), (b) 20 g/L $CHBr_3$ + blank, (c) 20 g/L $CHBr_2Cl$ + $CHBr_3$ + blank

4.1.3 Stage 3-Varian CP 3800 GC/1200 MS + CP-selected 624 CB Column, Chemistry Lab

A new column targeting for THMs - Varian CP-selected 624 CB 60 m * 0.25 mm * 1.4 μm - was bought and used in this stage. The oven temperature program was found from the application note provided by the supplier, shown in Table 4.3. Since it is impossible to cool a GC oven below 30 °C in the laboratory environments (the room temperature is 25 °C), the initial temperature was increased from 20 °C to 30 °C. Correspondingly to make up this change, the ramp rate 8 °C /min was reduced to 7 °C /min. Pentane was continually used as solvent because its boiling point (36.1 °C) is lower than other readily available solvents. Another solvent, methanol, was also used as a comparison.

Experimental runs in this stage were conducted based on the operation order proposed in stage 2. The exactly same runs were conducted in both pentane- and methanol- derived samples. Their corresponding FID results are shown in Figure 4.3 and Figure 4.4, respectively.

As shown in Figure 4.3 and Figure 4.4, it was found that the four THMs could be easily found in both pentane- and methanol- derived samples, and the peak resolution was also very good. However, one problem was found: there were 6 extra peaks in the pentane derived samples (Figure 4.3) and 5 extra peaks in the methanol derived samples (Figure 4.4). Four common peaks exist in both figures, which illustrated that contaminants were probably involved. To find out sources of contamination as well as further confirm THMs peaks, MS analysis was needed.

Table 4.3: Adjusted GC conditions for THMs in stage 3

Conditions	Parameters	For THMs
Column	Stationery Phase	Varian CP-selected 624 CB
	Dimensions	60 m * 0.25 mm * 1.4 μm Length * Diameter * Film Thickness
	Maximum Allowable Oven Temperature Programmed	280 °C
	Oven Temperature Program	20 °C hold 4 min; 8 °C /min to 80 °C, hold 4 min; 17 °C /min to 220 °C, hold 12 min; Total 35.38 min
Solvent	Derivative/Washing Solvent	Trial 1: Pentane/(Ether + Hexane) Trial 2: Methanol//(Ether + Hexane)

(The underlined numbers were from application note, and changed based on the actual conditions in the analytical lab.)

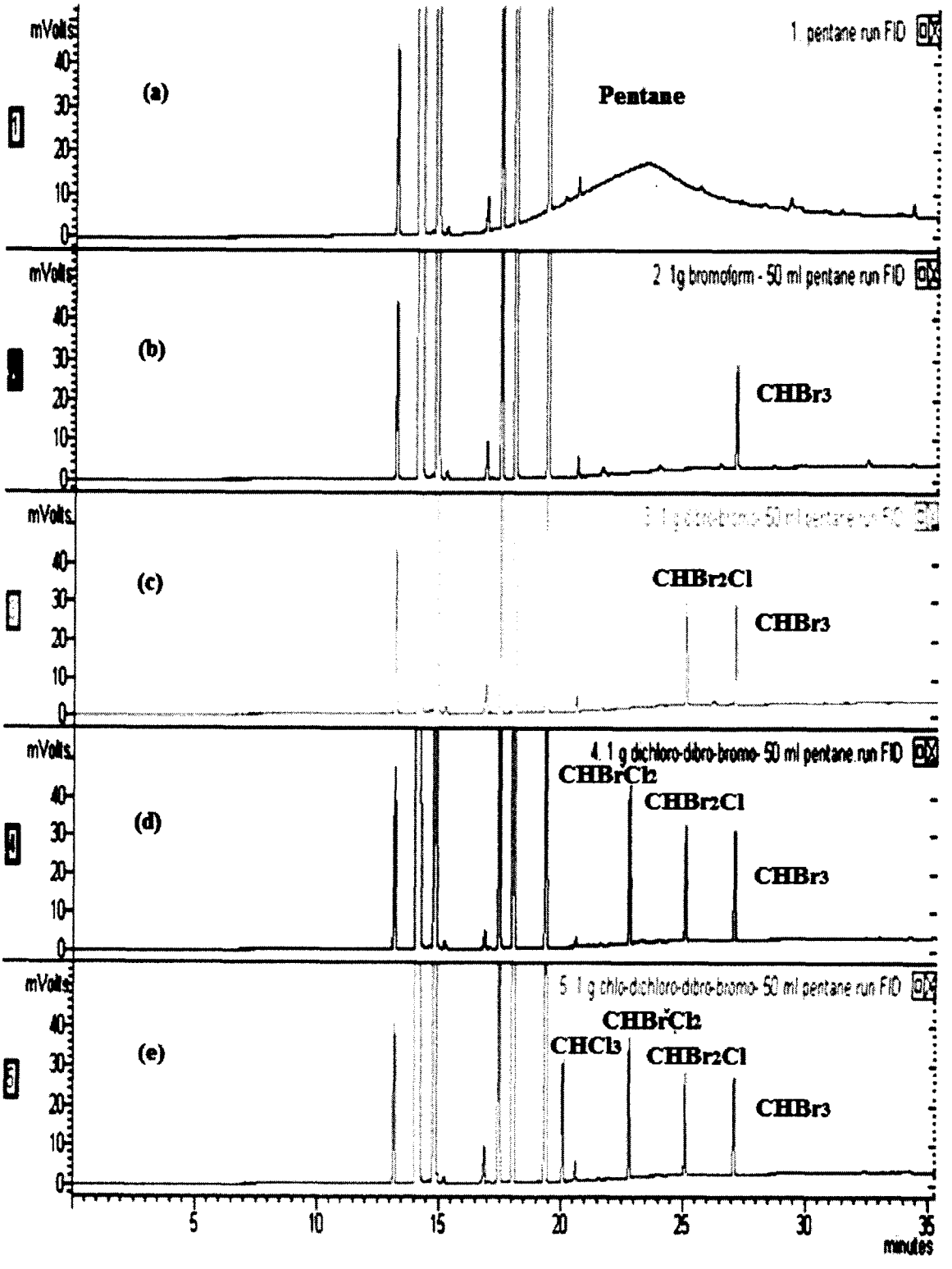


Figure 4.3: Stage 3 - FID results for (a) pentane, (b) 20 g/L $CHBr_3$ + pentane, (c) 20 g/L $CHBr_2Cl$ + $CHBr_3$ + pentane, (d) 20 g/L $CHBrCl_2$ + $CHBr_2Cl$ + $CHBr_3$ + pentane, (e) 20 g/L $CHCl_3$ + $CHBrCl_2$ + $CHBr_2Cl$ + $CHBr_3$ + pentane

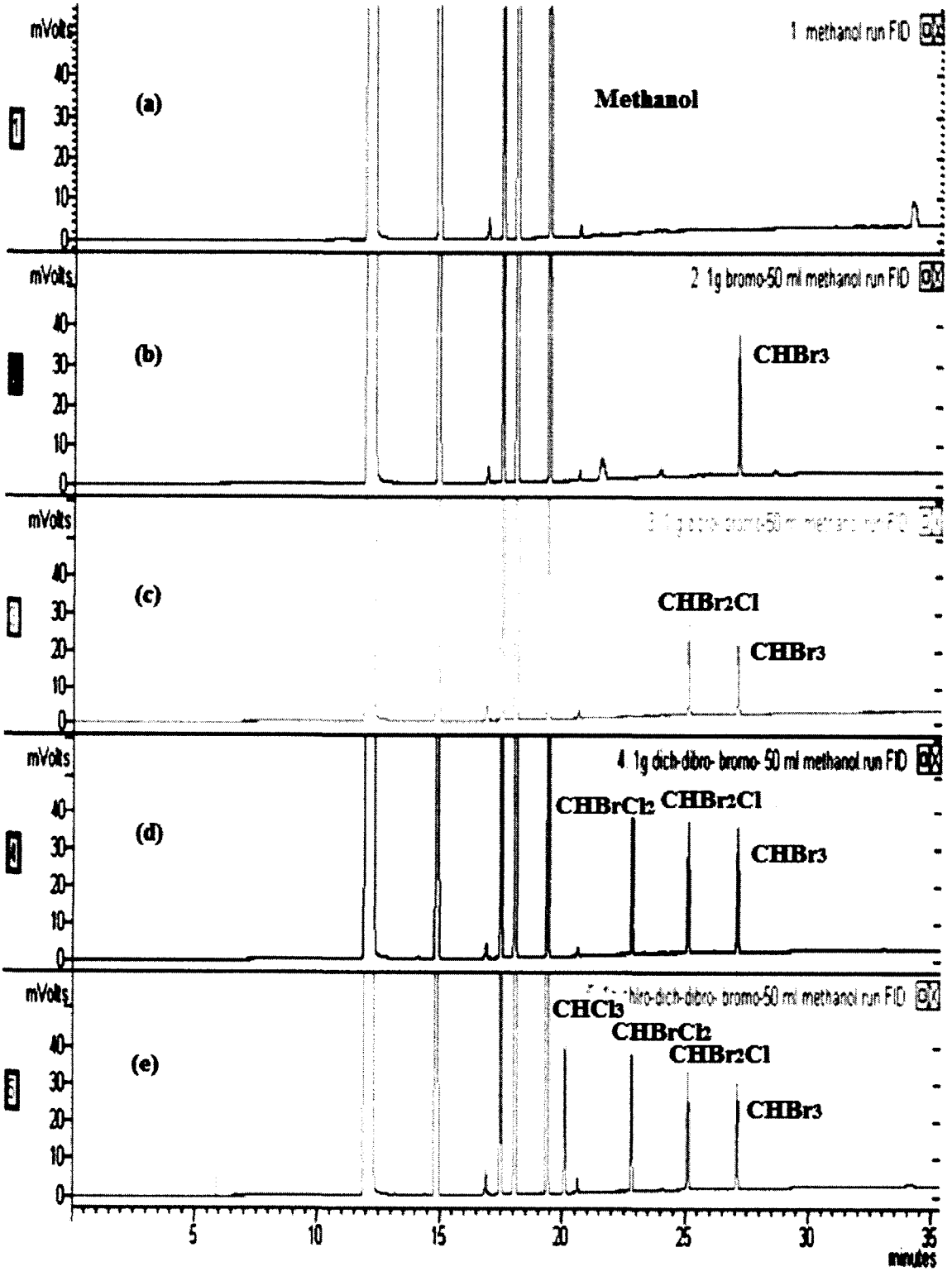


Figure 4.4: Stage 3 - FID results for (a) methanol, (b) 20 g/L $CHBr_3$ + methanol, (c) 20 g/L $CHBr_2Cl$ + $CHBr_3$ + methanol, (d) 20 g/L $CHBrCl_2$ + $CHBr_2Cl$ + $CHBr_3$ + methanol, (e) 20 g/L $CHCl_3$ + $CHBrCl_2$ + $CHBr_2Cl$ + $CHBr_3$ + methanol

4.1.4 Stage 4-Varian 1200 MS Confirmation for 20 g/L THMs

The solvent blank (Figure 4.3a and Figure 4.4a) and THMs + blank (Figure 4.3e and Figure 4.4e) were analyzed by a full-scan MS mode (25-400 m/z), where identical column and the same GC conditions were used as that in FID tests. The MS results are shown in Figure 4.5.

From Figure 4.5, it was found that THMs eluted at the same retention time in both pentane- and methanol- derived samples. Retention times of THMs obtained in the above two chromatograms are summarized in Table 4.4. It seemed that both pentane and methanol could be used for derivation of THMs, but considering the alcohol groups in methanol might interfere with results obtained, pentane was chosen for further analysis.

Table 4.4: Retention time of THMs in both pentane- and methanol-derived samples

Target Compounds	Retention Time (min)	
	Pentane	Methanol
Blank	10.642	10.635
$CHBr_3$	24.816	24.815
$CHBr_2Cl$	22.887	22.886
$CHBrCl_2$	20.495	20.499
$CHCl_3$	17.351	17.357

The peaks appeared in Figure 4.5 were identified by searching the library of spectra in the computer. The spectra of the solvents used are shown in Figure 4.6.

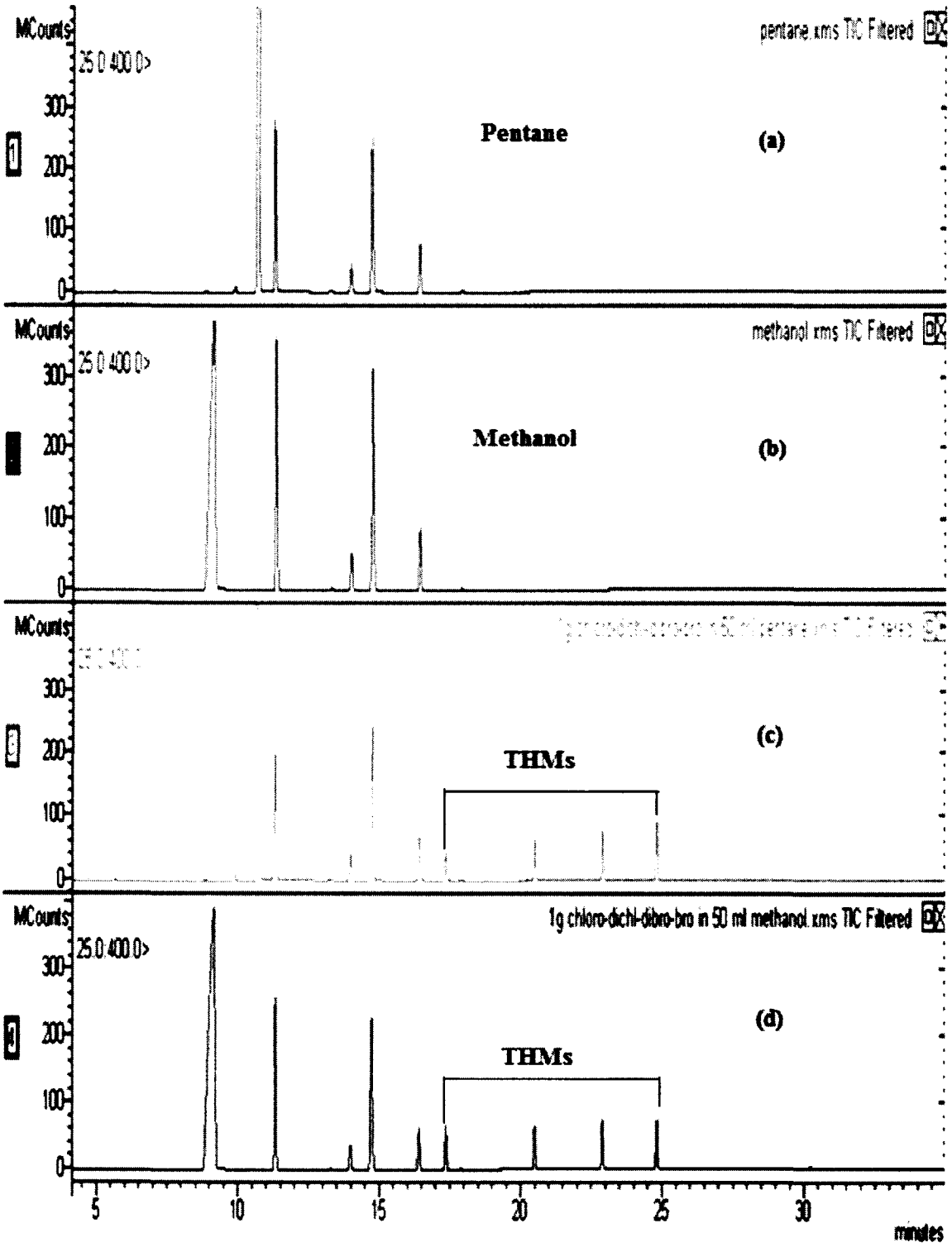
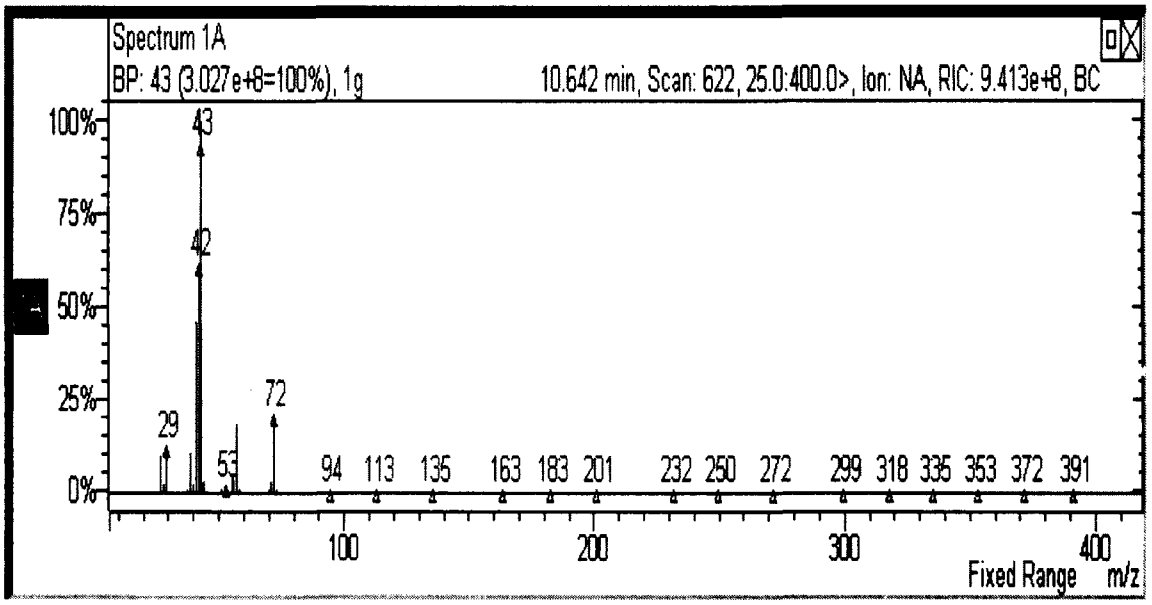


Figure 4.5: Full-scan MS analysis for (a) pentane, (b) methanol, (c) 20 g/L THMs + pentane, and (d) 20 g/L THMs + methanol

Pentane (C_5H_{12}, 72.15 g/mol, 10. 642 min)



Methanol (CH_4O, 32.04 g/mol, 9.147 min)

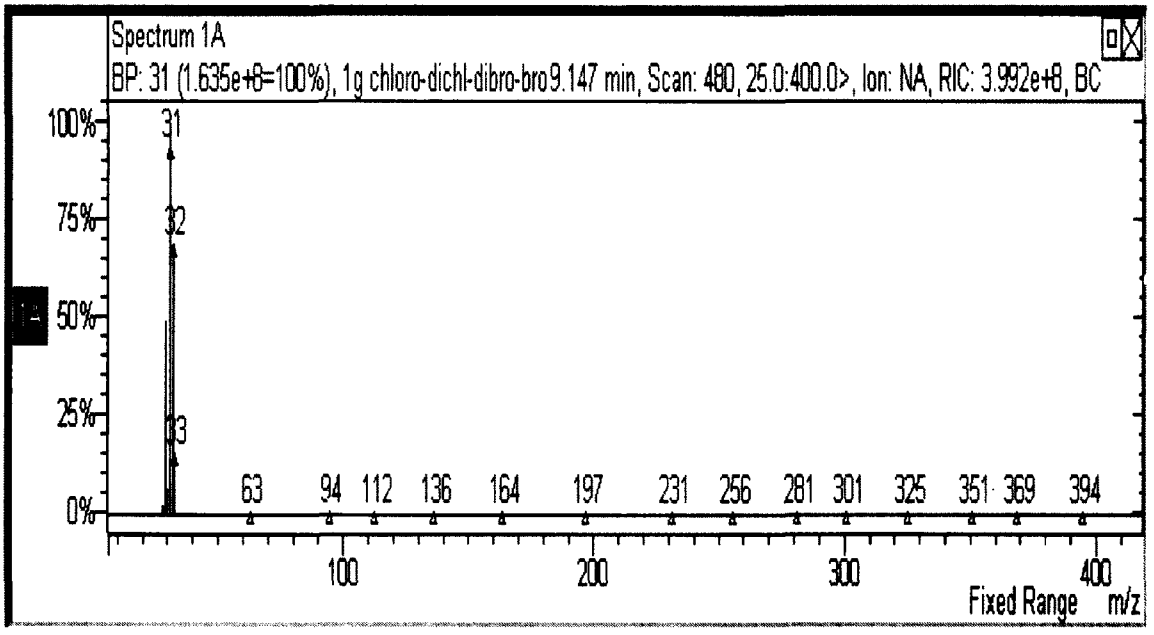
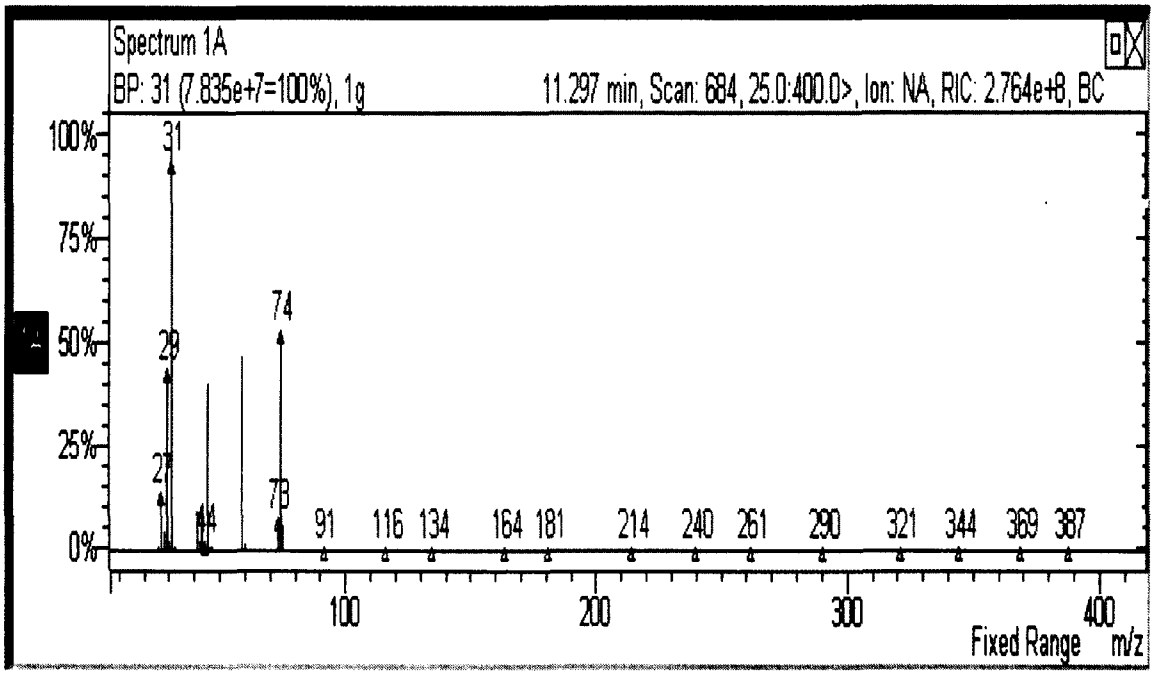


Figure 4.6: MS spectra of pentane and methanol

The four common "contamination" peaks that appeared in both pentane and methanol blank were identified as ethyl ether, 3-methylpentane, hexane, and methylcyclopentane, shown in Figure 4.7 and Figure 4.8.

Ethyl ether ($C_4H_{10}O$, 74.12 g/mol, 11.297 min)



3-methylpentane (C_6H_{14}, 86.18 g/mol, 13.985 min)

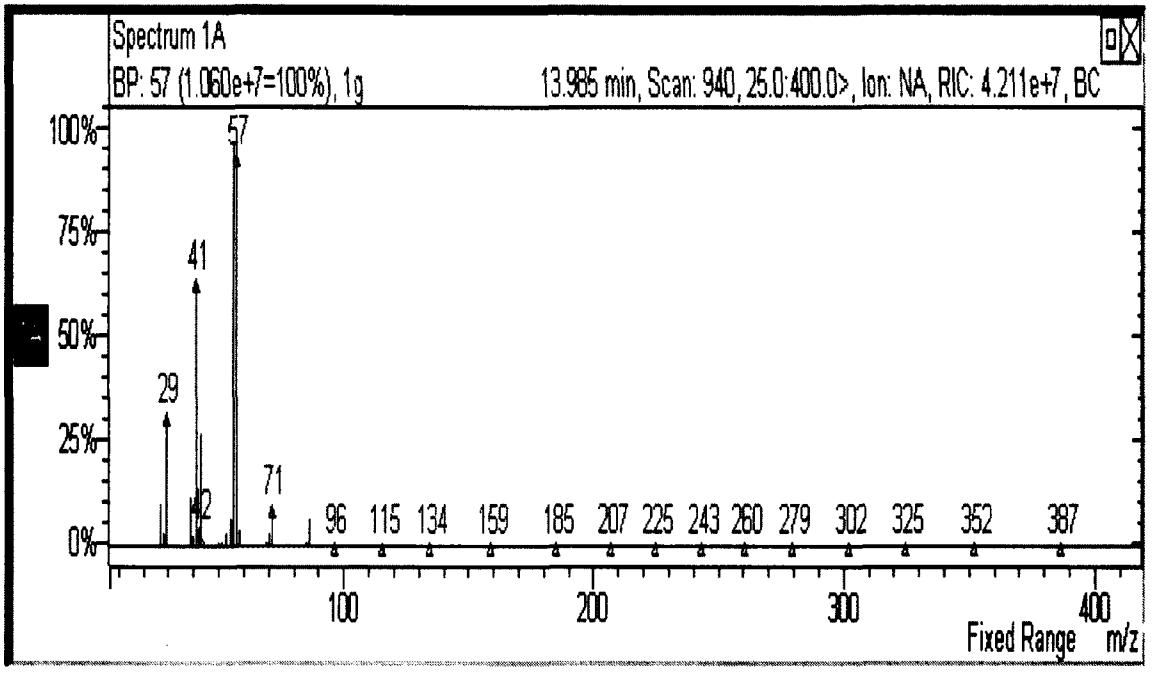
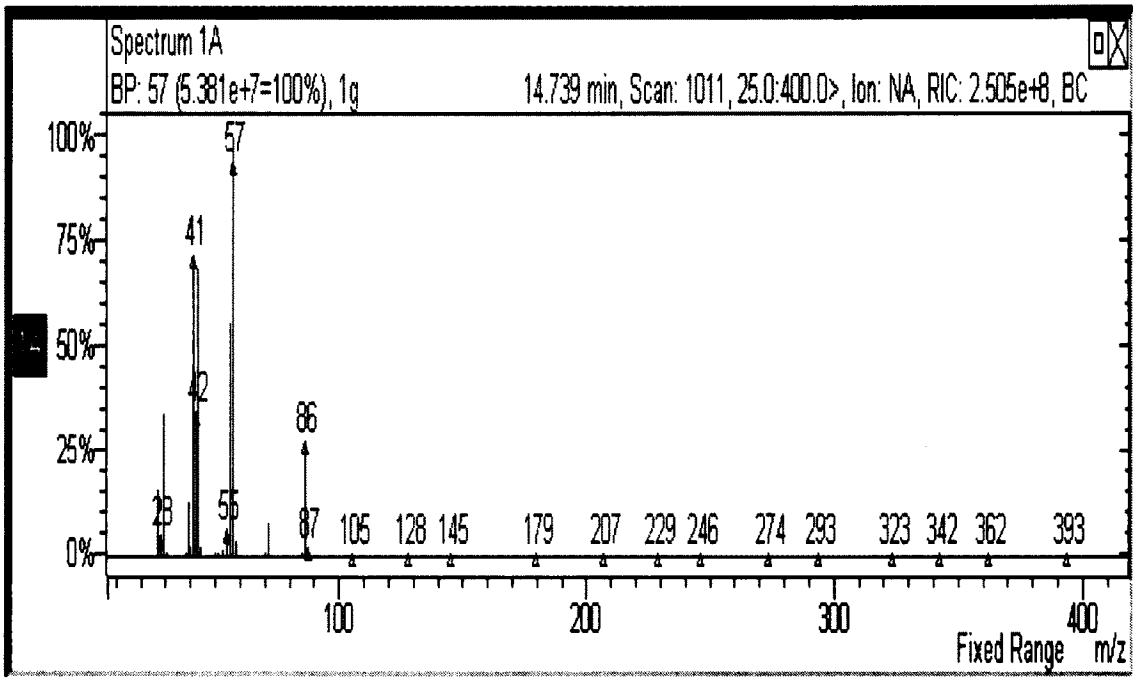


Figure 4.7: MS spectra of ethyl ether and 3-methylpentane

Hexane (C_6H_{14}, 86.18 g/mol, 14.739 min)



Methylcyclopentane (C_6H_{12}, 84.16 g/ml, 16.411 min)

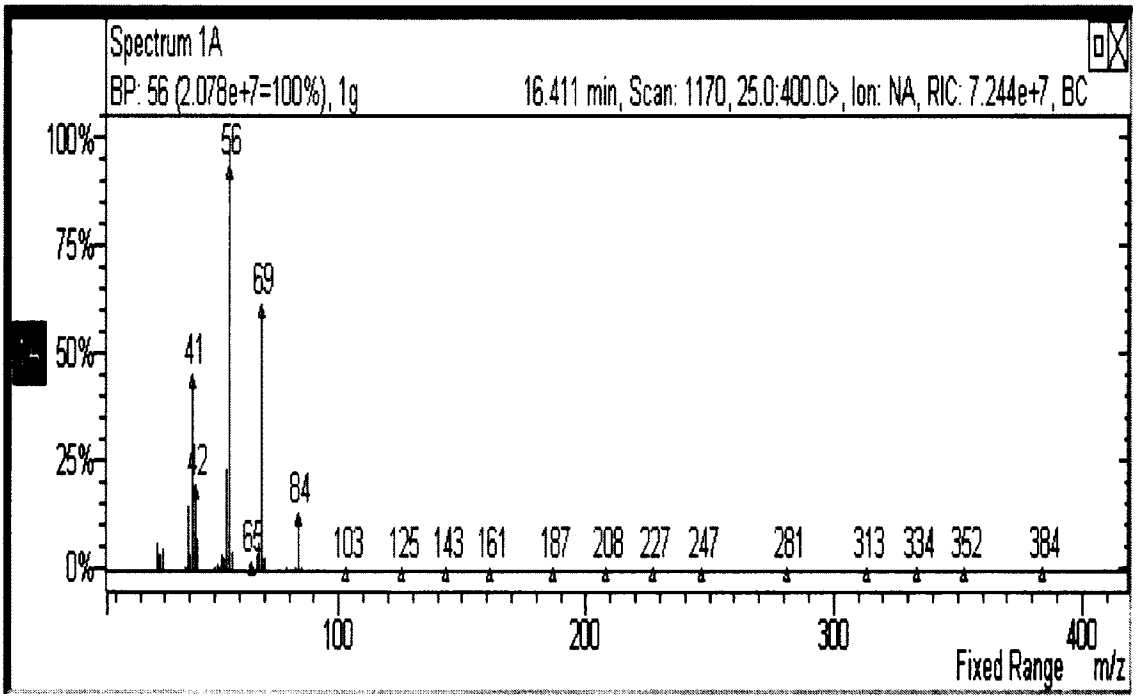


Figure 4.8: MS spectra of hexane and methylcyclopentane

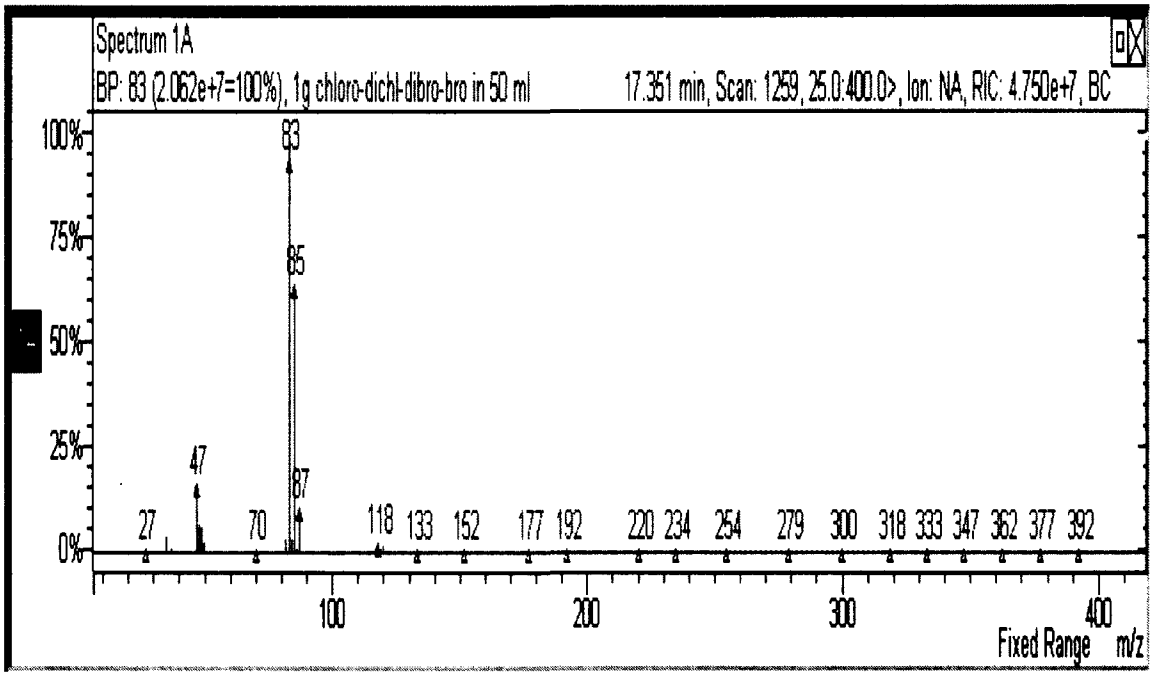
All the four "contaminant" peaks were from washing solvents. The auto-sampler was pre-washed and post-washed by these solvents before and after sampling. During these processes, these solvents might be mixed with the sample and injected into the column. To avoid this contamination, the washing solvents should be used as the same as the derivative solvents-therefore in future THMs runs pentane was used as the washing solvent.

The final four peaks in Figure 4.5 were the four THMs, confirmed by MS. Their spectra are shown in Figure 4.9 and Figure 4.10. The ions that have 100% intensity in these MS spectra are the main ions fragmented from the molecular ion. The fragment processes for THMs are shown in Table 4.5.

Table 4.5: Main fragment ions of THMs

THMs	MW (g/mol)	Lost Ion (g/mol)	Exact Mass of the main fragment ion (g/mol)
$CHCl_3$	119.38	Cl^-, 35.453	119.38 - 35.453 = 83.927
$CHBrCl_2$	163.8	Br^-, 79.904	163.8 -79.904 = 83.896
$CHBr_2Cl$	208.28	Br^-, 79.904	208.28 - 79.904 = 128.376
$CHBr_3$	252.73	Br^-, 79.904	252.73 -79.904 = 172.826

Chloroform ($CHCl_3$, 119.38 g/mol, 17.351 min)



Bromodichloromethane ($CHBrCl_2$, 163.8 g/mol, 20.495 min)

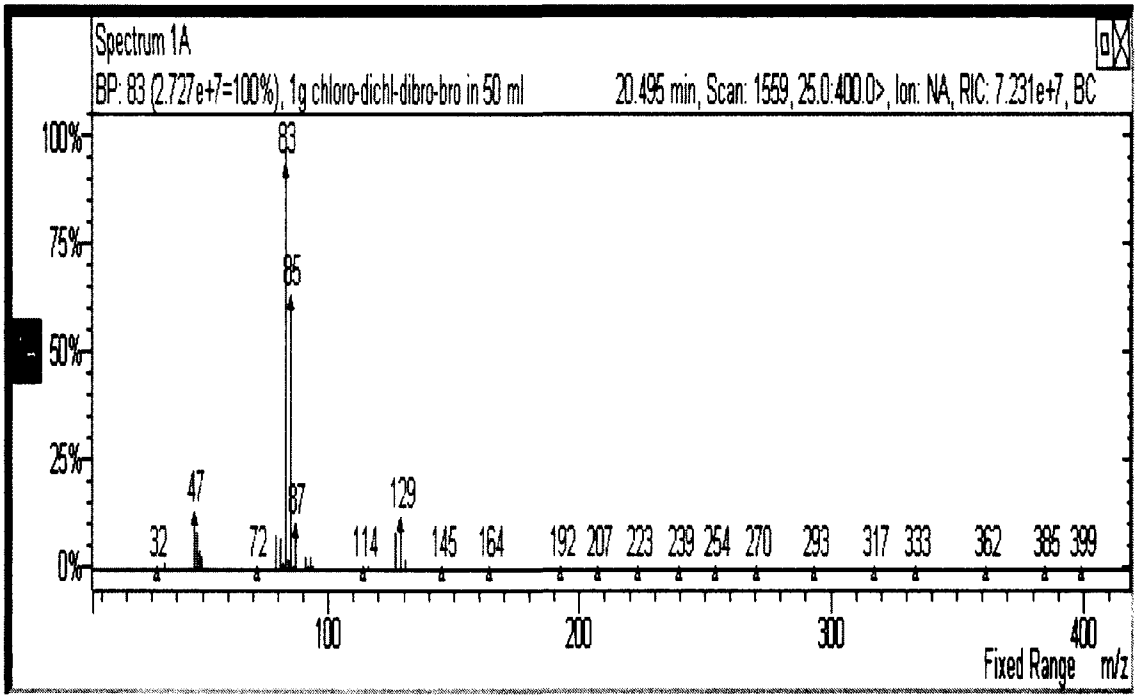
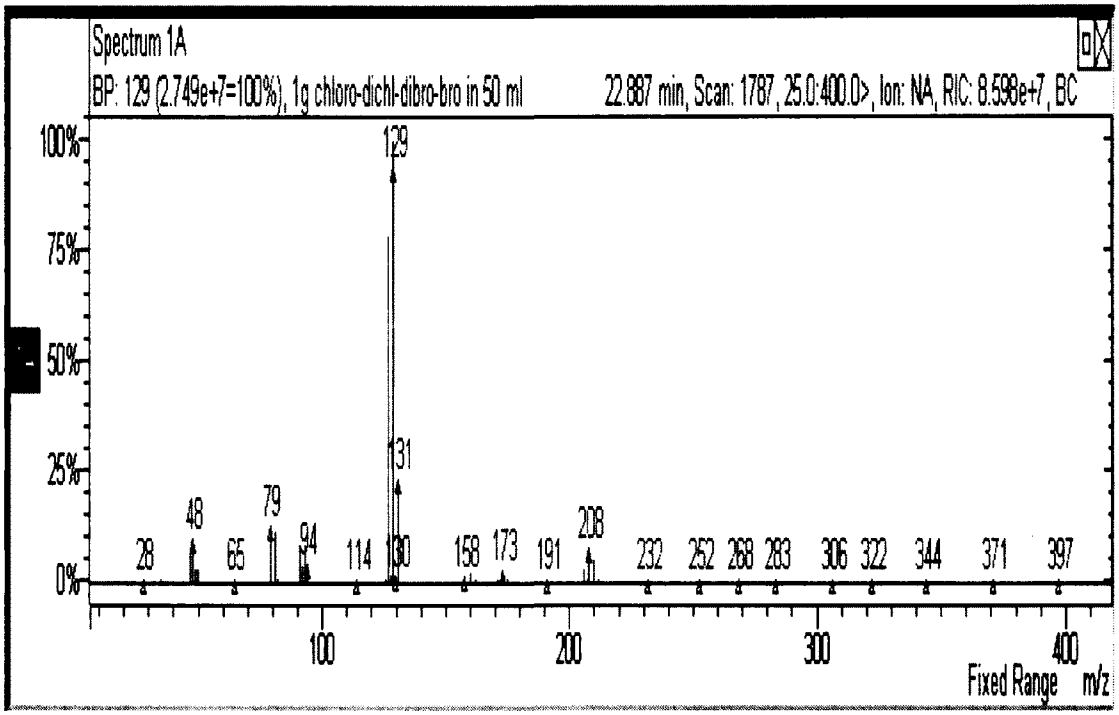


Figure 4.9: MS spectra of chloroform and bromodichloromethane

Dibromochloromethane ($CHBr_2Cl$, 208.28 g/mol, 22.887 min)



Bromoform ($CHBr_3$, 252.73 g/mol, 24.816 min)

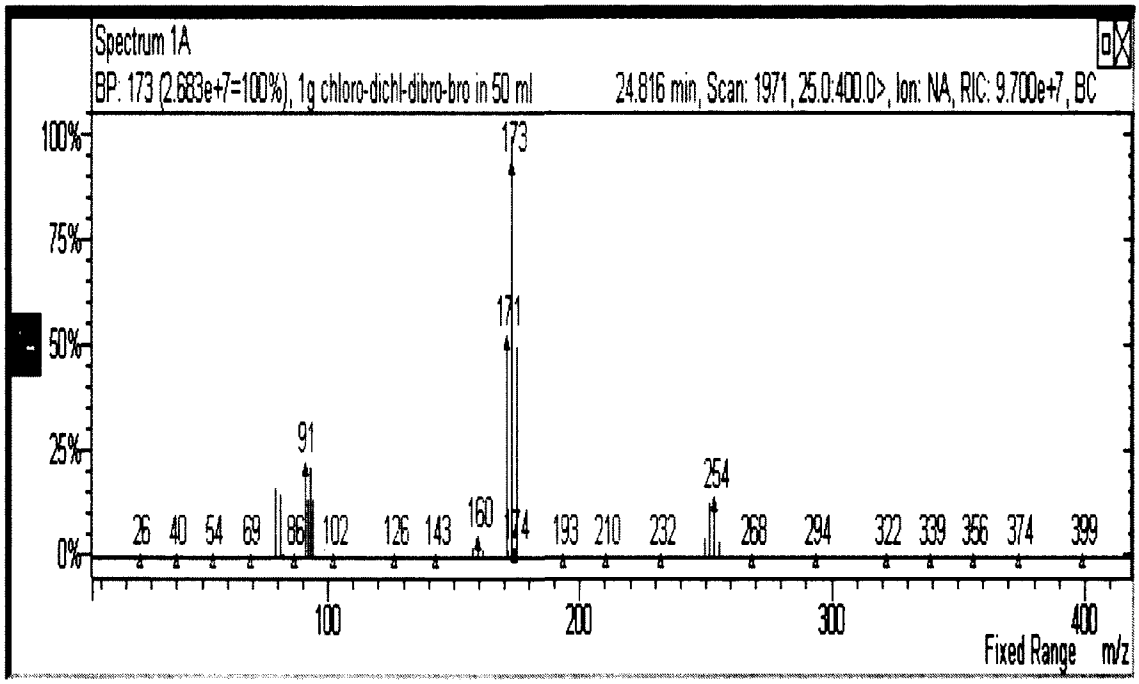


Figure 4.10: MS spectra of dibromochloromethane and bromoform

4.1.5 Stage 5-Method Validation at the Target Concentration Range

Once the THMs at a high concentration (20 g/L) were confirmed, further method validation need to be conducted for the target concentration ranges (1-10 mg/L). 10 ppm chloroform was chosen as a representative.

To avoid interference of solvent (pentane, 72.15 g/mol), the mass range in the full-scan mode was adjusted from 25-400 m/z to 75-400 m/z, and the starting time of MS analysis was set to 16.7 min, just before the eluting time of chloroform. Results of the full-scan analysis at 16.7-33 min are shown in Figure 4.11.

Comparison of Figure 4.11a (pentane) with Figure 4.11b (chloroform + pentane), the peak of 10 ppm chloroform was easily identified. Its retention time was found at 17.351 min, the same time as before. However, the intensity of this peak was very weak, which made the quantification difficult. Generally, the computer connected with GC/MS could automatically calculate the area under a peak identified. However, if the intensity of a peak was too weak, the computer might not be able to identify it. Manual detection and measurement is a possible solution, however it is possible that the accuracy would significantly decrease. Therefore, to achieve quantification of a small peak, the intensity of this peak need to be increased first.

Selected Ion Monitoring (SIM) mode in MS analysis can definitely accomplish this task. In this mode, MS only focus on several targeted ions and the sensitivity and selectivity of MS are considerably increased. The mass ranges of the target ions for the four THMs were decided according to Table 4.5 where the ions that have 100% intensity in the THMs spectra

were chosen. The corresponding selected ions ranges for the four THMs were: 82-84 m/z for chloroform and bromodichloromethane, 127-129 m/z for dibomochloromethane, and 171-173 m/z for bromoform.

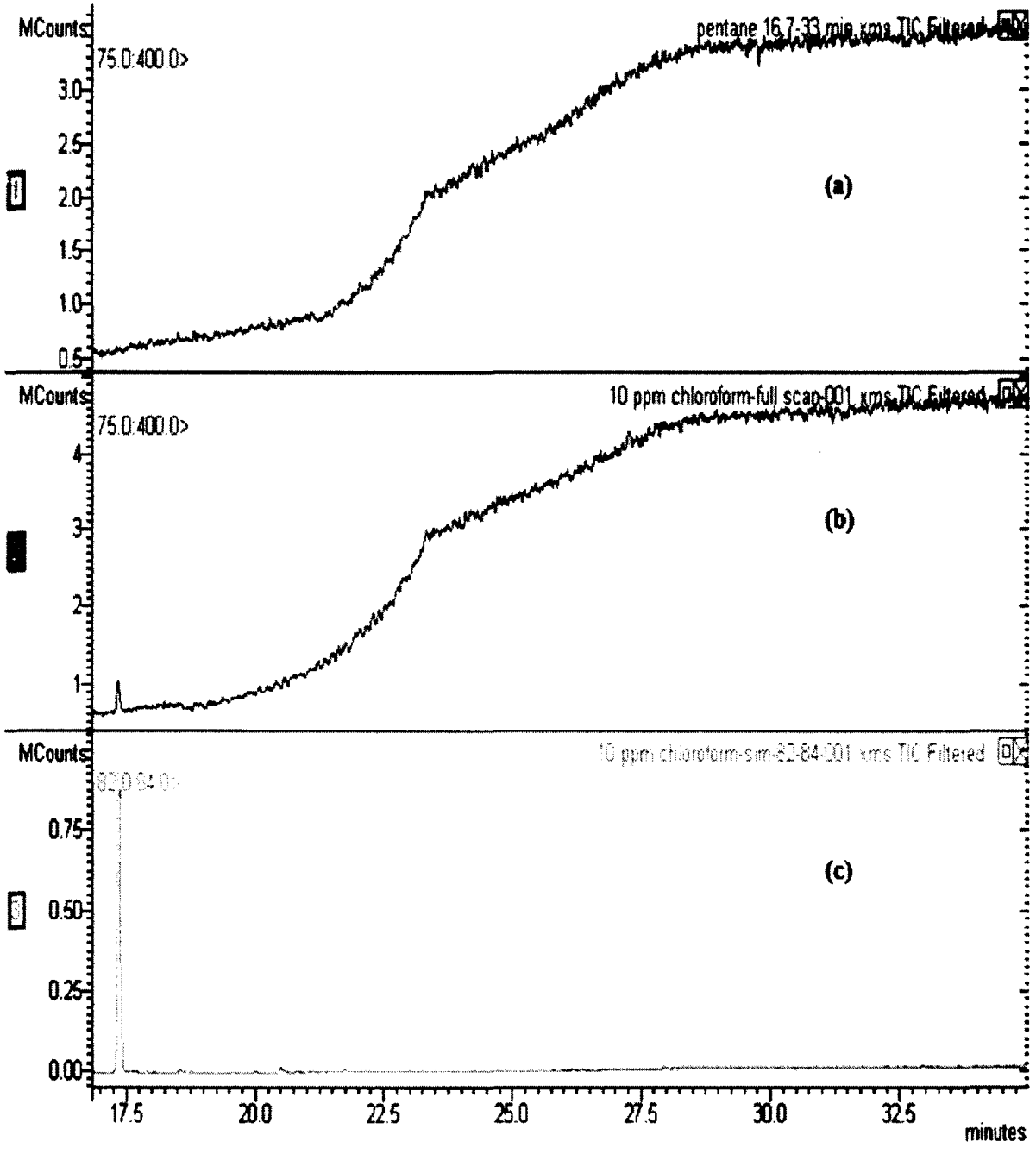


Figure 4.11: MS analysis for (a) pentane in full-scan mode, (b) 10 ppm chloroform in full-scan mode, (c) 10 ppm chloroform in SIM mode

The result of SIM analysis for 10 ppm chloroform was already shown in Figure 4.11c. Comparison of the peak in SIM mode (the third chromatogram) with that in full-scan mode (the second chromatogram), it was obvious that using SIM mode significantly increased the intensity of the chloroform peak. The computer could not only identify it, but also calculate the area under the peak. The retention time of this peak was identified at 17. 357 min and area under this peak was: $4.192 * 10^6$.

4.1.6 Stage 6-Summary of GC/MS Conditions Used for THMs

Based on the exploration of the previous five stages, the optimal GC/MS conditions for THMs were finally decided and summarized in Table 4.6.

4.2 Method Development for 17β-estradiol (E2)

Method development for 17β-estradiol (E2) included four stages: stage 1-2 solvent selection and peak identification, stage 3 peak confirmation and method validation, and stage 4 a summary for the method that was finally used for E2. As mentioned in Section 3.1.3, two calibration methods could be done for selected EDCs/pharmaceuticals: direct analysis like THMs (diluted standard solutions were directly dissolved in methanol and analyzed by GC/MS), and processed analysis (selected EDCs/pharmaceuticals mixed in distilled water went through a series of treatment processes - jar testing, acidification, extraction, evaporation, and methanol derivative, then analyzed by GC/MS). Direct analysis was conducted first because it could be easily operated and save time.

Table 4.6: Summary of the optimal GC/MS conditions for THMs

Conditions	Parameters	For THMs
Equipment	GC/MS	Varian CP3800 GC/1200 MS + Auto-sampler
Default Settings	Carrier Gas	Helium
	H_2, Air, N_2 Flow	30, 300, 29 mL/min
	Injector Temperature	250 °C
	Detector Temperature	300 °C
	Inject Volume	1 μL
Standards	Four THMs	Chloroform, bromodichloromethane, dibromochloromethane, bromoform
Column	Stationery Phase	CP-Select 624 CB
	Dimensions	60 m * 0.25 mm * 1.4 μm Length * Diameter * Film Thickness
	Oven Temperature Program	30 °C, hold 4 min; 7 °C /min to 80 °C, hold 4 min; 17 °C/min to 220 °C, hold 12 min; Total 35.38 min
Solvent	Derivative/Washing Solvent	Pentane
Injection	Split Ratio	1:10
Calibration	MS Analysis	SIM mode

4.2.1 Stage 1- Solvent Selection and Peak Identification Based on THMs Method

Since the solubility information of 17β-estradiol (E2) could not be found from the MSDS data sheet provided by Sigma-Aldrich, different solvents were investigated. Pentane as a solvent used for THMs was first attempted, but it was found that 17β-estradiol (E2) was almost insoluble in pentane. Then a commonly used organic solvent- diethyl ether was tried and the 17β-estradiol (E2) partially dissolved in this solvent. To check the sensitivity of GC/MS for detection of slightly dissolved 17β-estradiol (E2), a sample was prepared and directly analyzed by MS.

The method for 17β-estradiol (E2) was developed based on that of THMs. Since the melting point (176-180 °C, no boiling point available) of 17β-estradiol (E2) is higher than that (146-150 °C) of bromoform, 17β-estradiol (E2) might need longer time to elute. Therefore, the holding time at the final temperature (220 °C) in the oven temperature program was adjusted from 12 min to 30 min, resulting in the total time of 53 min. The result of the full-scan (75-400 m/z) MS analysis was shown in Figure 4.12. It was shown that except the solvent (ether) itself, no any peak appeared. This might be because the inappropriate solvent, oven temperature program or column was used.

Methanol as another frequently used solvent was further tried. It was found that 17β-estradiol (E2) could be completely dissolved in methanol. Therefore, methanol was eventually chosen as derivative solvent for 17β-estradiol (E2).

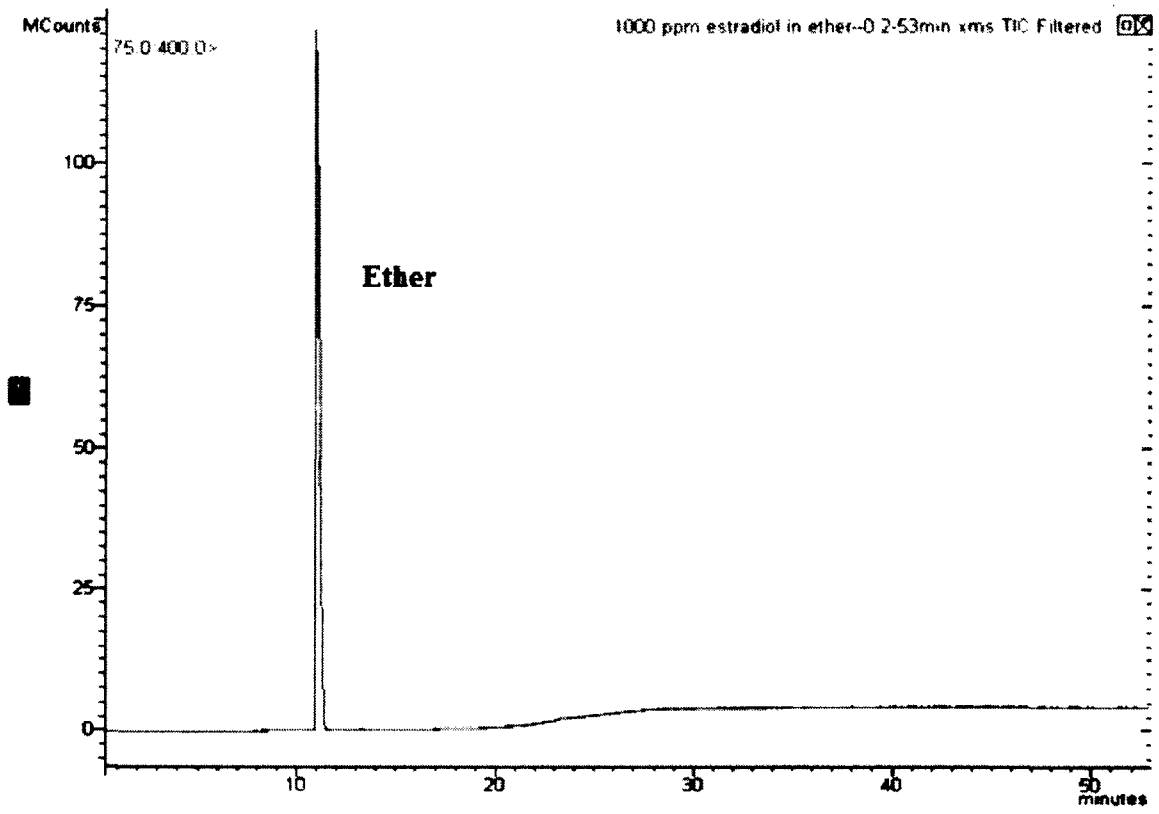


Figure 4.12: Full-scan MS analysis of 17β-estradiol (E2) in diethyl ether

From Figure 4.12, it was found that prolonged retention time had no effect on detection of 17β-estradiol (E2). One reason might be that the oven temperature was not high enough to vaporize 17β-estradiol (E2). Therefore, the maximum allowable operating temperature (265 °C) was used for the final temperature. The initial temperature was also increased from 30 °C to 80 °C. The specific program was proposed as follows: 80 °C, hold for 4 min; 17 °C/min to 265 °C, hold 12 min. The total running time was 26.88 min. Under this condition, two concentrations (2000 ppm and 200 ppm) of 17β-estradiol (E2) in methanol were analyzed. Results are shown in Figure 4.13.

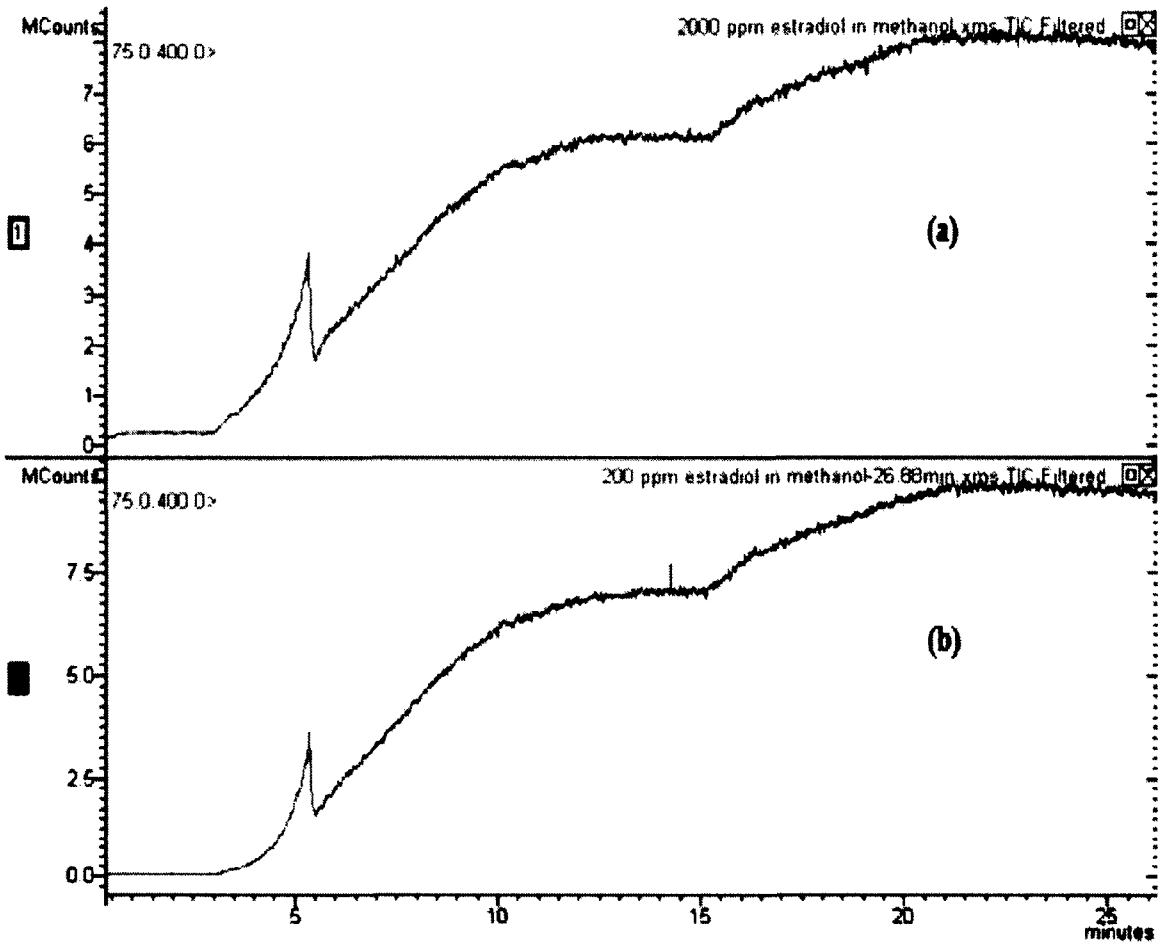


Figure 4.13: Stage 1- Full-scan MS analysis for 17β-estradiol (E2) in methanol (a) 2000 ppm, (b) 200 ppm

From Figure 4.13, two identical peaks were found in both chromatograms, but they were neither 17β-estradiol (E2) nor methanol according to the results searched by the library of spectra. Theoretically, they were also impossible to be methanol, since the mass of methanol (32.04 g/mol) was not in the measured mass range (75-400 m/z). Poor resolution of peaks and significantly increased baseline were also found in this figure.

All these problems mentioned above illustrated that the oven temperature program needed to be further adjusted and/or the column might need to be changed. Since THMs are

very volatile while 17β-estradiol (E2) is rather stable, the column specific for THMs was probably unsuitable for 17β-estradiol (E2), and vice versa.

4.2.2 Stage 2-Peak Identification Based on the Method Found in Literature

Based on the above points, the column that was unsuitable for THMs in stage 2 of THMs method development was used for 17β-estradiol (E2): Varian CP8944 Factor Four™ Capillary Column VF-5ms, 30 m * 0.25 mm * 0.25 μm, maximum oven temperature programmed- 350 °C. Oven temperature program was constructed based on the method developed by [58]. In case of the deviation caused by the different column used from the literature, the holding time at the final temperature was slightly increased from 3.3 min to 6.3 min. The specific program was: 150 °C, hold 1.5 min; 260 °C at the ramp rate of 50 °C /min, hold 5 min; 270 °C at the ramp rate of 10 °C /min, hold 6.3 min. Total running time was 16 min. The splitless mode was chosen [58].

100 ppm stock solution of 17β-estradiol (E2) and 10 ppm working solution were prepared with methanol. After three runs of methanol for the purpose of cleaning the equipment and column, both 100 ppm and 10 ppm 17β-estradiol (E2) in methanol were analyzed by GC/FID. Results are shown in Figure 4.14. Triplicate injections were run for both samples to ensure reproducibility. It was shown that the two peaks appeared around 12 min were probably 100 ppm and 10 ppm 17β-estradiol (E2). Further confirmation was needed.

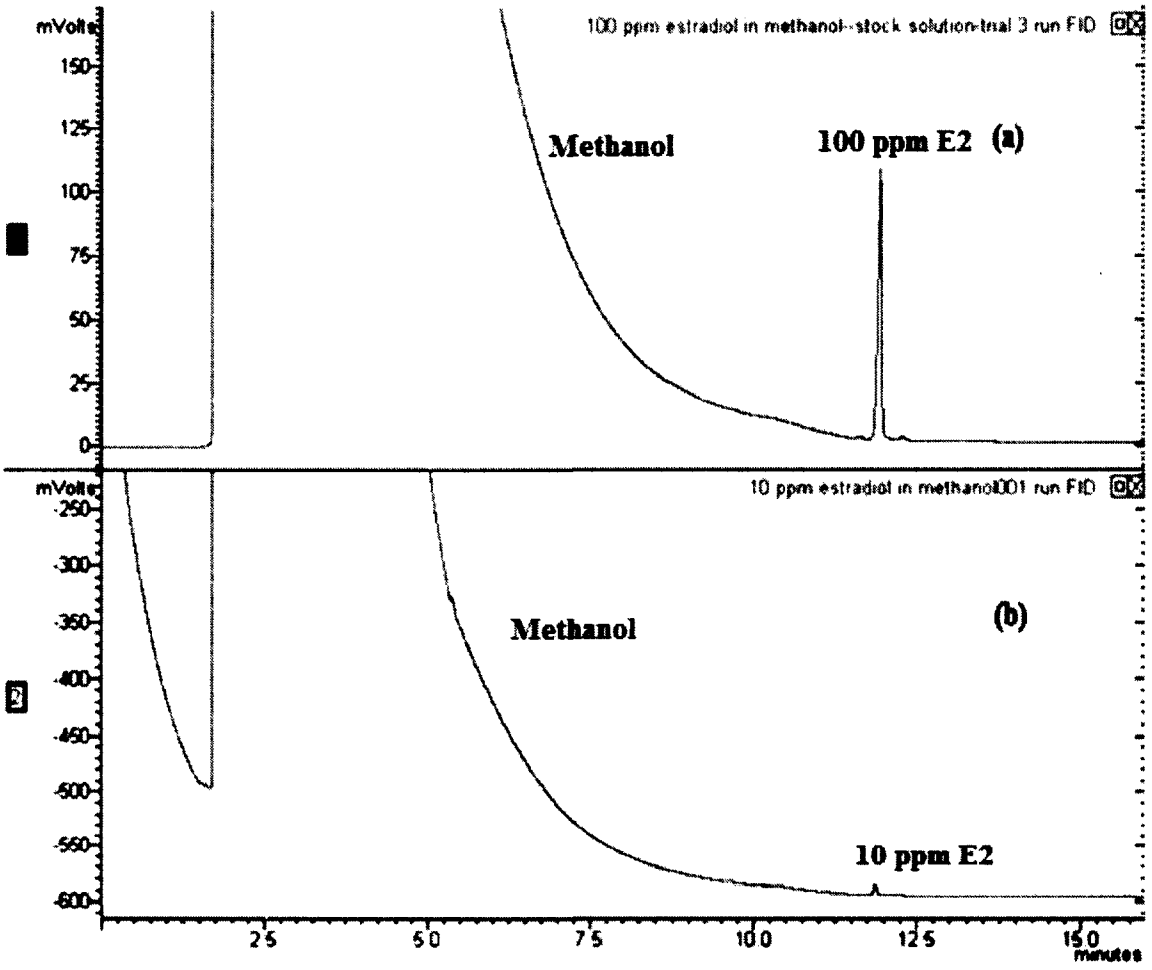
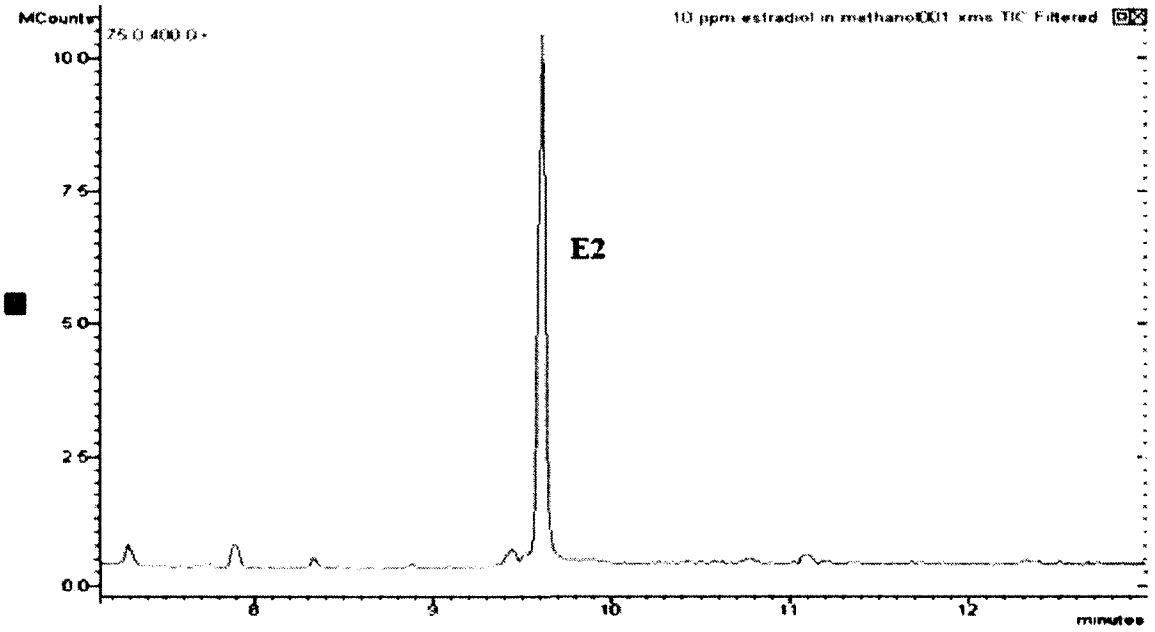


Figure 4.14: Stage 2- FID results for 17β-estradiol (E2) in methanol (a) 100 ppm, (b) 10 ppm

4.2.3 Stage 3-Peak Confirmation and Method Validation

To avoid the interference of solvent (methanol) and increase intensity of 17β-estradiol (E2) peaks, the starting time of MS analysis was set to 7 min. From Figure 4.14, it was known that 17β-estradiol (E2) appeared before 13 min, so the total running time could be further shortened. By reducing the holding time at the final temperature from 6.3 min to 3.3 min, the total time was reduced from 16 min to 13 min. Based on the new conditions, 10 ppm 17β-estradiol (E2) in methanol was analyzed by full-scan (75-400 m/z) MS.

Results are shown in Figure 4.15. It was known the peak of 10 ppm 17β-estradiol (E2) was confirmed at 9.615 min and its intensity was significantly increased compared to the FID result. The area under this peak was computed as 2.783 * 10^7. Therefore, direct analysis calibration for 17β-estradiol (E2) could be accomplished in full-scan MS mode.



17β-estradiol, E2 ($C_{18}H_{24}O_2$, 272.38 g/mol, 9.615 min)

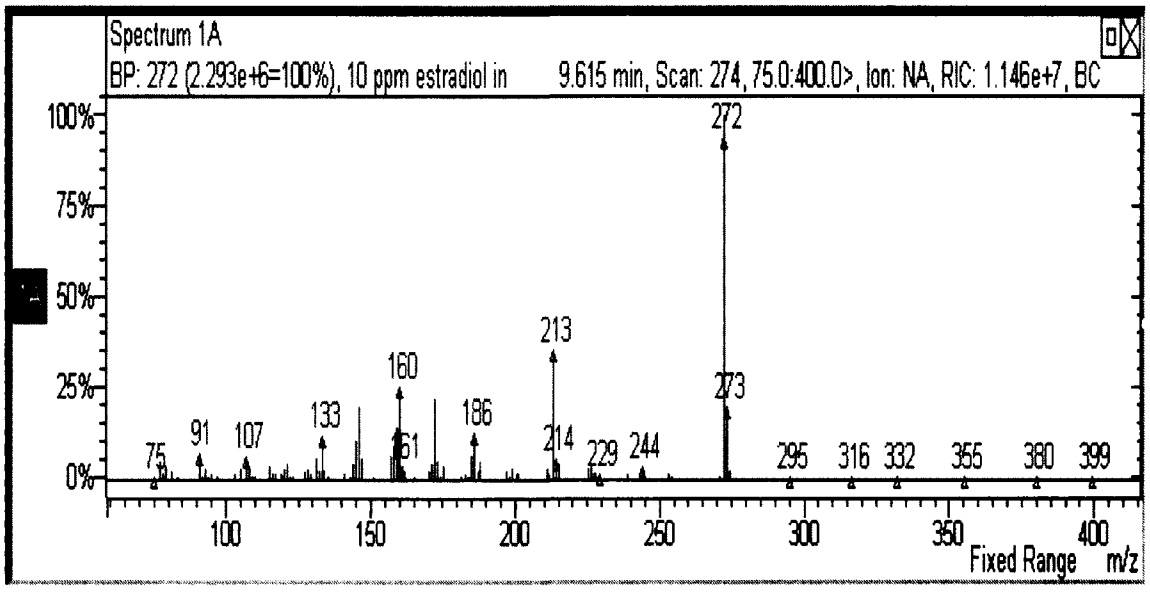


Figure 4.15: Full-scan MS direct analysis for 10 ppm 17β-estradiol (E2) and its spectrum

The processed analysis was then conducted based on the method developed by direct analysis. Result was shown in Figure 4.16. Comparison of Figure 4.16 with Figure 4.15, it was found that a much stronger intensity of 17β-estradiol (E2) peak was found in processed analysis (250 MCounts) than in direct analysis (10.5 MCounts). The area under the peak in Figure 4.16 was computed as 2.826 * 10^9. In this way, although both direct analysis and processed analysis could achieve calibration for 17β-estradiol (E2), the processed standard calibration might be more practical since the samples would go through the same handling methods.

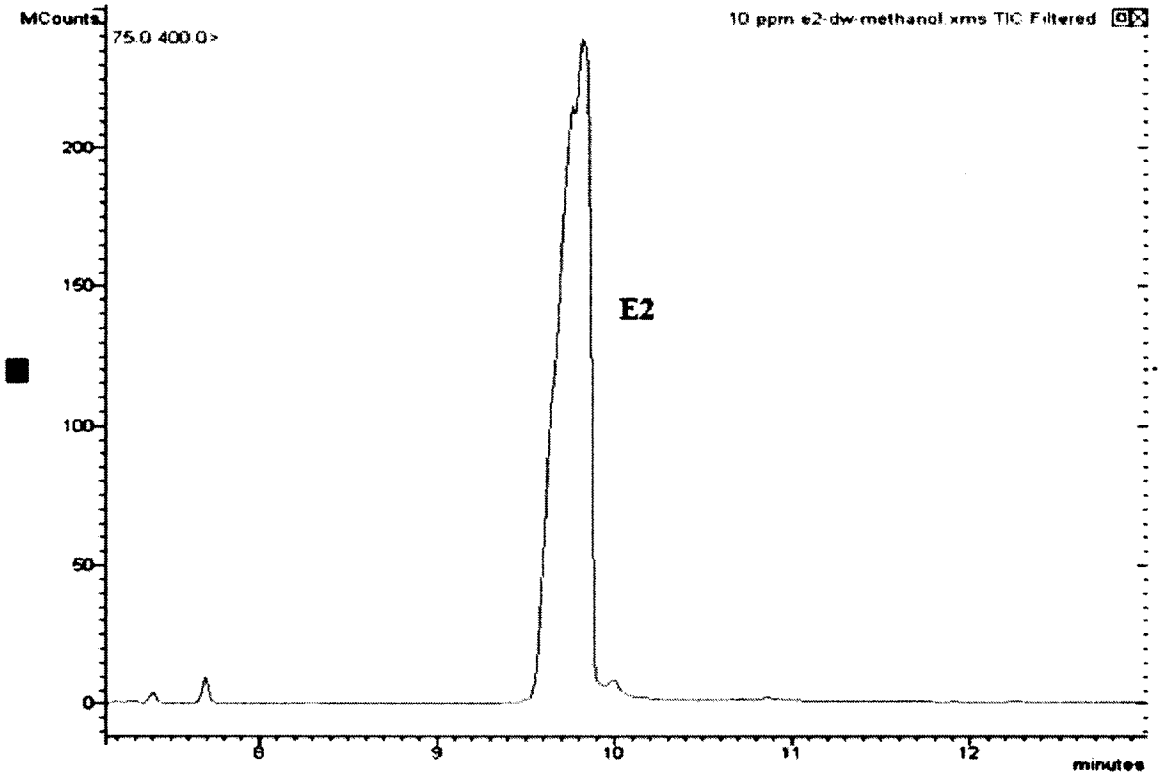


Figure 4.16: Full-scan MS processed analysis for 10 ppm 17β-estradiol (E2)

4.2.4 Stage 4-Summary of GC/MS Conditions for 17β-estradiol (E2)

Based on the above investigation, the optimal GC/MS conditions for 17β-estradiol (E2) were summarized in Table 4.7.

Table 4.7: Summary of the optimal GC/MS conditions for 17β-estradiol (E2)

Conditions	Parameters	For 17β-estradiol (E2)
Equipment	GC/MS	Varian CP3800 GC/1200 MS + Auto-sampler
Default Settings	Carrier Gas	Helium
	H_2, Air, N_2 Flow	30, 300, 29 mL/min
	Injector Temperature	250 °C
	Detector Temperature	300 °C
	Inject Volume	1 μL
Column	Stationery Phase	VF-5ms
	Dimensions	30m * 0.25mm * 0.25 μm Length * Diameter * Film Thickness
	Oven Temperature Program	150 °C, hold 1.5 min; 50 °C /min to 260 °C, hold 5 min; 10 °C /min to 270 °C, hold 3.3 min; Total 13.00 min
Solvent	Derivative/Washing Solvent	Methanol
Injection	Split Ratio	Splitless
Calibration	MS Analysis	Full-scan Mode (75-400 m/z) Direct Analysis + Processed Calibration

4.3 Method Development for Clofibric Acid and Ketoprofen

Method developments for both clofibric acid and ketoprofen were conducted based on method for 17β-estradiol (E2). Except oven temperature program, all the other GC conditions were kept the same. The specific processes were illustrated according to three stages: stage 1 and stage 2 are oven temperature program development and method validation for clofibric acid and ketoprofen, respectively; and stage 3 is a summary for GC/MS conditions used for both clofibric acid and ketoprofen.

4.3.1 Stage 1-Method Development for Clofibric Acid

The temperature program developed for 17β-estradiol (E2) - 150 °C, hold 1.5 min; 50 °C /min to 260 °C, hold 5 min; 10 °C /min to 270 °C, hold 3.3 min; total of 13.00 min - was attempted first, but except for the solvent peak no other new peaks were found. This might be due to the different properties between clofibric acid (a non-steroidal drug) and 17β-estradiol (E2, an estrogen steroid). Therefore, a specific oven temperature program for clofibric acid was needed.

One oven temperature program proposed by [50] for clofibric acid determination was then tried: 60 °C, hold 9 min; 150 °C at the ramp rate of 10 °C /min, hold 9 min; rate of 4 °C /min to 250 °C, hold 25 min; 10 °C /min to 300 °C, hold 5 min; total retention time of 46 min. However, still only the solvent peak was found. This might be caused by a different column and pretreatment method used in the literature. Clofibric acid was converted by [50] to methyl ester with trimethylsilydiazomethane before GC/MS analysis, where a HP 5ms column was used.

Given this situation, a completely new oven temperature program was built based on the above two programs: 150 °C, hold 9 min; rate of 10 °C /min to 250 °C, hold 25 min; total of 44 min. 100 ppm clofibric acid in methanol was then prepared and analyzed by GC/FID. It was found that one new peak was found, shown in Figure 4.17.

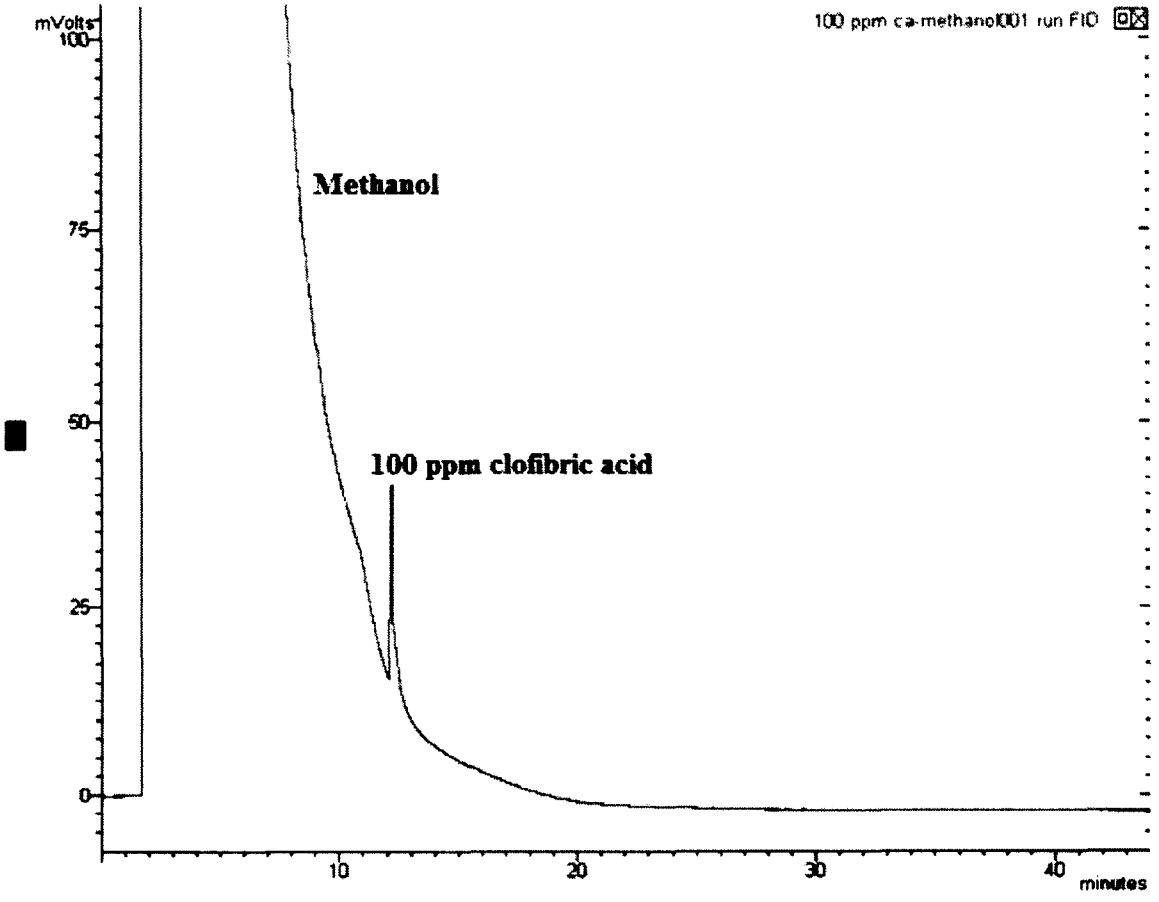
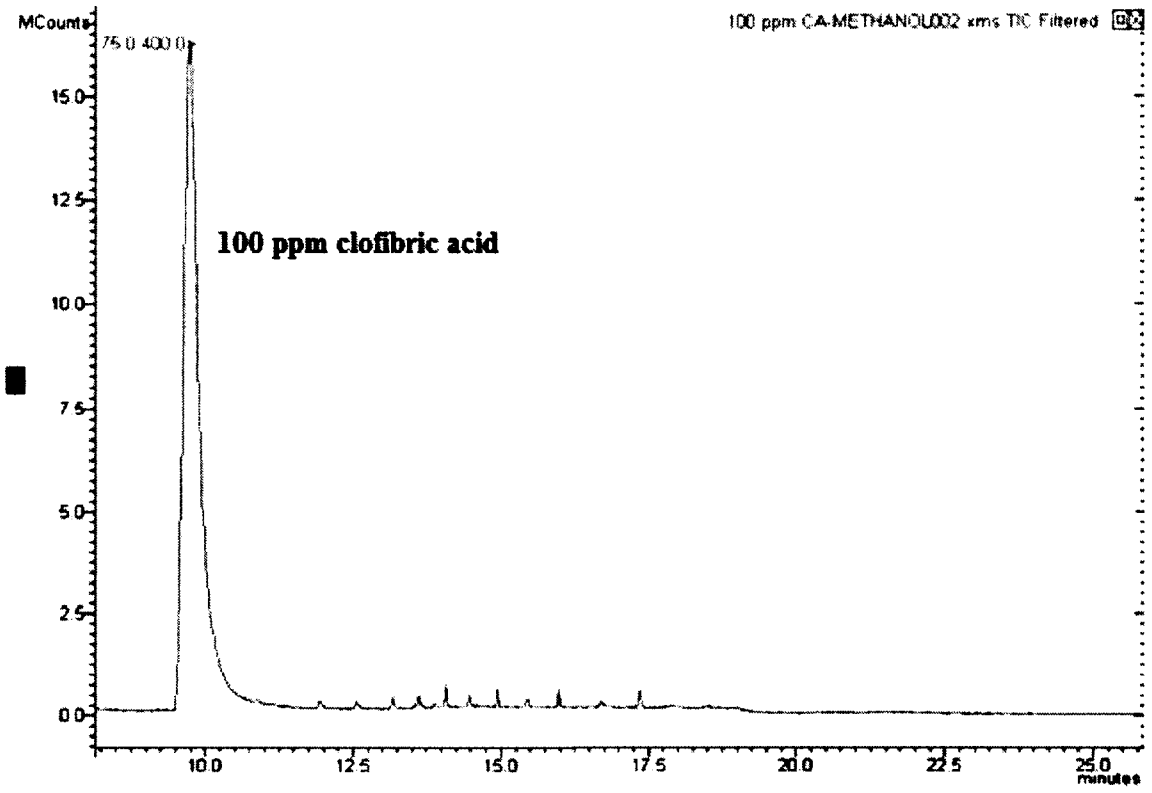


Figure 4.17: FID results for 100 ppm clofibric acid in methanol

The new peak need to be further confirmed. Nevertheless, before the confirmation, the total running time could be reduced from 44 min to 25.5 min by adjusting the holding time from 25 min to 6.5 min at final temperature 250 °C. The starting time of MS was set to 8 min to avoid the interference of methanol. The confirmation results of 100 ppm clofibric acid are shown in Figure 4.18.



Clofibric Acid ($C_{10}H_{11}ClO_3$, 214.645 g/mol, 9.746 min)

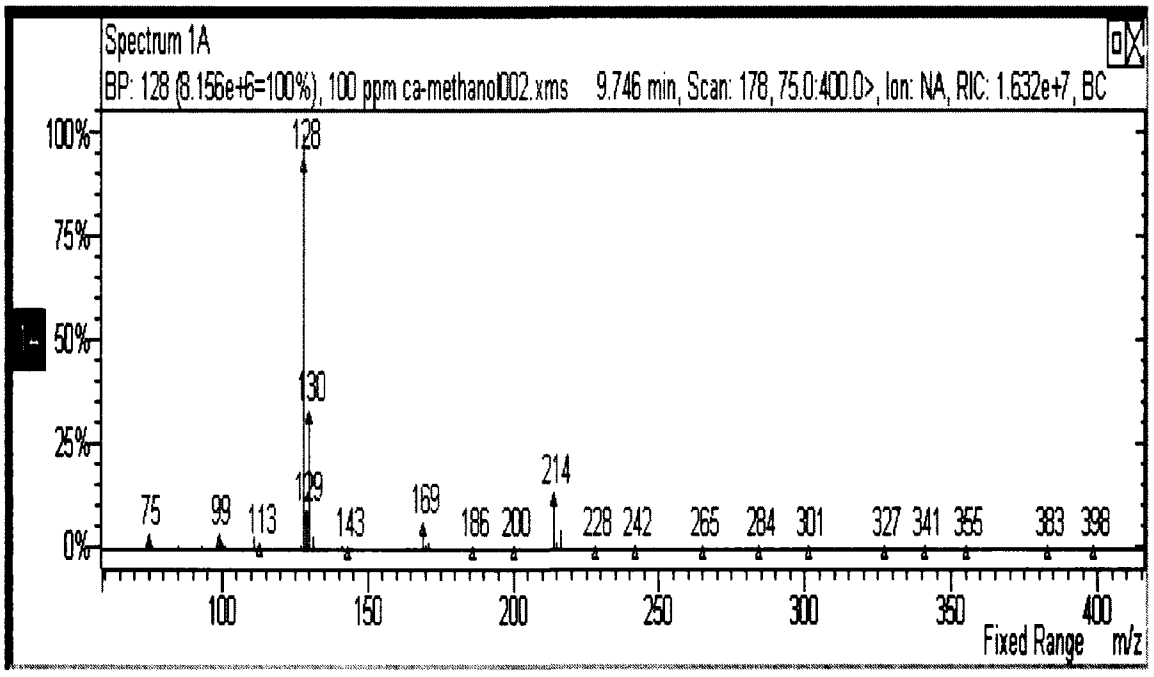


Figure 4.18: Full-scan MS direct analysis of 100 ppm clofibric acid and its spectrum

From Figure 4.18, it was found that the total running time still could be further reduced. The ramp rate was increased from 10 °C/min to 50 °C/min, and the holding time was reduced from 6.5 min to 3 min to achieve a total time of 14 min. A target concentration - 10 ppm clofibric acid was then prepared and analyzed by MS under this condition. Results are shown in Figure 4.19.

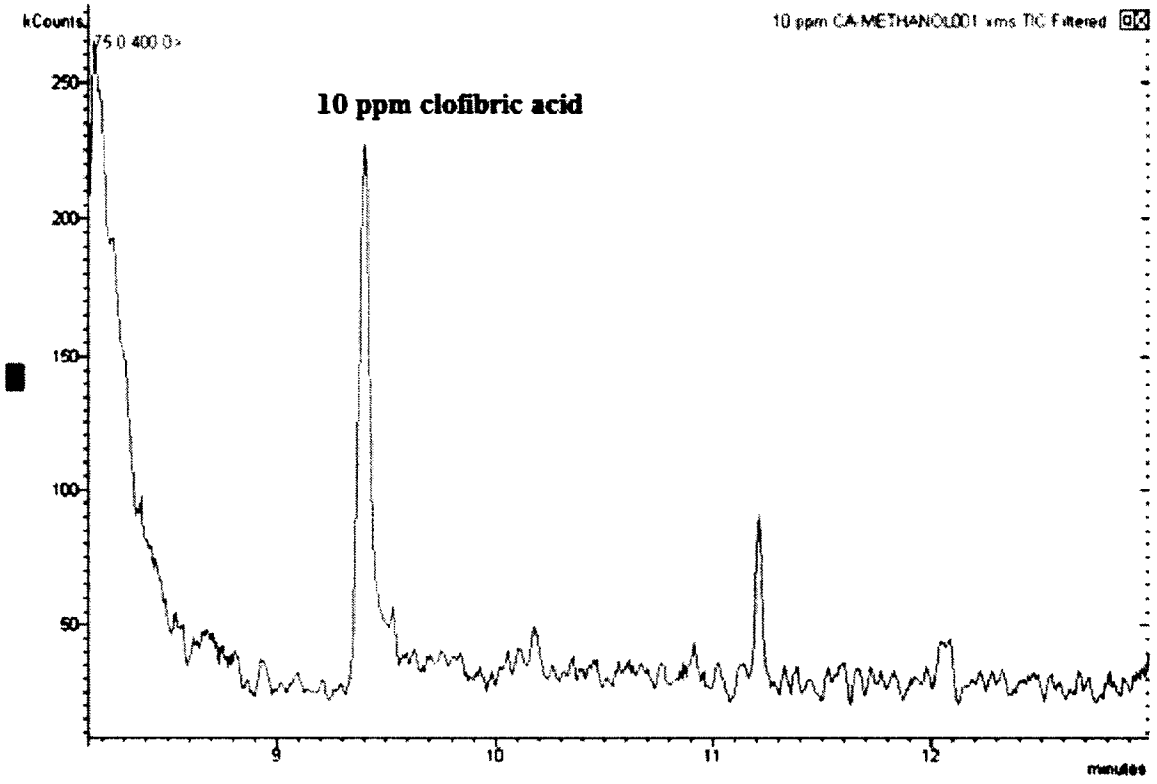


Figure 4.19: Full-scan MS direct analysis for 10 ppm clofibric acid

Although MS could identify this peak at 10 ppm (at 9.746 min, Figure 4.19), the intensity was so low that the computer could not quantify it. Other noisy peaks also interfered with the quantification of this peak. Therefore, the processed standard analysis for clofibric acid was needed. To avoid the interference by the peak before 9 min, the starting time of MS was reset from 8 min to 8.5 min. Result of processed analysis for 10 ppm clofibric acid was

shown in Figure 4.20. The intensity was significantly increased, and the area under the peak was computed as $1.707 * 10^9$.

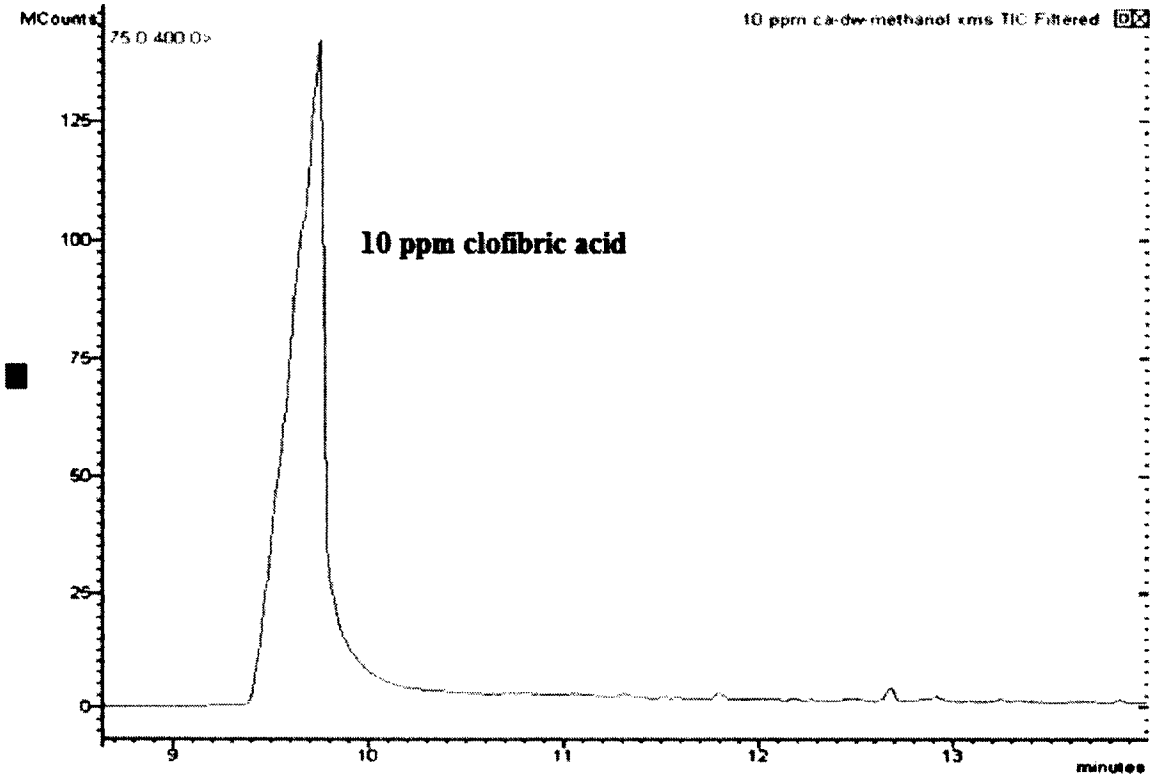
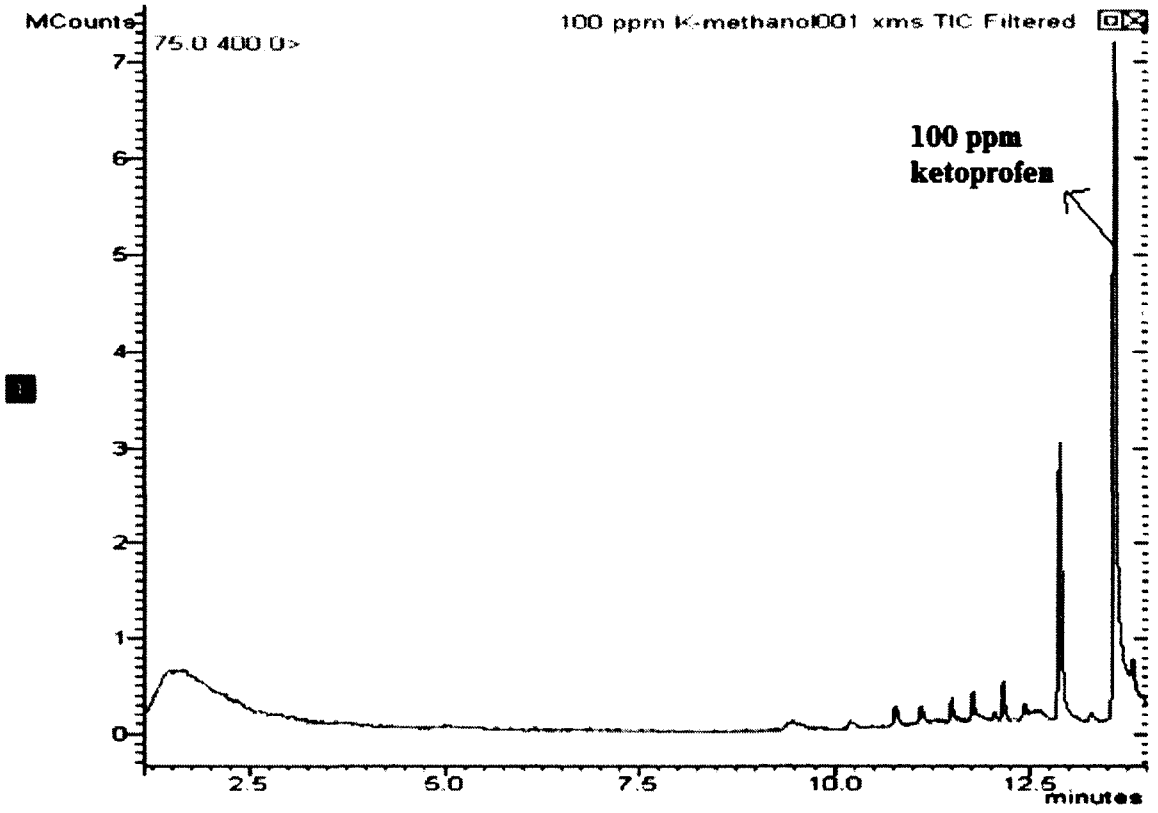


Figure 4.20: Full-scan MS processed analysis for 10 ppm clofibric acid

4.3.2 Stage 2-Method Development for Ketoprofen

Since ketoprofen and clofibric acid are both non-steroidal drugs, the method developed for clofibric acid was directly used for ketoprofen. 100 ppm stock solution of ketoprofen was prepared and analyzed by MS. The peak was confirmed at 13.647 min, shown in Figure 4.21. However, since the peak was located at the very end time of running, the oven temperature program needed to be adjusted. The peak appeared at around 13 min was clofibric acid. The ketoprofen runs were conducted directly after clofibric acid runs, so the remaining clofibric acid in the injector or column contaminated the ketoprofen samples.



Ketoprofen ($C_{16}H_{14}O_3$, 254.281 g/mol, 13.647 min)

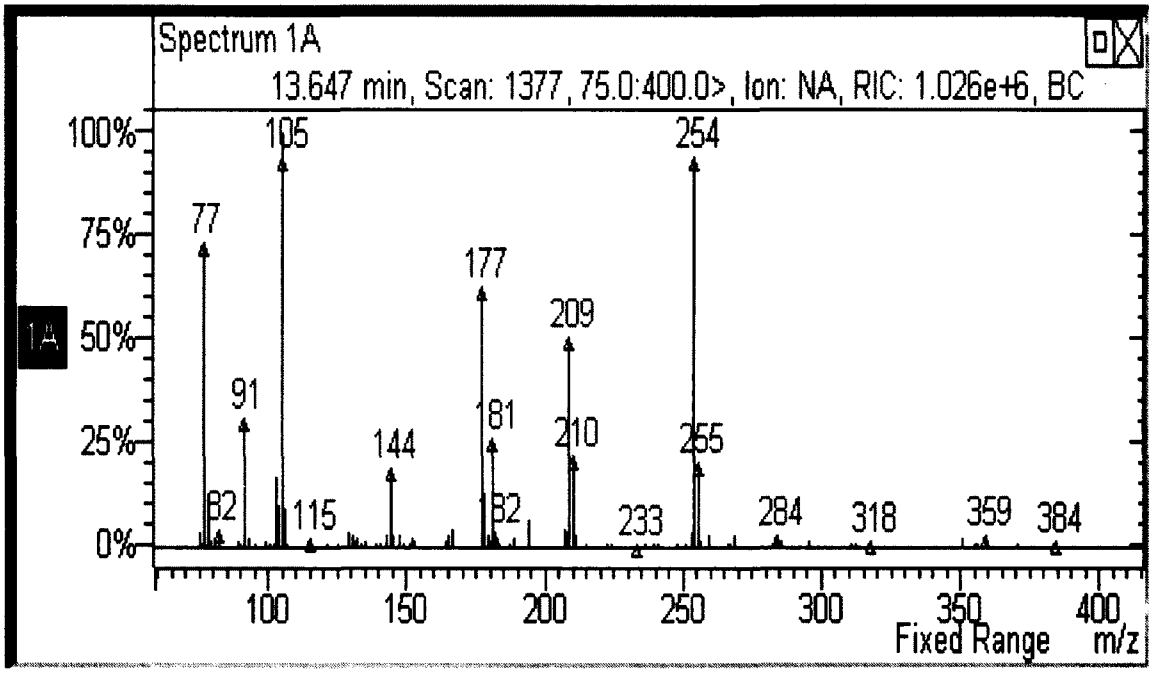


Figure 4.21: Full-scan MS direct analysis of 100 ppm ketoprofen and its spectrum

To avoid contamination, the starting time of MS was set to 13 min, and at least triplicate methanol washing samples were run. The holding time of the final temperature was prolonged from 3 min to 7 min, resulting in total time of 18 min. Both 100 ppm and 10 ppm ketoprofen were analyzed under this condition. Results are shown in Figure 4.22.

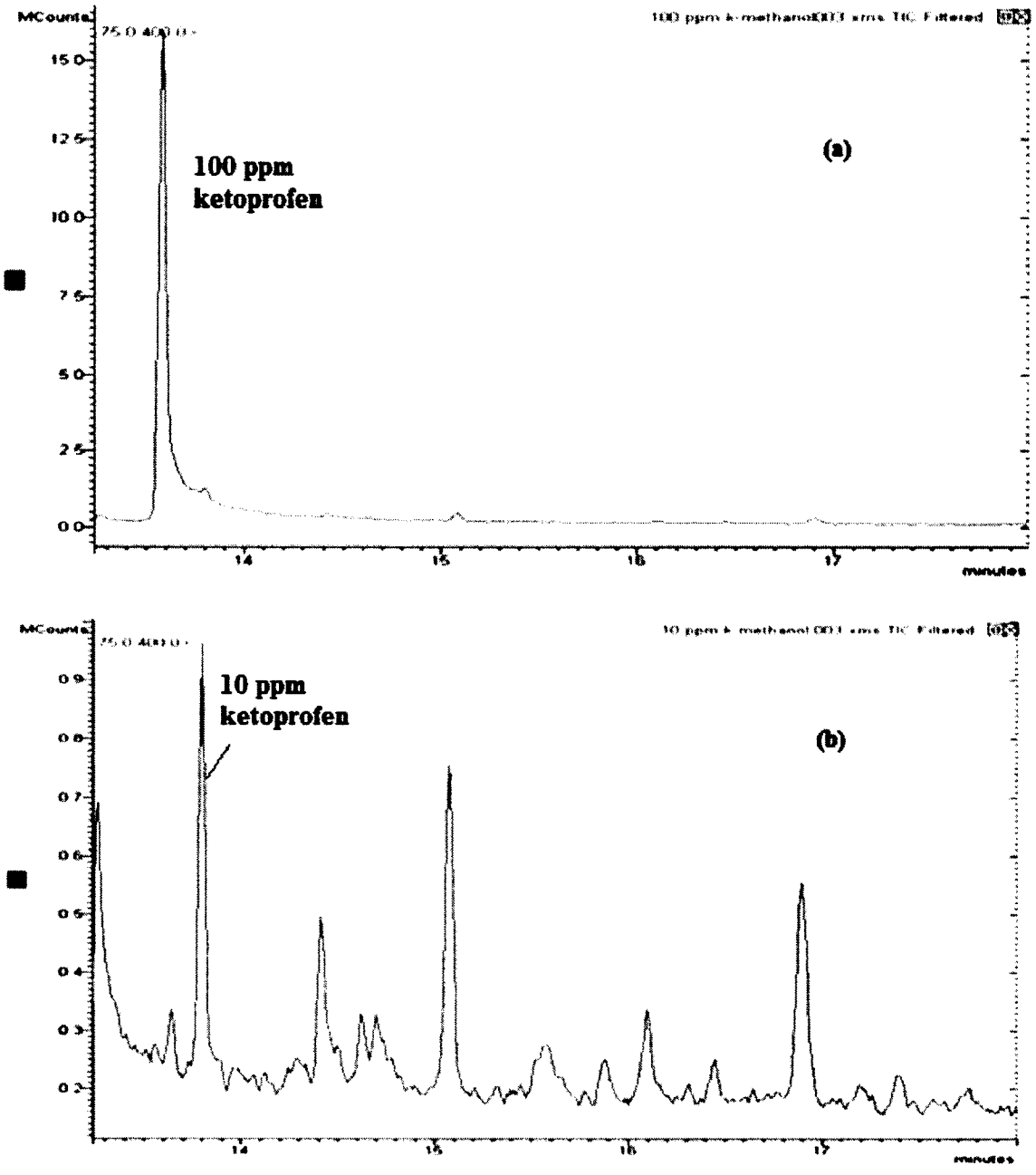


Figure 4.22: Full-scan MS direct analysis for ketoprofen (a) 100 ppm, (b) 10 ppm

It was found that the peak of 100 ppm ketoprofen had better resolution and higher intensity than that of 10 ppm. The area under the 10 ppm peak could not compute. Therefore, the processed standard analysis for ketoprofen was conducted, shown in Figure 4.23. Higher intensity of the peak identified by processed analysis helped the quantification of the computer. The area under this peak was computed as $1.653 * 10^9$.

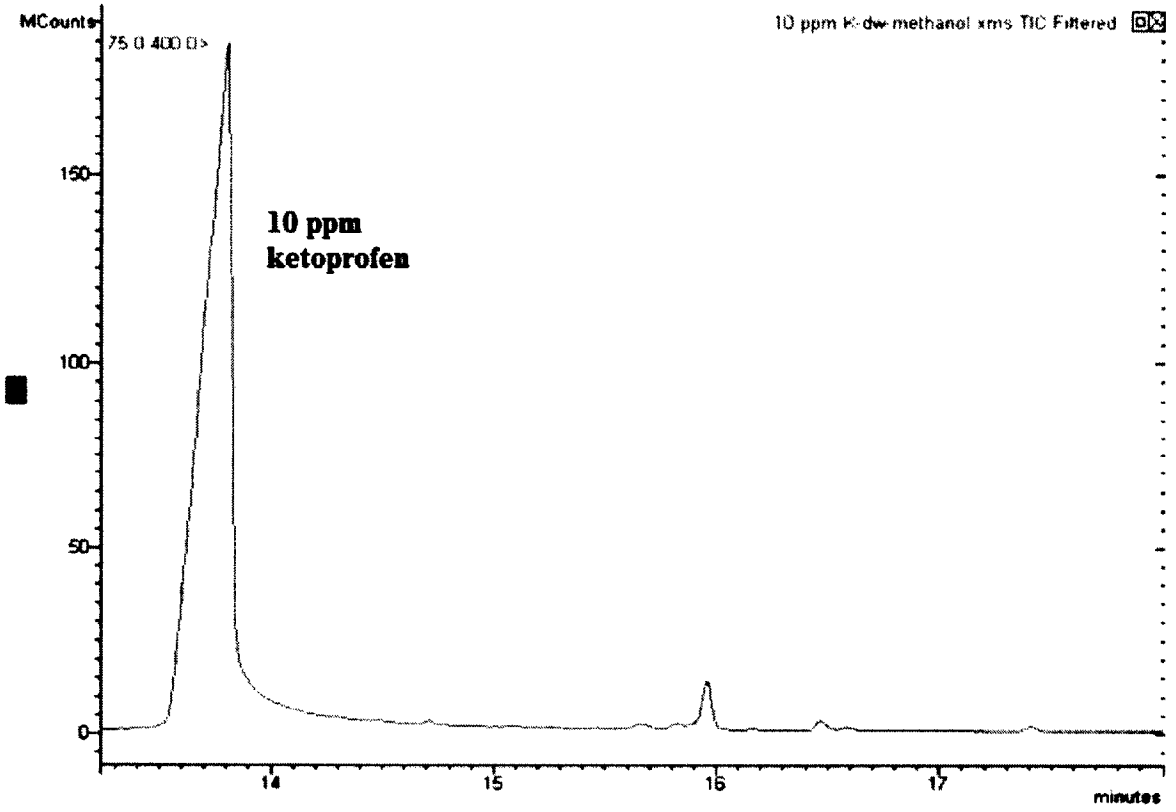


Figure 4.23: Full-scan MS processed analysis for 10 ppm ketoprofen

4.3.3 Stage 3-Summary of the Optimal GC/MS Conditions for Clofibric Acid and Ketoprofen

The finalized GC/MS conditions in stage 1 and stage 2 were summarized in Table 4.8.

Table 4.8: Summary of the optimal GC/MS conditions for clofibric acid and ketoprofen

Conditions	Parameters	Clofibric Acid	Ketoprofen
Equipment	GC/MS	Varian CP3800 GC/1200 MS	
Default Settings	Carrier Gas	Helium	
	H_2, Air, N_2 Flow	30, 300, 29 mL/min	
	Injector Temperature	250 °C	
	Detector Temperature	300 °C	
	Inject Volume	1 µL	
Column	Stationery Phase	VF-5ms	
	Dimensions	30m * 0.25mm * 0.25 µm Length * Diameter * Film Thickness	
	Oven Temperature Program	150 °C, hold 9 min; 50 °C /min to 250 °C, hold 3 min; Total 14.00 min	150 °C, hold 9 min; 50 °C /min to 250 °C, hold 7 min; Total 18.00 min
Solvent	Derivative/Washing Solvent	Methanol	
Injection	Split Ratio	Splitless	
Calibration	MS Analysis	Full-scan Mode (75-400 m/z) Processed Calibration	

4.4 Summary

Method development for THMs, 17β-estradiol (E2), clofibric acid and ketoprofen were achieved through a series of trial and error processes. To save time and reduce trial and error, four main GC conditions including column, oven temperature, split ratio, solvent were focused. Other conditions including injector temperature, inject volume, detector temperature, gas selection and flow rate were defaulted set according to the GC operating manual.

Column was selected based on availability, as well as the polarity and volatility of the target compounds. THMs are very volatile compounds, so the column-CP-Select 624 CB, 60 m * 0.25 mm * 1.4 μm that is specific for identifying volatile organic compounds were used; while for the extremely stable selected EDCs/pharmaceuticals, the column-VF-5ms, 30m * 0.25mm * 0.25 μm was used.

Oven temperature program, split ratio, and solvent were tried based on consulting with GC operating manuals, experienced GC users, and previous analysis information for the target compounds. Boiling point of a target compound could also help build the oven temperature program. To ensure that a compound could be detected, the initial temperature in the oven temperature program has to be lower than the boiling point of the target compound, and the final temperature has to be certain that the target compound have eluted from the column. Adjustment of ramping rate could help improve the separation efficiency and peak resolution. A series of trial and error processes were needed.

For each trial, the analyzing procedures for a target compound by GC/MS were:

1. Preliminary identification of standard peaks at a higher concentration (such as 20 g/L for THMs and 100 ppm for the selected EDCs/pharmaceuticals) by GC/FID, achieved by the comparison of the standard sample runs with the blank runs.
2. MS confirmation for the identified peaks by FID (higher concentrations)
3. MS identification and quantification for standards at a target concentration (10 ppm)

Standards of the target compounds were prepared according to two ways to achieve calibration: direct analysis (diluted standard solutions were directly analyzed by MS) and processed standards (standards were mixed in water samples, went through acidification, extraction, evaporation, derivation, and finally analyzed by MS). It was found that THMs can only be calibrated by direct analysis because even by direct analysis, volumes of THMs standard samples decreased very rapidly as time going, not to mention the time-consuming processed standards. However, processed standards calibration was generally preferred because the sample handling processes were used for samples. Therefore, 17β-estradiol (E2), clofibric acid and ketoprofen were calibrated using processed standards.

Two MS modes, full-scan mode and selected ion monitoring (SIM) mode were used for peak analysis. Full-scan was first conducted for all the target compounds, it was found that 17β-estradiol (E2), clofibric acid and ketoprofen could be easily identified and quantified. However, THMs were identified but could not be quantified because of low intensity of peaks, so SIM was tried and it was found that the intensities of THMs peaks were significantly increased. Therefore, THMs were quantified by MS at SIM mode.

Chapter 5

WASTEWATER DISINFECTION BY PAA/NaClO: RESULTS AND DISCUSSION

The results and discussions in this section include preliminary tests, THMs calibration, and wastewater DBPs analysis.

5.1 Preliminary Tests

Preliminary tests were undertaken to look for comparative differences between PAA and NaClO (herein referred to simply as PAA/NaClO) dosages and contact time on the following parameters-pH, specific UV_{254} absorbance (SUVA), TOC, COD, and NH_3-N in wastewater (WW) versus distilled water (DW). As PAA/NaClO dosages and/or contact time increases, the change of these parameters could help indicate the oxidation potential and the DBPs formation potential of PAA versus NaClO.

5.1.1 pH Test

pH was studied in this project because it has been reported to have a great influence on water and wastewater disinfection efficiency and DBP formation [1, 45, 47]. The initial pH was set to 6.7-7.0 (the pH range of WW) for both DW and WW. After 60 min, pH was measured for different dosages of PAA/NaClO treated DW/WW. Results are shown in Figure 5.1.

It was found that as dosages increased, PAA reduced pH of DW significantly (3.75-6.69, Figure 5.1a) but had almost no influence on pH of WW (6.86-7.03, Figure 5.1b), while NaClO increased pH of both DW (7.08-8.09, Figure 5.1a) and WW (7.16-7.53, Figure 5.1b).

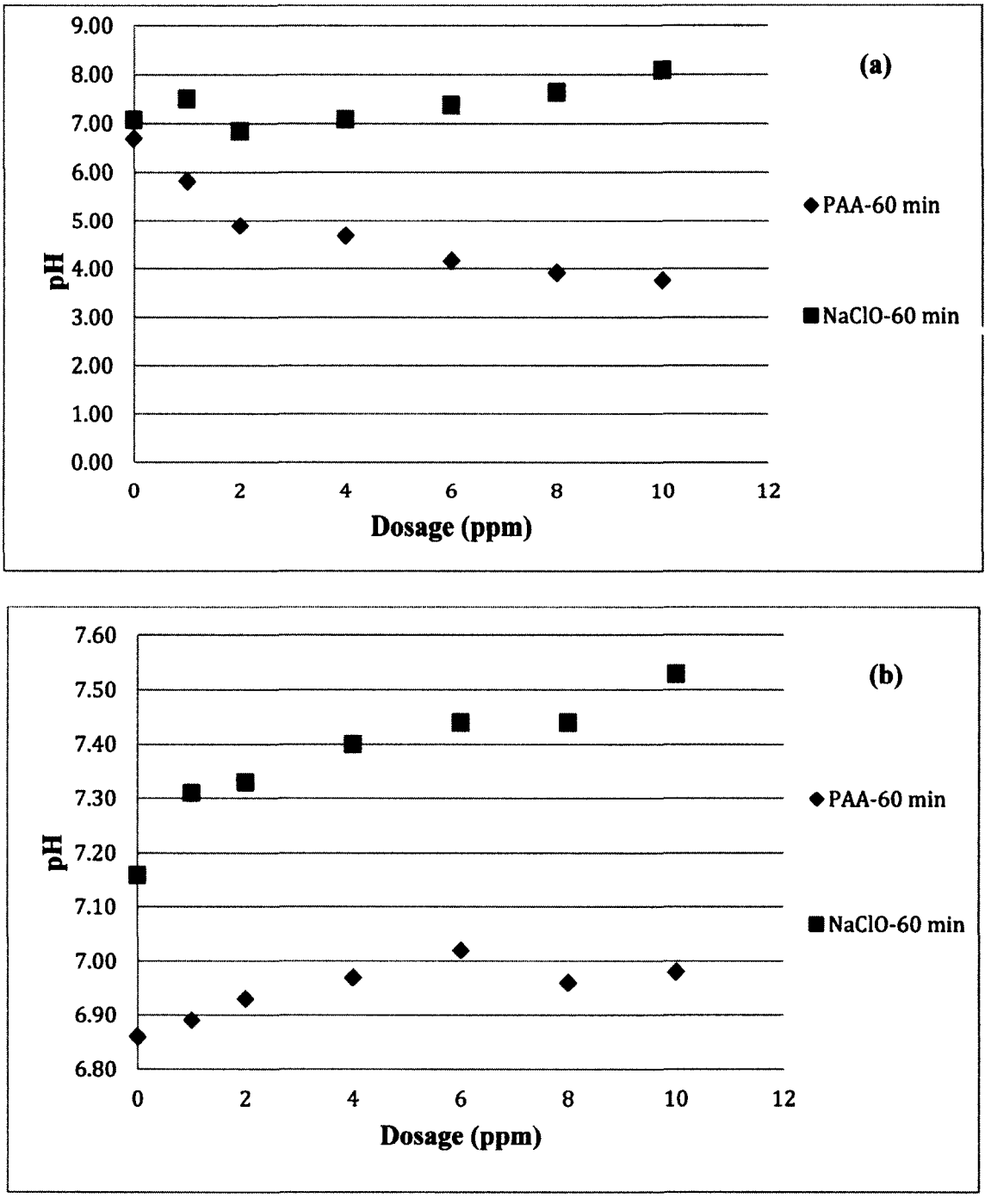


Figure 5.1: pH results for (a) PAA/NaClO in distilled water (DW), (b) PAA/NaClO in wastewater (WW)

The reduced pH by PAA and increased pH by NaClO was due to the acidity (pH = 3.5-4) of PAA and basicity (pH = 10.8-11.5) of NaClO. The smaller pH change in PAA/NaClO treated WW compared to DW was because of the buffering effect of contaminants in WW.

5.1.2 TOC and COD Tests

Total organic carbon (TOC), is a parameter commonly used for wastewater characteristics analysis, and plays an important role in quantifying the organic matter in wastewater. When PAA or NaClO is added in raw wastewater, the organic matter will react with PAA or NaClO to form DBPs [1, 37]. It was found that removing amounts of TOC could reduce DBP precursors and DBP formation [66]. Therefore, TOC was measured in this project to evaluate the change of DBP formation potential by the addition of PAA/NaClO.

Similar to TOC, chemical oxygen demand (COD) as a parameter indirectly measuring the organic matter in wastewater, could also indicate the potential of DBP formation. For both PAA and NaClO treated DW/WW, TOC and COD were measured at 10, 15, 30, 60 min. Results of the effects of different dosages of PAA/NaClO on TOC and COD in both DW and WW are shown in Figure 5.2 and Figure 5.3, respectively.

The impacts of different PAA/NaClO dosages and contact time on TOC and COD in DW (Figure 5.2a and Figure 5.3a) were found respectively similar. Increasing contact time had no or very little difference on TOC and COD in PAA/NaClO treated DW. However, the impact of PAA/NaClO dosages on TOC and COD was obvious. Increasing PAA dosages

resulted in a proportional increase of TOC and COD, while NaClO dosages had almost no impact on these two parameters.

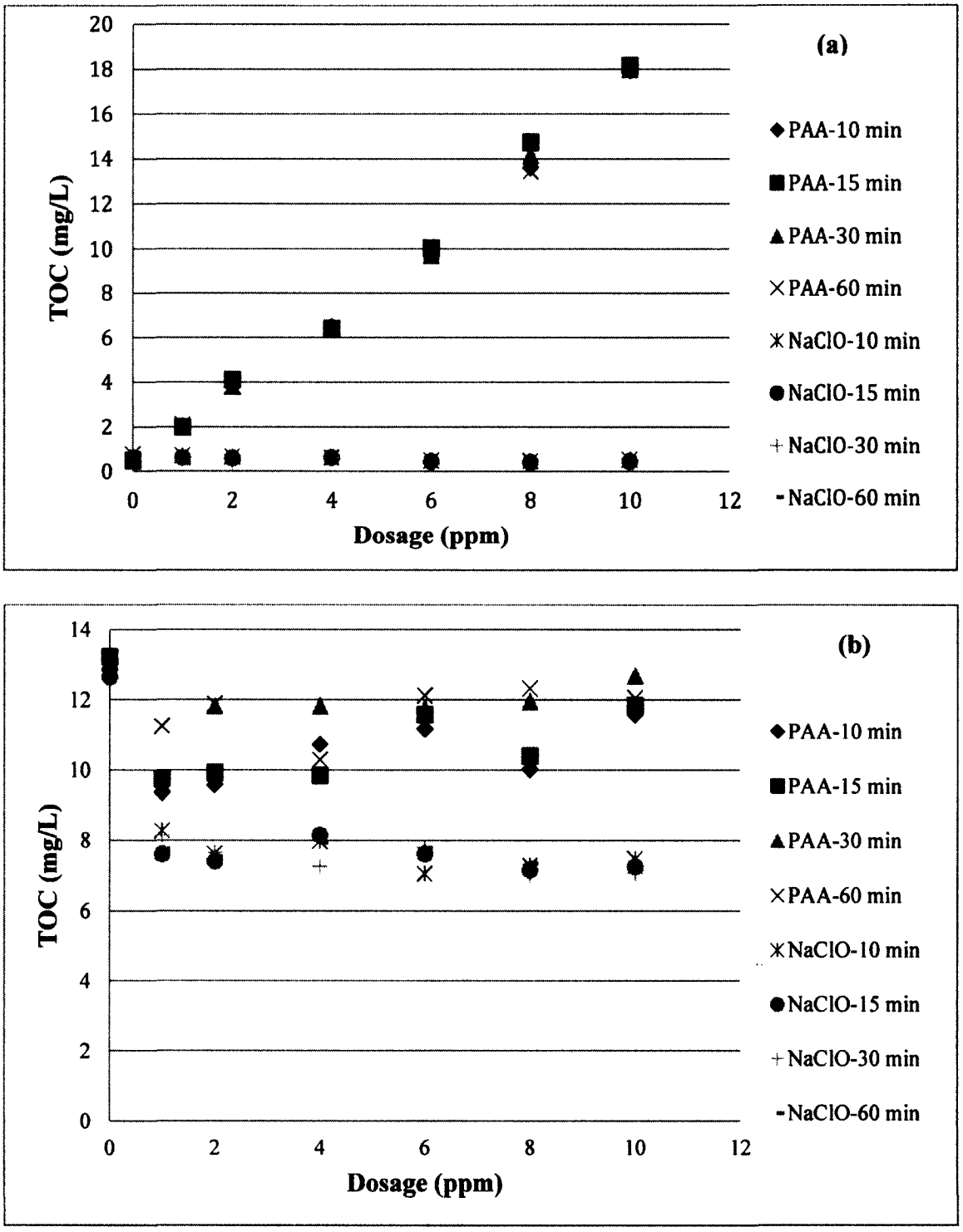


Figure 5.2: TOC results for (a) PAA/NaClO in distilled water (DW), (b) PAA/NaClO in wastewater (WW)

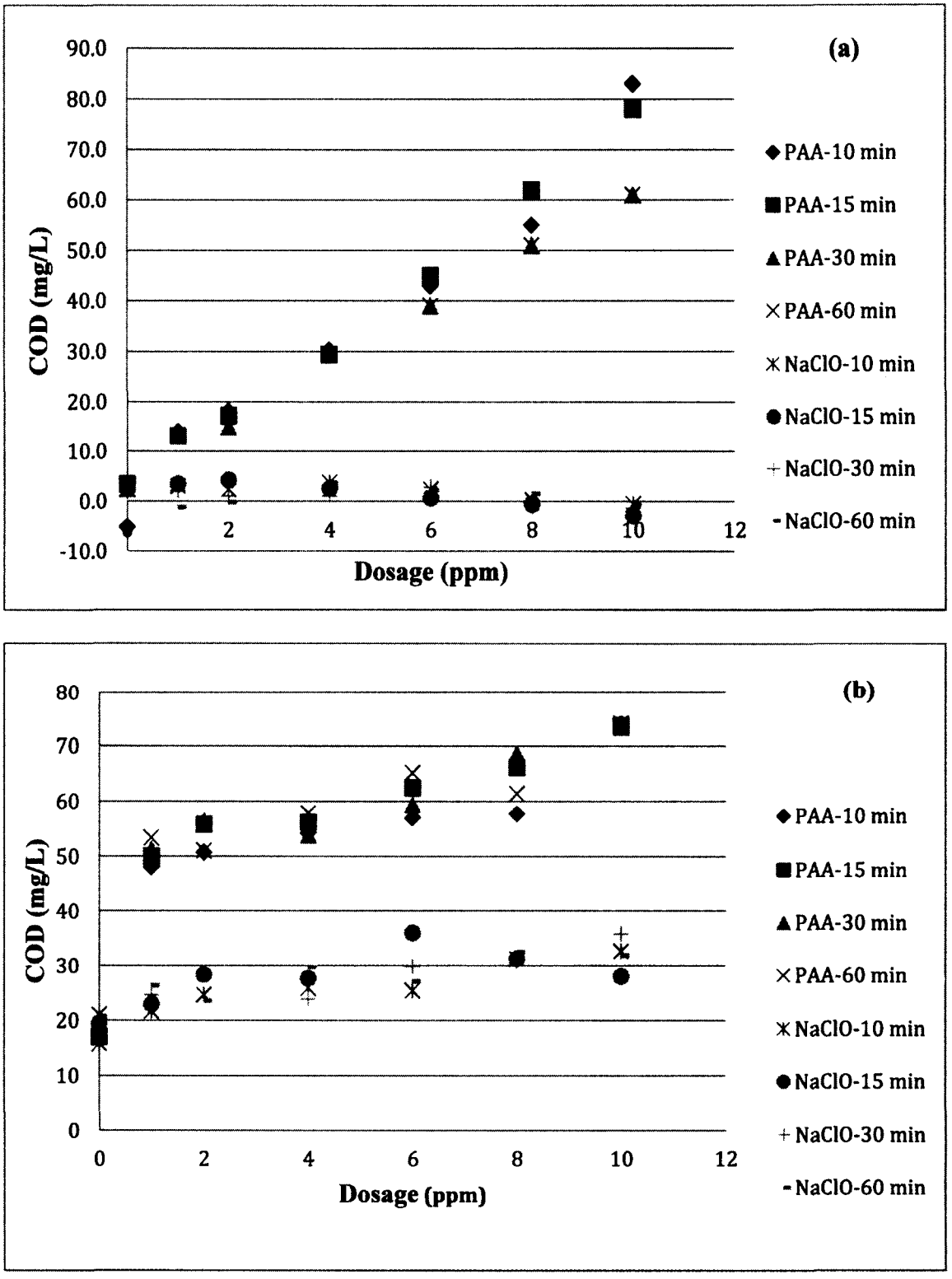


Figure 5.3: COD results for (a) PAA/NaClO in distilled water (DW), (b) PAA/NaClO in wastewater (WW)

TOC and COD in DW was significantly increased by PAA but was not influenced by NaClO. This could be explained since organic carbon atoms are an inherent make-up of the chemical formula of PAA (CH_3COOOH) which also contains acetic acid in its solution mixture (CH_3COOH). On the other hand, NaClO does not contain organic carbon and therefore TOC and COD were not affected by NaClO dosages as expected. To validate this explanation, theoretical TOC and COD values at different dosages of PAA were calculated (see Appendix A). The calculated values had similar results to the experimental values.

The impacts of different PAA/NaClO dosages and contact time on TOC in WW (Figure 5.2b) were different. It was found that TOC values in both PAA and NaClO treated WW were less than that in the raw WW although PAA seemed slightly increased TOC while NaClO had almost no influence as dosages increased. The reduction of TOC by PAA was not expected since PAA was reported to increase TOC significantly in WW [12], which indicated that errors involved in the TOC analysis. The errors could be caused by the loss of volatile organic substances in PAA treated WW when conducting TOC analysis [67]. However, different wastewater samples should be further conducted and reproduced to investigate more accurate TOC trends. Increasing contact time had no or very little influence on TOC in both PAA and NaClO treated WW.

For COD in WW (Figure 5.3b), PAA/NaClO performed similarly. Increasing contact time had almost no influence on COD in WW, while increasing PAA/NaClO dosages lead to an increase of COD in both PAA and NaClO disinfected WW. Nevertheless, the increase of COD in PAA disinfected WW was more consistent and more significant than that in NaClO disinfected WW, where COD after 6 ppm began to decrease as dosage increased. Similar to

DW, the increase of COD in WW by PAA was more significantly than that by NaClO. Nevertheless, the extent of COD to increase in WW was reduced because of the existence of contaminants.

5.1.3 SUVA Test

Specific UV_{254} absorbance (SUVA) is a parameter that uses UV_{254} absorbance and dissolved organic carbon (DOC-TOC values after filtration) to analyze wastewater. The equation for calculating SUVA provided by U.S. EPA method 415.3 was as follows [68]:

$$\text{SUVA (L/mg-M)} = UV_{254}\,(cm^{-1}) / \text{DOC (mg/L)} * 100\ \text{cm/M} \qquad \text{(Equation 5.1)}$$

SUVA was chosen in this project because SUVA could indicate the water treatability and DBPs formation potential by PAA/NaClO. Previous studies [69-70] found that SUVA correlated well with the aromaticity of the organic carbon, and high aromaticity was associated with DBPs formation potential. Therefore, a higher SUVA indicates a higher potential to produce DBPs.

UV_{254} absorbance in DW/WW after adding different dosages of PAA/NaClO was measured at 10 and 60 min. Results of UV_{254} absorbance in DW/WW as well as SUVA in DW are shown in Appendix A. The TOC values were used for DOC in the equation. Results of the effects of different dosages of PAA/NaClO on SUVA in WW are shown in Figure 5.4.

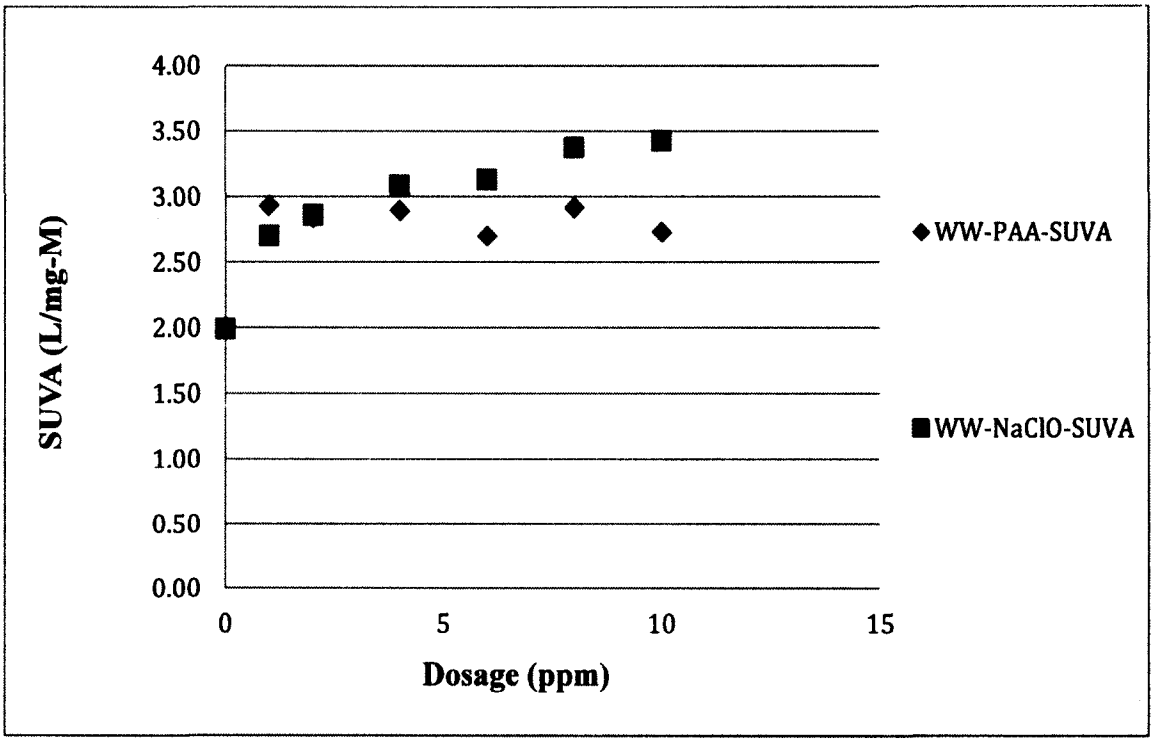


Figure 5.4: SUVA results after 60 min for PAA/NaClO in wastewater (WW)

Comparison of SUVAs in PAA/NaClO disinfected WW with SUVA in raw WW, it was found that both PAA and NaClO increased SUVA in WW, and as dosages increased, PAA had almost no influence on SUVA in WW, while NaClO kept increasing SUVA in WW. These phenomena illustrated DBPs formation potential in WW could be increased by both PAA/NaClO, and increasing NaClO dosage could further increase the potential of DBP formation. However, since there were errors existed in TOC values, the calculated SUVA values might not be accurate. In addition, different wastewater samples need to be further investigated.

5.1.4 NH_3-N Test

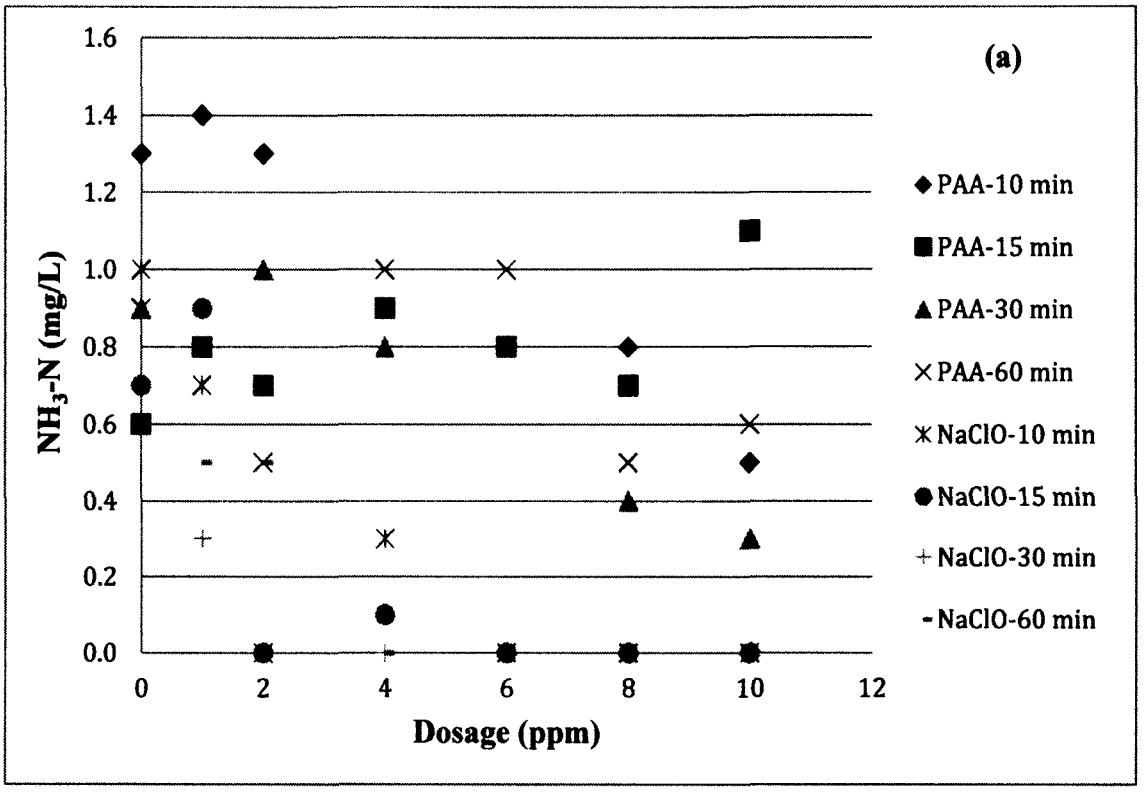
Ammonia (NH_3-N) is a nutrient which is commonly found in wastewater. It is toxic to aquatic life because it can exert an oxygen demand and promote microbiological growth. In

addition ammonia in the unionized form is directly toxic to many aquatic species [71]. One way to remove ammonia in wastewater is by chlorination. Chlorine cannot only oxidize ammonia to form chloramines, it also can lead to breakpoint chemistry to achieve the removal of ammonia through conversion to NCl_3 which offgases from solution [1, 3, 44]. As a promising alternative to chlorine, PAA has not been targeted to study its possible reactions with ammonia, therefore ammonia removal by PAA still needs investigation.

The effects of different dosage of PAA/NaClO at 10, 15, 30, and 60 min on NH_3-N in both DW and WW were investigated. Results are shown in Figure 5.5.

As shown in Figure 5.5a, there was no obvious trend for NH_3-N in PAA/NaClO treated DW. However, the range (0-1.4 mg/L) of NH_3-N indicated that interferences were involved in the DW. The most possible reason is that the distillation system was renewed during the experimental periods, resulting that the tested DW was not pure.

For NH_3-N in PAA/NaClO treated WW (Figure 5.5b), it was found that both PAA and NaClO reduced NH_3-N in raw WW, and the reduction by NaClO disinfection was more significant than PAA. 92.3% - 94.6% removal of NH_3-N was achieved by NaClO, while PAA reduced NH_3-N by 64.1% - 66.5% (Appendix A). However, considering that the reduction of NH_3-N by NaClO should increase as dosage increases before breakpoint (weight ratio of Cl_2 : NH_3 is around 7.6 [2]), errors involved in the ammonia analysis by NaClO. The results of NH_3-N reduction by PAA were also not expected since PAA was reported not to react with NH_3 [12]. Nevertheless, very few studies on NH_3-N reduction by PAA were conducted. Therefore, further study on NH_3-N reduction by both PAA and NaClO should be conducted.



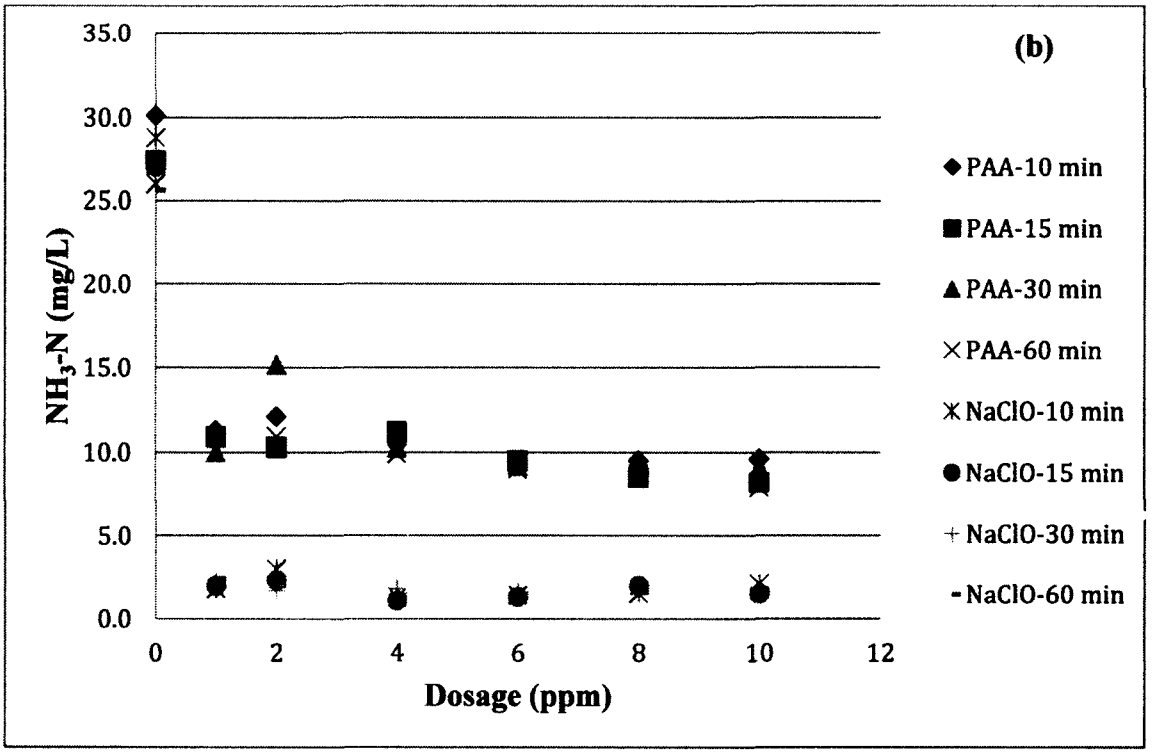


Figure 5.5: NH_3-N results for (a) PAA/NaClO in distilled water (DW), (b) PAA/NaClO in wastewater (WW)

5.2 THMs Calibration

THMs standards were calibrated only by direct analysis because it was found that THMs were very temperature- and time- sensitive. It was found that when one sample was running, the volumes of other samples decreased very rapidly although they were kept in a 4 °C refrigerator. This was probably caused by the volatility of THMs. To prevent THMs volatilization from resulting in an inaccurate result, samples were sealed carefully and put in an ice bucket until analysis.

As illustrated in Chapter 4, the intensity of THMs peaks in the full-scan mode of MS was so weak that the areas under these peaks (calibration) could not be automatically computed. To increase the peak intensity, selected ion monitoring (SIM) mode, where only the target ions were monitored, was used. Calibration of the four THMs-chloroform, bromodichlromethane, dibromochloromethane, and bromoform by direct-SIM analysis are shown in Figure 5.6, Figure 5.7, Figure 5.8, and Figure 5.9, respectively. The full-scan analysis of these four THMs was also conducted as comparisons, shown in Appendix B.

The actual calibration curves for chloroform, bromodichlromethane, dibromochloromethane, and bromoform are shown in Figure 5.10 and Figure 5.11. Each of the THM calibration curves had good correlation coefficients R^2, which demonstrates good linearity of the methods. Their corresponding calibration equations are:

$$y = 166863\ x + 3E{+}06,\ R^2 = 0.9165 \qquad \text{(Equation 5.2)}$$

$$y = 165740\ x + 493503,\ R^2 = 0.9393 \qquad \text{(Equation 5.3)}$$

$$y = 100602\ x + 272473,\ R^2 = 0.9884 \qquad \text{(Equation 5.4)}$$

$$y = 164994\ x + 37792,\ R^2 = 0.9497 \qquad \text{(Equation 5.5)}$$

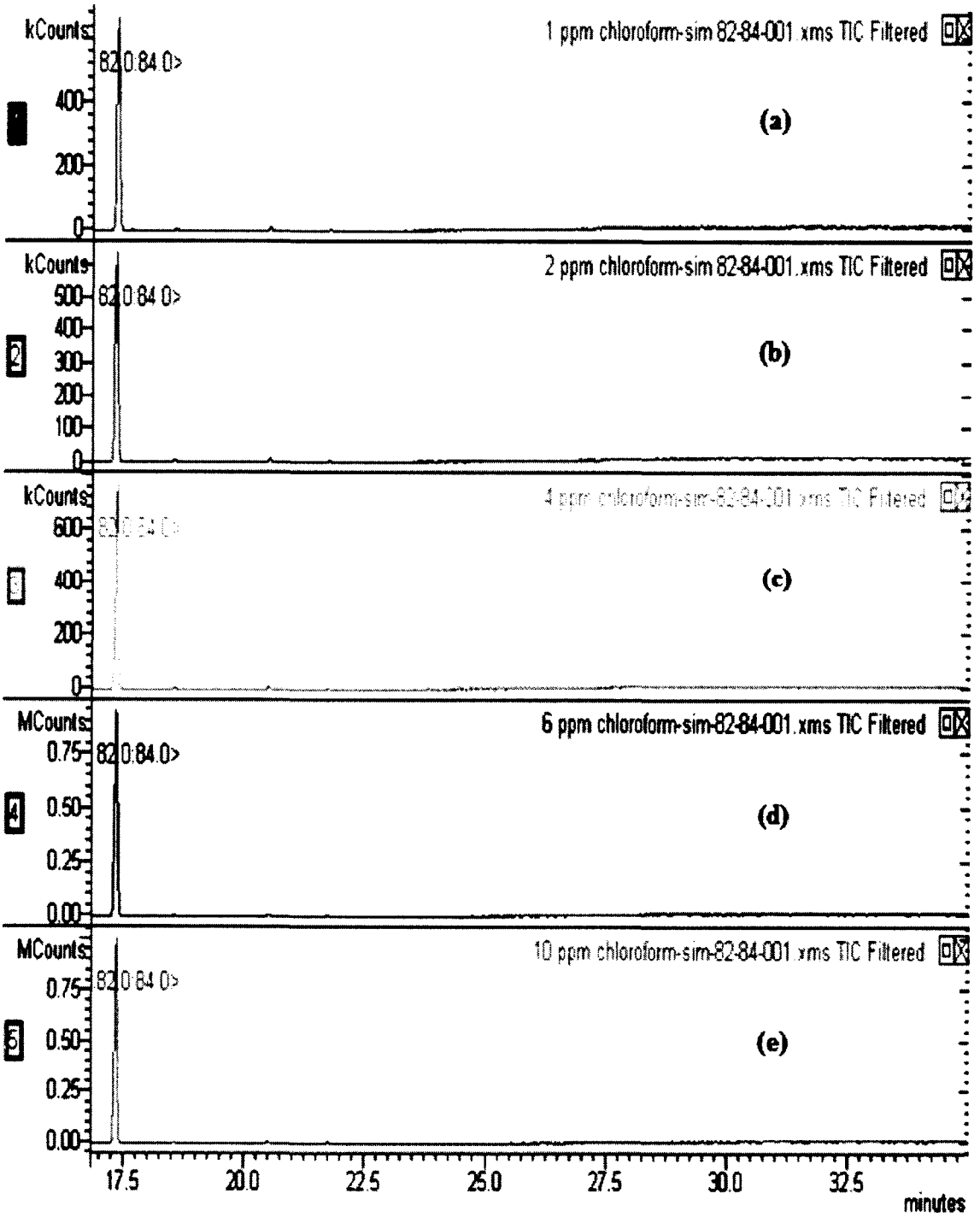


Figure 5.6: Selected ion monitoring (SIM, 82-84 m/z) calibration for chloroform: (a) 1 ppm, (b) 2 ppm, (c) 4 ppm, (d) 6 ppm, (e) 10 ppm

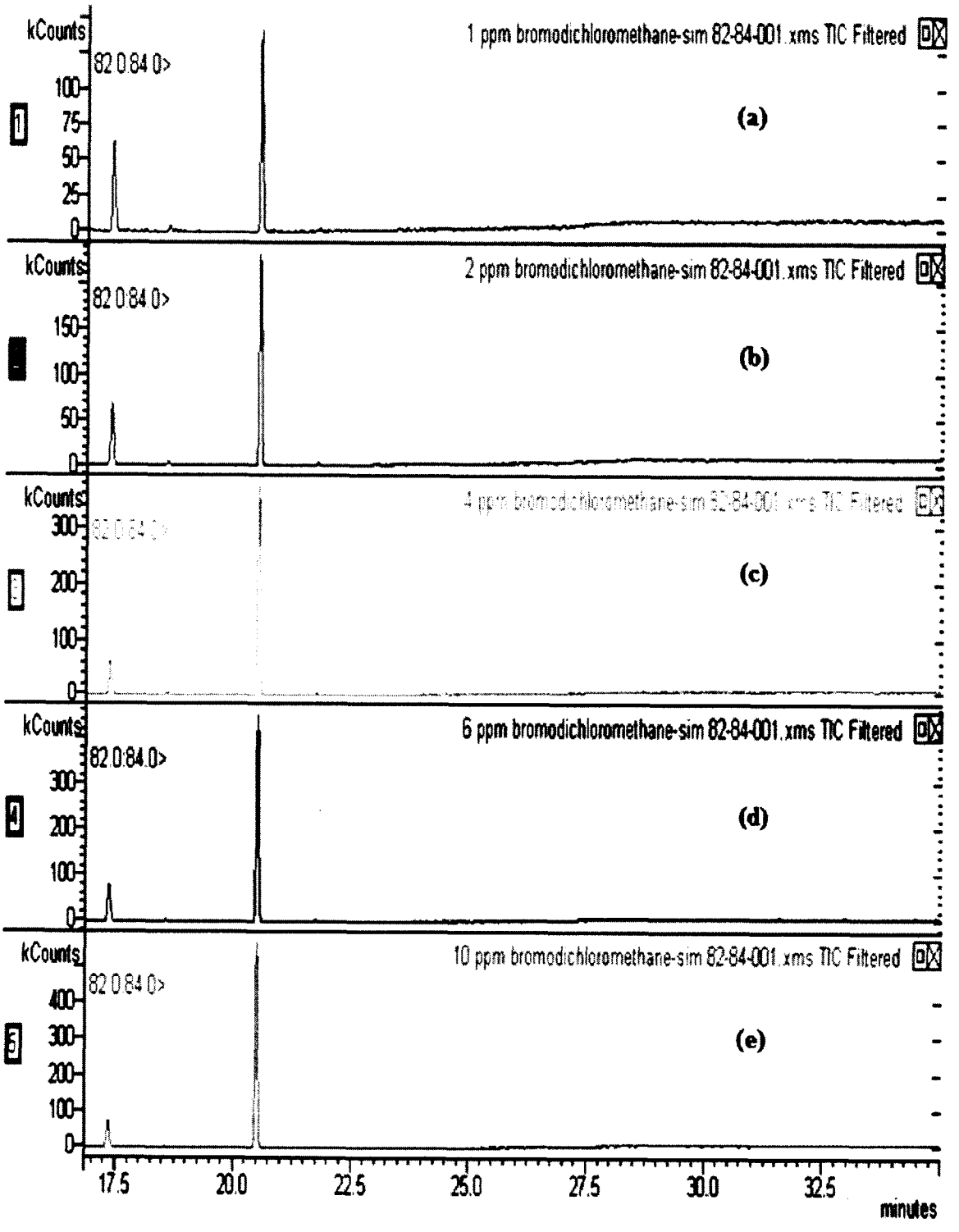


Figure 5.7: Selected ion monitoring (SIM, 82-84 m/z) calibration for bromodichloromethane: (a) 1 ppm, (b) 2 ppm, (c) 4 ppm, (d) 6 ppm, (e) 10 ppm

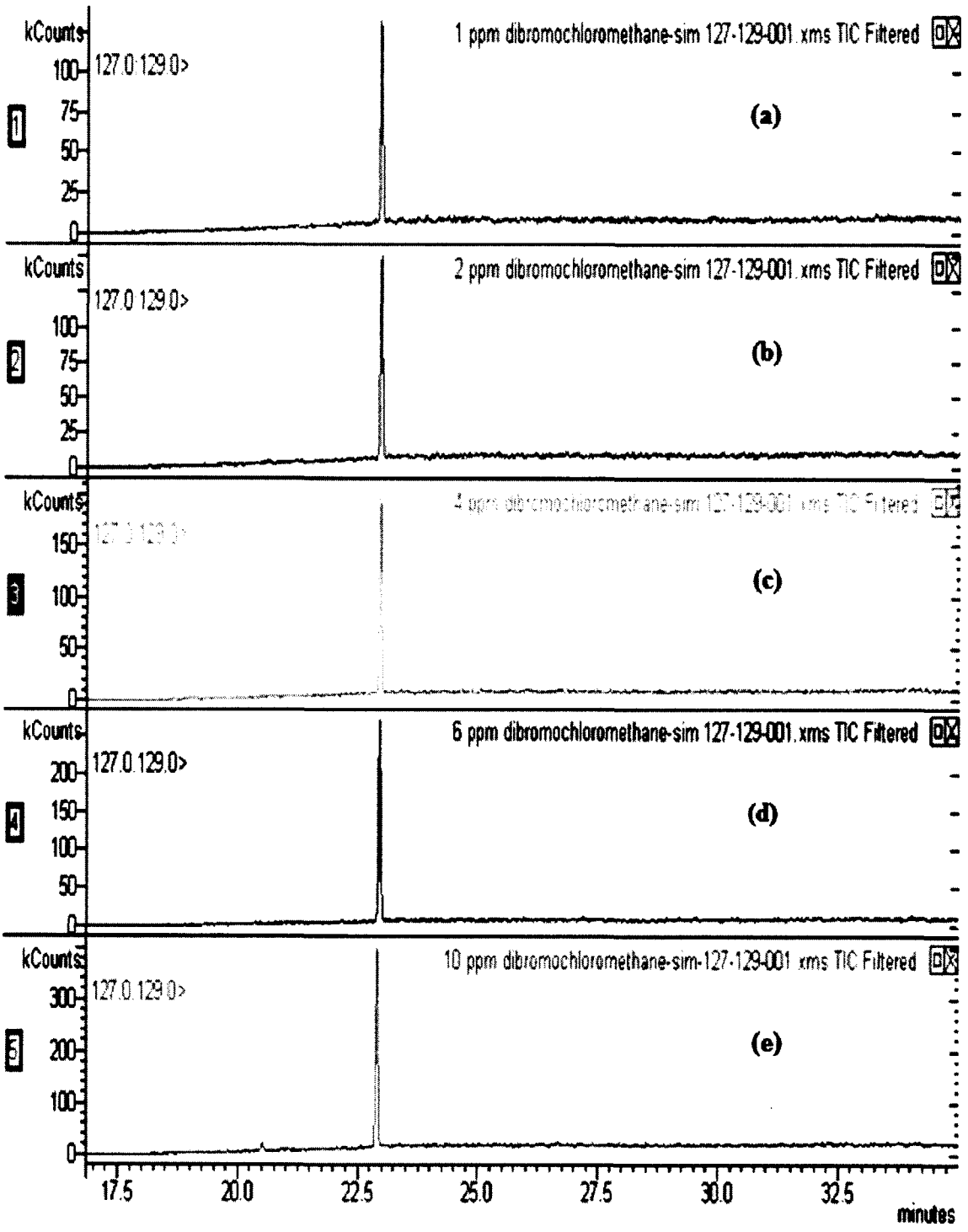


Figure 5.8: Selected ion monitoring (SIM, 127-129 m/z) calibration for dibromochloromethane: (a) 1 ppm, (b) 2 ppm, (c) 4 ppm, (d) 6 ppm, (e) 10 ppm

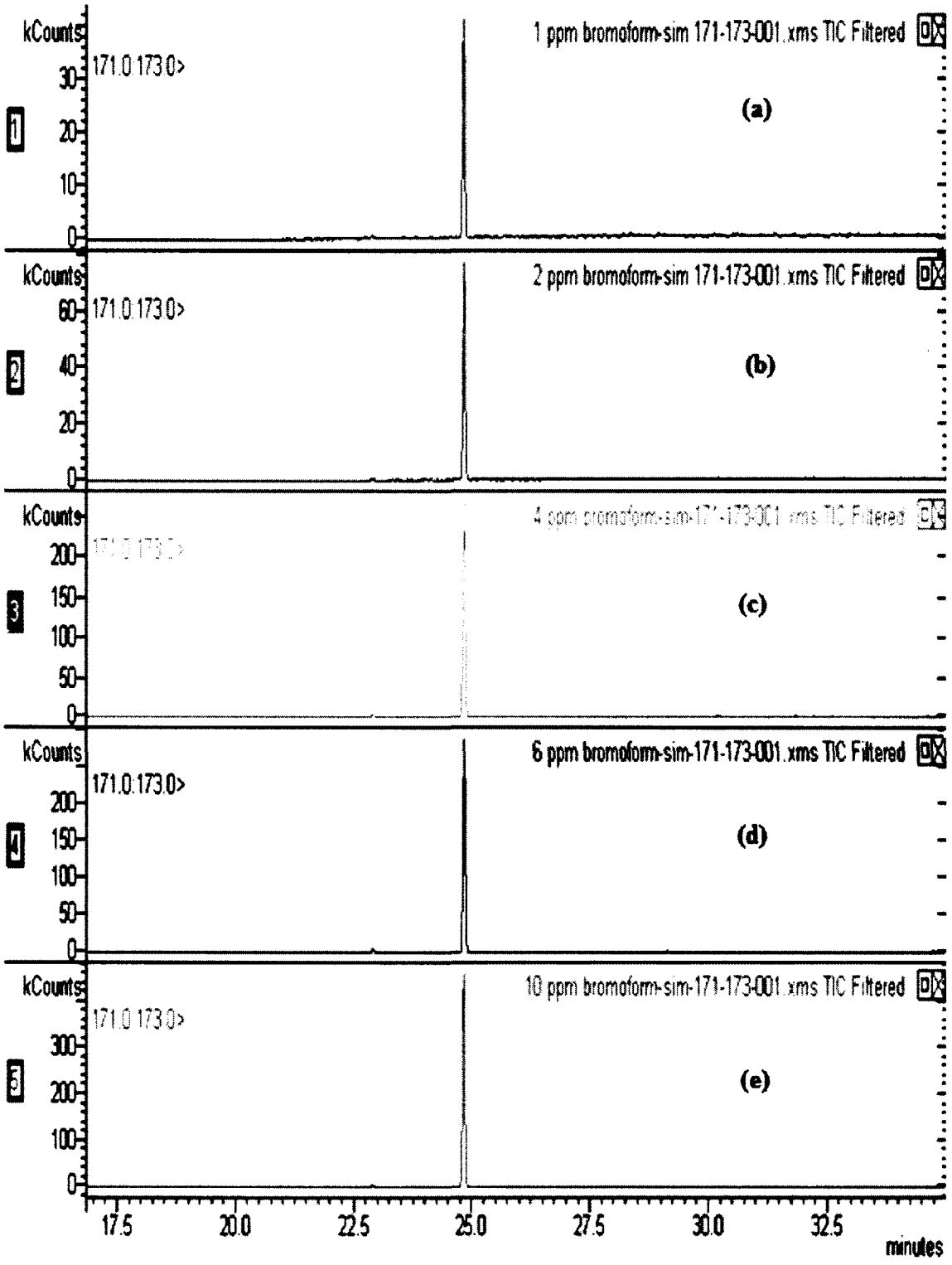


Figure 5.9: Selected ion monitoring (SIM, 171-173 m/z) calibration for bromoform: (a) 1 ppm, (b) 2 ppm, (c) 4 ppm, (d) 6 ppm, (e) 10 ppm

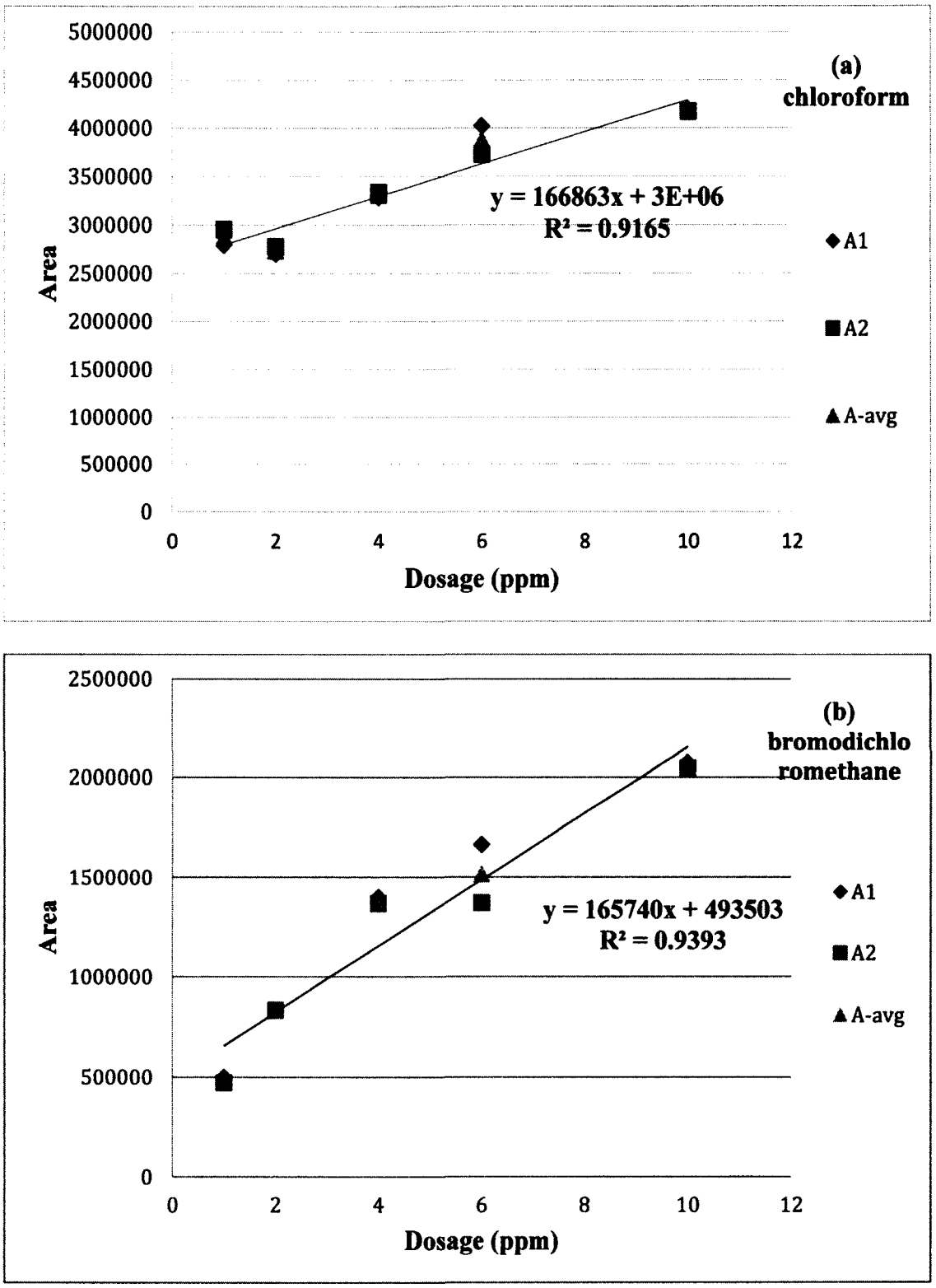
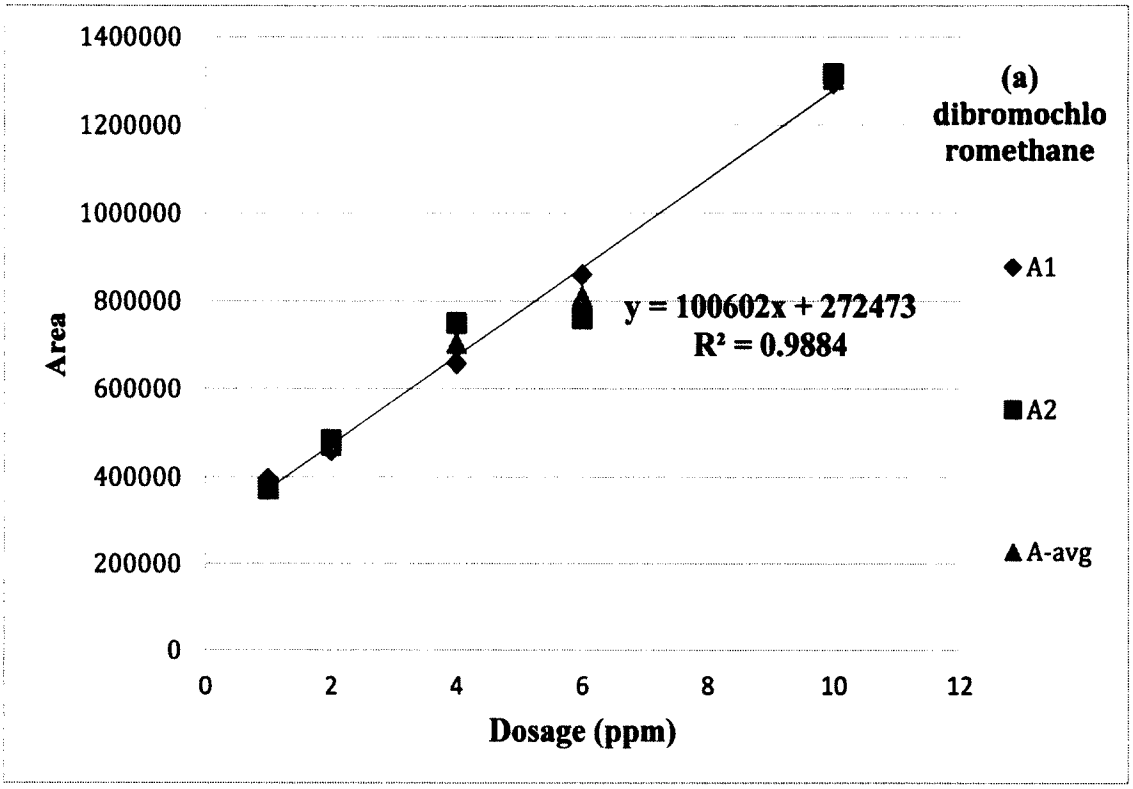


Figure 5.10: Calibration curves for THMs: (a) chloroform, (b) bromodichloromethane



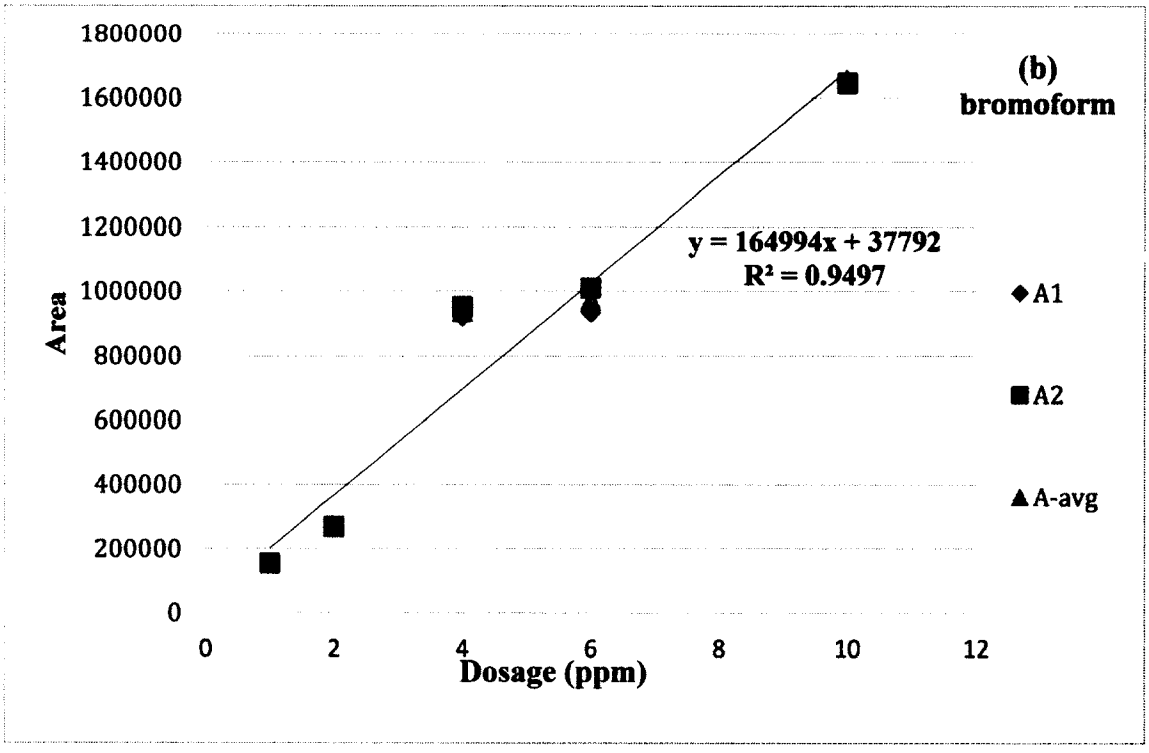


Figure 5.11: Calibration curves for THMs: (a) dibromochloromethane, (b) bromoform

5.3 Results and Discussions for Wastewater Disinfection by PAA/NaClO

Wastewater sampling and analysis was started in a broad perspective to investigate possible DBPs formation during wastewater disinfection with PAA. This analysis was conducted simultaneously when developing the method for THMs. Since the only available MS was broken down in the beginning, GC/FID was preliminarily used for PAA-induced DBPs analysis. When MS was ready for use, a comparative study of DBPs formation by PAA versus NaClO was performed, followed by the THMs analysis in PAA/NaClO-disinfected wastewater based on the specific method developed.

5.3.1 GC/FID Analysis for DBPs Formed in PAA Disinfected Wastewater

Initially the GC (Varian CP3800) in Environmental Lab was used with an Agilent J&W DB-1 column 15 m * 0.32 mm * 0.25 μm for PAA-induced DBPs analysis. The oven temperature program was set as follows [16]: 40 °C, hold 2 min; 250 °C at 10 °C/min hold 10 min; a total running time of 33 min. Splitless injection was applied, and the derivative solvent and washing solvents were ether and a mixture of ether/hexane, respectively.

10 ppm PAA was added into 1000 mL wastewater and reacted for 10 min. 250 mL of PAA-disinfected samples was collected, treated by a series of processes (as discussed in Section 3.2, and finally analyzed by GC/FID. Results are shown in Figure 5.12.

Ten small peaks (1-10 in Figure 5.12), including two peaks at 9-10 min and eight peaks at 18.5-26 min, were found. These peaks could be compounds that originally existed in wastewater and/or DBPs formed by PAA. Unfortunately, GC/FID was not able to identify

them because standards of these compounds were unavailable. Therefore, a comparative run for raw wastewater was needed.

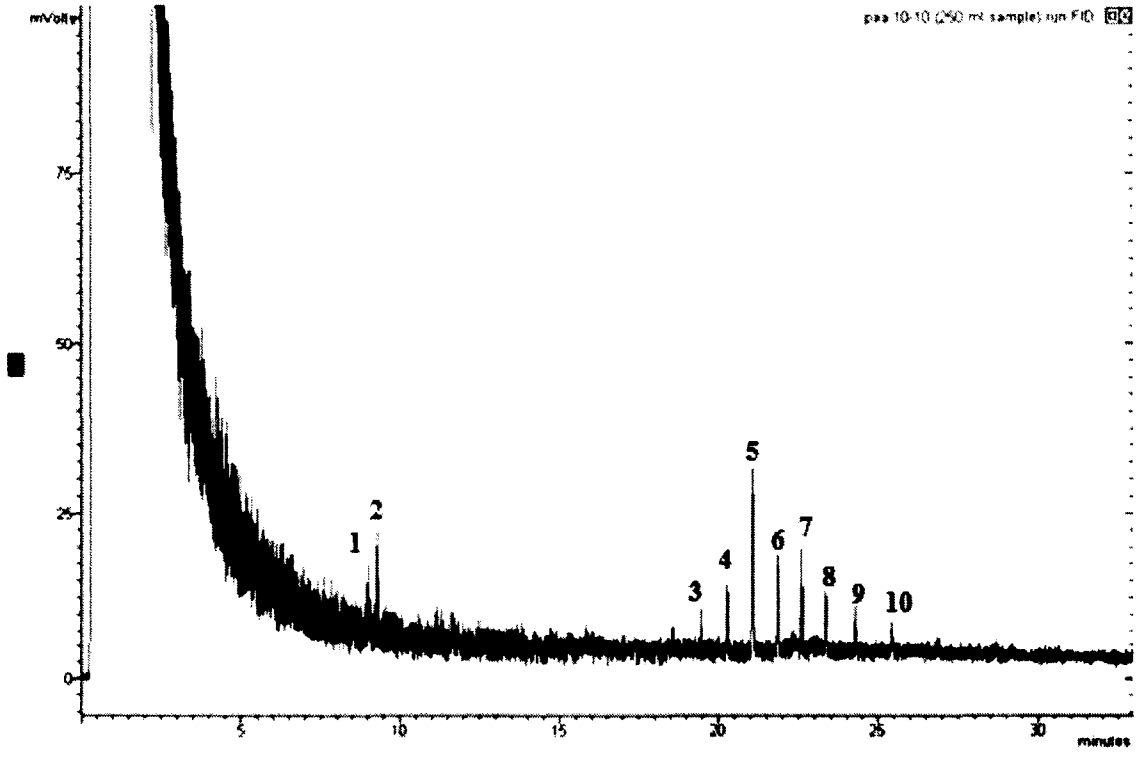


Figure 5.12: Trial 1- FID results for wastewater (January 2011) disinfection by 10 ppm PAA at 10 min contact time (PAA + WW)-GC in Environmental Engineering Lab

The GC used for detection of the above peaks had some drawbacks, including the absence of an auto-sampler and USB interface for data acquisition, therefore another superior Varian CP3800 GC in the Chemistry Lab was used at this point. VF-5ms 30m * 0.25mm * 0.25 μm Column was used. The GC oven temperature was the same as before. 250 mL samples of both raw wastewater and PAA-disinfected wastewater were prepared, treated and analyzed by GC/FID. Results are shown in Figure 5.13.

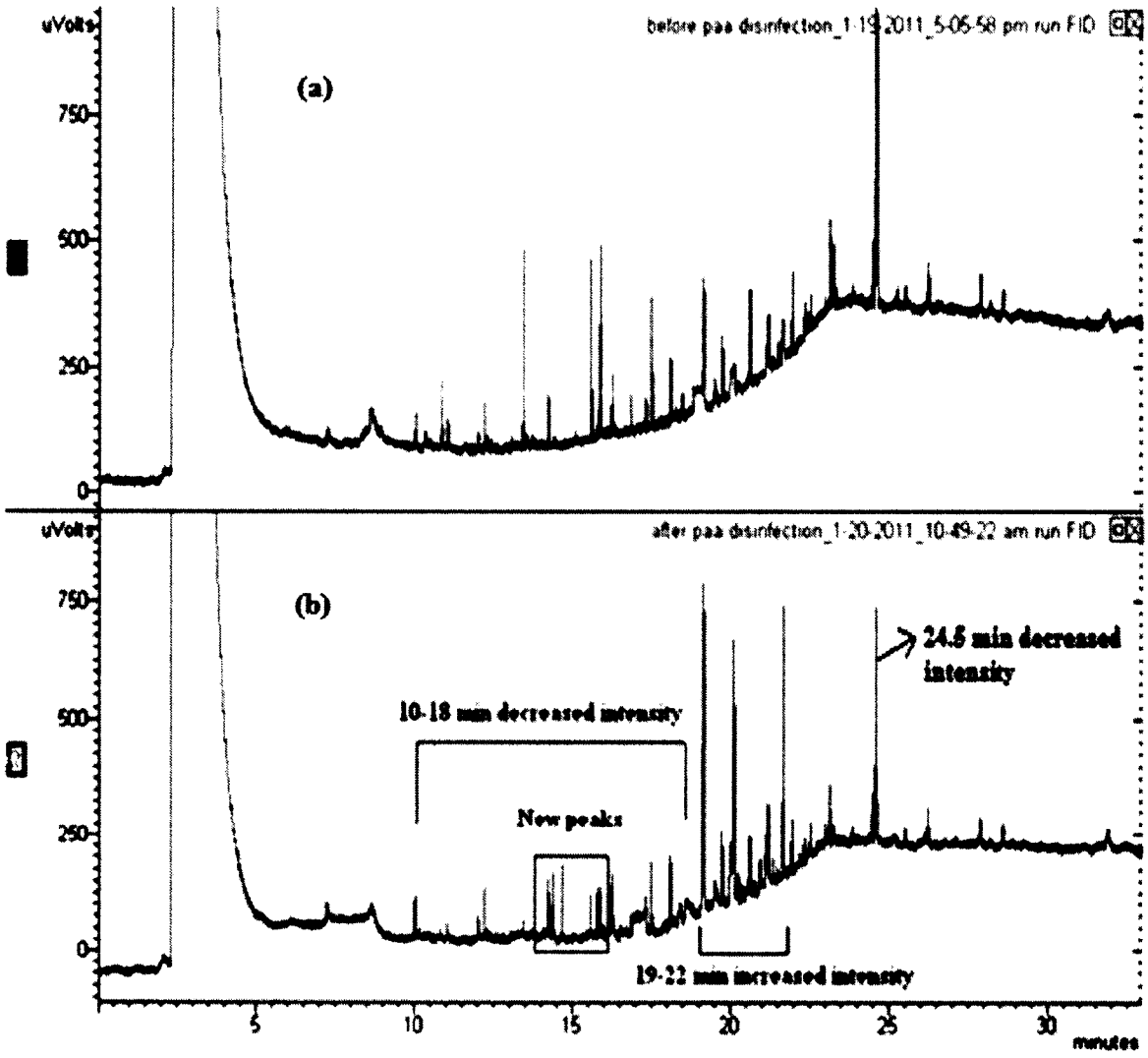


Figure 5.13: Trail 2 - FID results for (a) raw wastewater (WW, January 2011), (b) 10 ppm - 10 min PAA disinfected wastewater (PAA + WW) - VF-5ms column

By comparison of the two chromatograms in Figure 5.13, it was observed that the 10 ppm PAA addition in wastewater decreased the intensity of peaks at 10-18 min and 24.5 min, while the intensity of peaks at 19-22 min was increased. In addition, several new peaks were found at 14-16 min. These new peaks were probably DBPs formed by PAA disinfection. This was an exciting finding. However, reproducibility for these peaks was still needed to confirm findings.

Another set of raw wastewater and 10 ppm-10 min PAA disinfected wastewater was analyzed by GC/FID, shown in Figure 5.14.

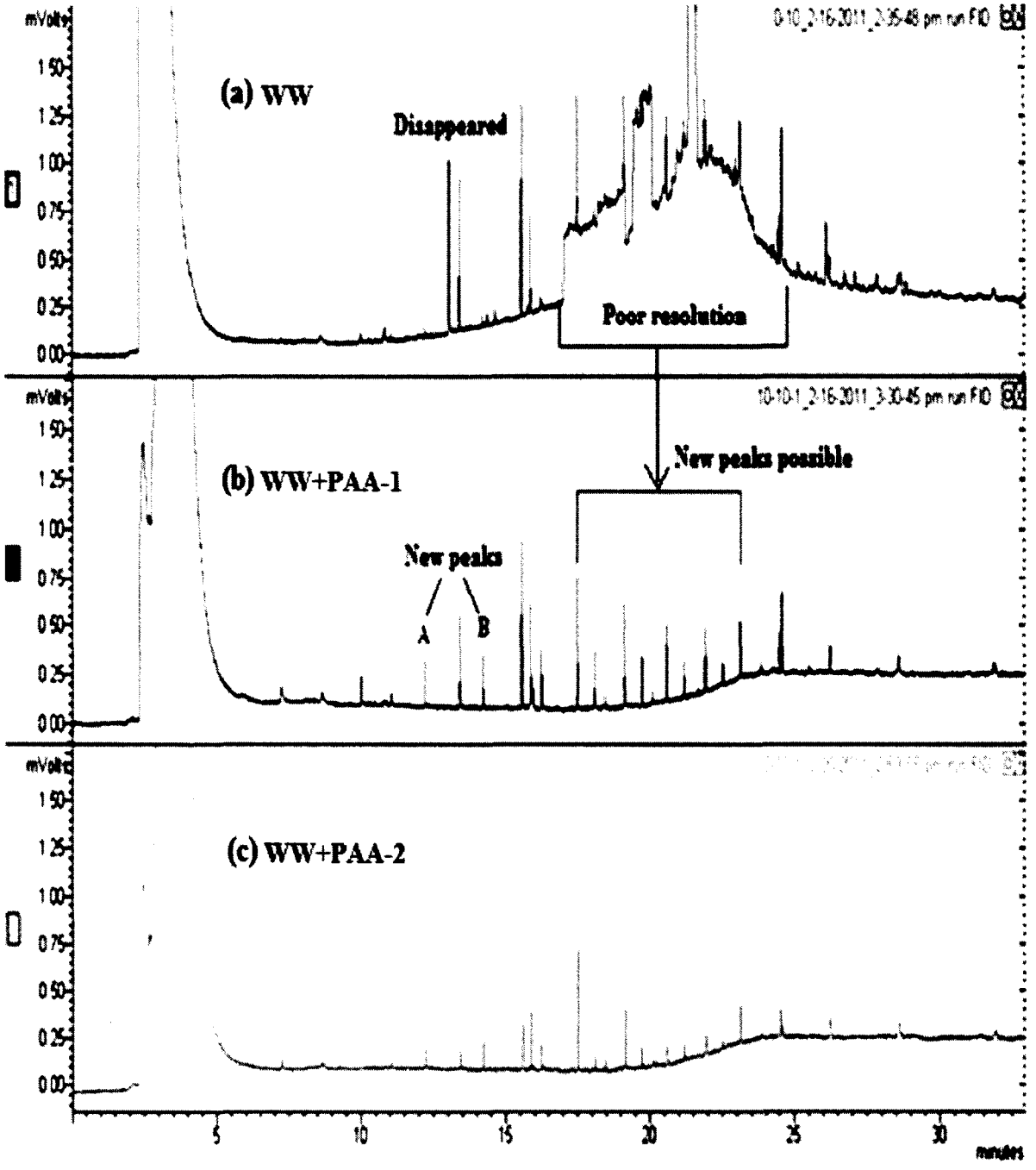


Figure 5.14: Trial 3 - FID results for (a) raw wastewater (WW, February 2011), (b) 10 ppm-10 min PAA disinfected wastewater (PAA + WW, sample 1), (c) 10 ppm-10 min PAA disinfected wastewater (PAA + WW, sample 2) - VF-5ms column

Two PAA-disinfected wastewater samples-sample 1 (Figure 5.14b) and sample 2 (Figure 5.14c), were conducted on two different days. A great reproducibility of peaks was found. By comparing PAA + WW (Figure 5.14b and 5.14c) with WW (Figure 5.14a), it is clear that the peak at 14 min (Figure 5.14a) disappeared and that two new peaks A and B (Figure 5.14b) were found at 12.3 min and 14.3 min, respectively. There may also be new peaks at 17.5-23.5 min (Figure 5.14b), but it was difficult to confirm because the peak resolution in the chromatogram of WW (Figure 5.14a) was poor.

Unfortunately, these GC samples were not run through MS for peak identification because MS was still unavailable at that time.

5.3.2 MS Analysis for DBPs Formed in PAA/ NaClO Disinfected Wastewater

Once MS was ready for use (about three months later), a set of raw wastewater and PAA-disinfected wastewater samples were analyzed by MS, trying to confirm the peaks detected by GC/FID. The previous column used (Agilent J&W DB-1 column) was no longer in use because earlier tests found it was not possible to obtain reliable results (see Section 4.1.1), therefore a new column (VF-5ms, 30 m * 0.25 mm * 0.25 μm) was used. Other conditions were kept same. A NaClO disinfected wastewater sample at the same conditions was also conducted as a comparative study to PAA. Results are shown in Figure 5.15.

Comparison of Figure 5.15b (WW+PAA) with Figure 5.15a (WW), two new peaks were found at 15 min (peak-A in Figure 5.15b) and 15.35 min (peak-B in Figure 5.15b). Comparison of Figure 5.15c (WW+NaClO) with Figure 5.15a (WW), one new peak was found at 15 min (peak-A in Figure 5.15c). A change of peak intensity was also noted: peaks

at 13-14 min in Figure 5.15a disappeared; peak intensity at 14.5 min in Figure 5.15c was also increased by NaClO. Unfortunately, no results were shown by searching MS library of spectra, and it is impossible to manually analyze the spectra of these peaks due to the complexity of WW. Therefore, possible DBPs peaks A and B were not identified.

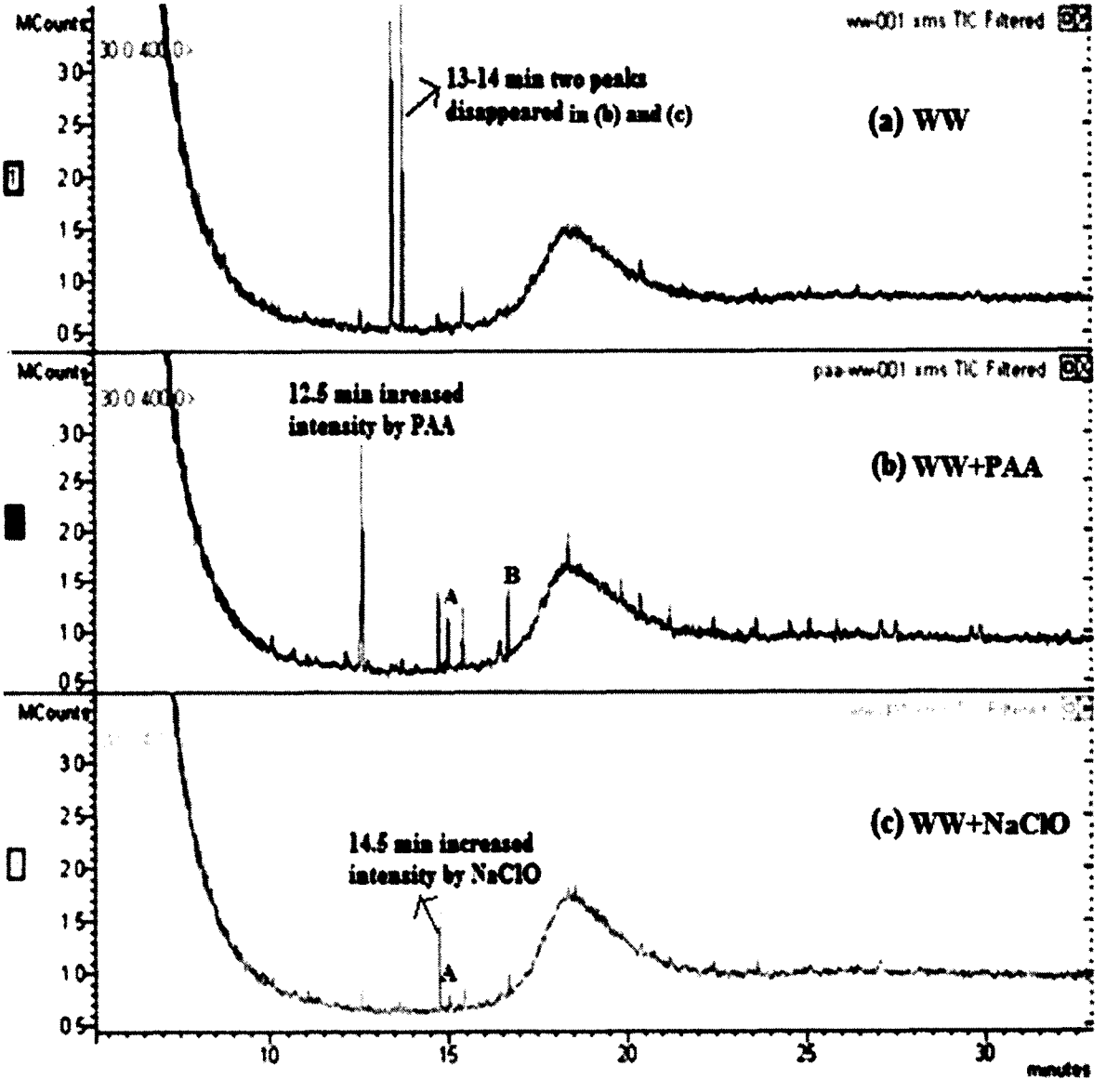


Figure 5.15: Trial 4 - MS analysis for (a) raw wastewater (WW, June-July 2011), (b) 10 ppm-10 min PAA disinfected wastewater (PAA + WW), (c) 10 ppm-10 min NaClO disinfected wastewater (NaClO + WW) - VF-5ms column

Comparison the spectra of PAA+WW in both Figure 5.14 and Figure 5.15, it was found that although the same column (VF-5ms 30m * 0.25mm * 0.25 μm) was used for both FID and MS analysis, different spectra were found and the DBPs peaks detected were also different. The reason was probably the completely different wastewater samples collected. The wastewater samples used in GC/FID analysis (Figure 5.14) were collected in winter (January-February 2011), while the samples for MS analysis (Figure 5.15) were collected in summer time (June-July 2011). The characteristics of wastewater change rapidly even in one day, not to mention in different seasons. Different temperature as well as the possible seasonal events such as snow in winter might lead to much more significant change of wastewater characteristics, and thus resulted in difficulty detecting the previous peaks found by GC/FID.

In addition to the above factors, detecting different unknown DBPs (which have a lack of standards) in one spectrum based on one method might be too difficult to start. Therefore, the most abundant groups of known DBPs- THMs were chosen to specifically study THMs formation during wastewater disinfection by PAA compared to NaClO.

5.3.3 MS Analysis for THMs in PAA/ NaClO Disinfected Wastewater

According to the method developed for THMs, the column (Varian CP-selected 624 CB 60 m * 0.25 mm * 1.4 μm), oven temperature program, and other conditions targeting for THMs were used. The raw wastewater and PAA/NaClO disinfected wastewater samples were analyzed by MS, shown in Figure 5.16.

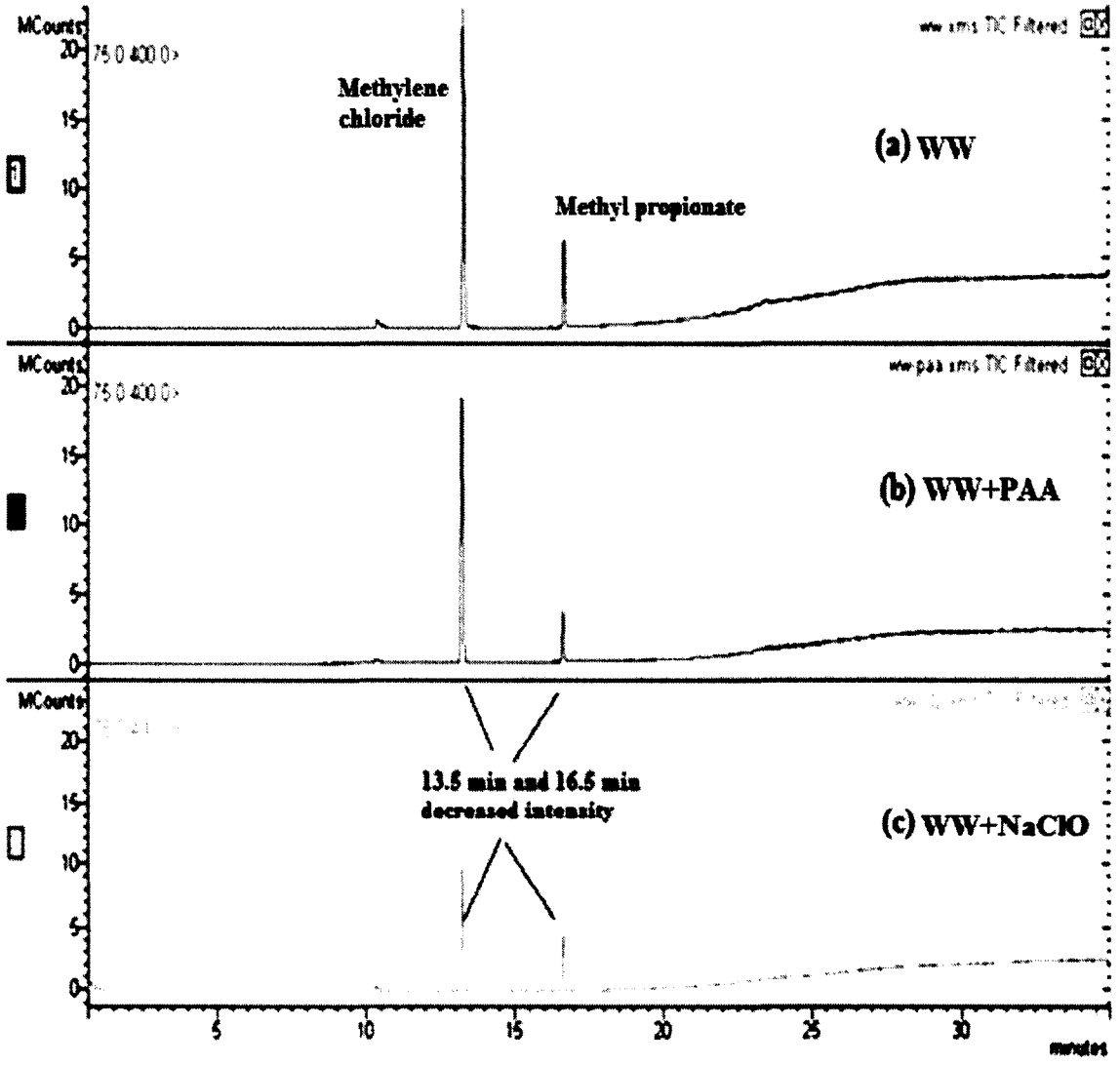


Figure 5.16: Trial 5 - MS analysis for THMs in (a) raw wastewater (WW, December 2011-January 2012), (b) 10 ppm-10 min PAA disinfected wastewater (PAA + WW), (c) 10 ppm-10 min NaClO disinfected wastewater (NaClO + WW) - CP-selected 624 CB column

It was expected that THMs should be detected by running this test because by using the same method, THMs standards were calibrated (see Section 5.2). However, from Figure 5.16, no THMs were detected. The two peaks consistently existed in both raw wastewater and PAA/NaClO disinfected wastewater was identified as methylene chloride at 13.295 min, and methyl propionate at 16.676 min. The intensity of these two peaks was decreased by both PAA and NaClO.

Other sets of experiments were tried again, but still no THMs were found. This phenomenon indicated that other errors were probably involved because THMs standards could be identified and even quantified (see Section 4.1.1 and Section 5.2), THMs in the samples should also be detected at least.

To check where the errors happened, the sample preparation processes applied in THMs standards calibration and wastewater sample analysis were compared. Standards were prepared by serial dilution with pentane and directly analyzed by MS, which only took around 30 min. However, a sample went through at least 3 hours treatment processes including quenching, acidification, extraction, evaporation, and derivation before being analyzed by MS. Among these processes, evaporation was definitely the process that THMs were volatilized, because at the pressure (120-150 mbar, see Section 3.2.6) of the evaporator for drying ethyl acetate (last at least 1 hour), THMs as extremely volatile compounds could be easily evaporated. To solve this problem in the future, directly extraction of THMs by pentane should be conducted to avoid evaporation.

5.4 Summary

The impact of different PAA/NaClO dosages and contact time on parameters including pH, SUVA, TOC, COD, and NH_3-N in wastewater (WW) compared to distilled water (DW) were investigated.

In DW, the acidity of PAA and basicity of NaClO determined the final pH in DW, therefore as expected the pH in DW decreased by PAA and increased by NaClO. SUVA, TOC and COD are directly related to the amount of organic carbon in the structure of

PAA/NaClO, so SUVA, TOC and COD in DW were proportionally increased as PAA dosages increased, but were not affected by NaClO. Similarly, since no nitrogen in the structures of either PAA/NaClO or DW, both PAA/NaClO had no influence on NH_3-N in DW.

In WW, the buffering effects of contaminants reduced the effect of PAA/NaClO on the five parameters. PAA had little or no influence on pH in WW, while NaClO slightly increased pH in WW as dosages increased. Both PAA and NaClO have potential to produce DBPs since the increased COD and SUVA in PAA/NaClO disinfected WW were found. However, since errors involved in TOC analysis, SUVA values might be not accurate. And NH_3-N trends in both PAA and NaClO treated WW were not expected. Therefore, more tests and different wastewater samples need to be further studied.

For wastewater disinfection by PAA/NaClO, different trials were conducted in a broader perspective to investigate possible DBP formation by PAA. Since no MS was available at the beginning, the detected peaks that might be DBPs from PAA disinfection were unable to be identified. After MS was ready to use, several trials were further conducted and analyzed at the same GC/MS conditions by MS. Unfortunately, the previously detected peaks did not show up in MS. This was probably caused by the completely different wastewater samples collected. The new DBPs peaks detected by MS were also not identified by searching MS library of spectra.

Since detecting DBPs in a broad perspective was difficult to achieve, target compounds-THMs were selected to further study the DBP formation in PAA/NaClO disinfected wastewater. THMs were calibrated by direct analysis because they are very

volatile and might be vaporized during the processes calibration. Just for this reason, THMs in wastewater samples were not detected since the handling processes of wastewater sample were exactly same as that of processes calibration. THMs were probably volatized after evaporation process. To solve this problem, direct extraction of THMs by solvent-pentane should be conducted in the future.

Chapter 6

OXIDATION OF SELECTED EDCs/PHARMACEUTICALS BY PAA/NaClO: RESULTS AND DISCUSSION

Based on the methods developed in Chapter 4, calibration curves for 17β-estradiol (E2), clofibric acid, and ketoprofen were constructed, followed by the investigation on oxidation efficiency of PAA/NaClO and formation of oxidation products. The raw data for the figures and tables provided in this section are presented in Appendix C.

6.1 Calibration for 17β-estradiol (E2), Clofibric Acid, and Ketoprofen

Calibrations (the linearity of peak area response versus concentration) for 17β-estradiol (E2), clofibric acid, and ketoprofen were studied at the concentration range of 1, 2, 4, 6, and 10 ppm. The calibration curves, evaluated by the correlation coefficient R^2, were constructed based on the average area of triplicate runs at each concentration. The method detection limits (MDL), the minimum concentration that is greater than zero could be measured with 99% confidence [68, 72], for each standard were also calculated.

6.1.1 17β-estradiol (E2) Calibration

17β-estradiol (E2) was calibrated in two ways: direct analysis and processed analysis, shown in the Figure 6.1 and Figure 6.2, respectively. Their calibration curves were constructed and shown in Figure 6.3.

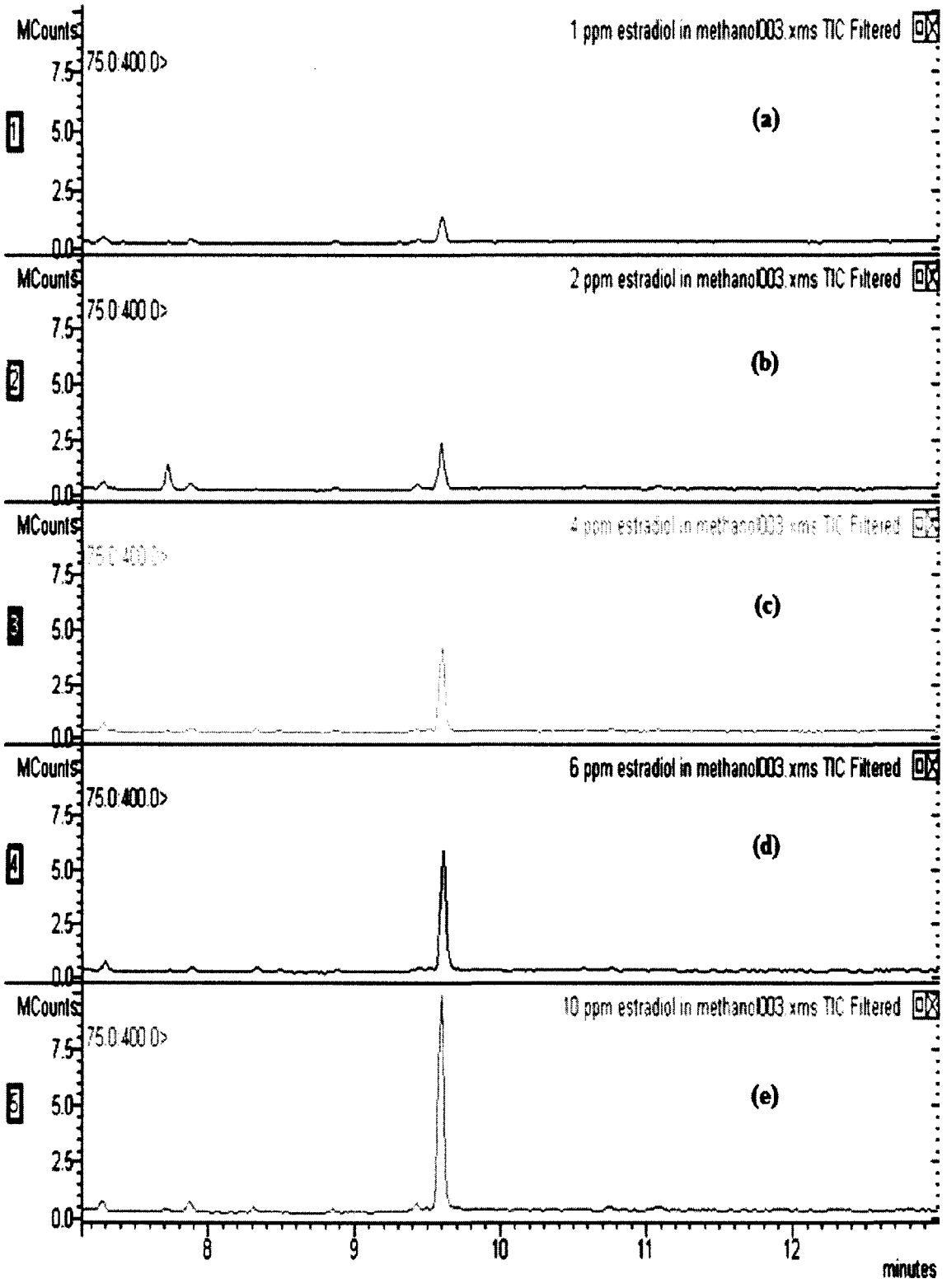


Figure 6.1: 17β-estradiol (E2) direct analysis calibration- E2 in methanol: (a) 1 ppm, (b) 2 ppm, (c) 4 ppm, (d) 6 ppm, (e) 10 ppm

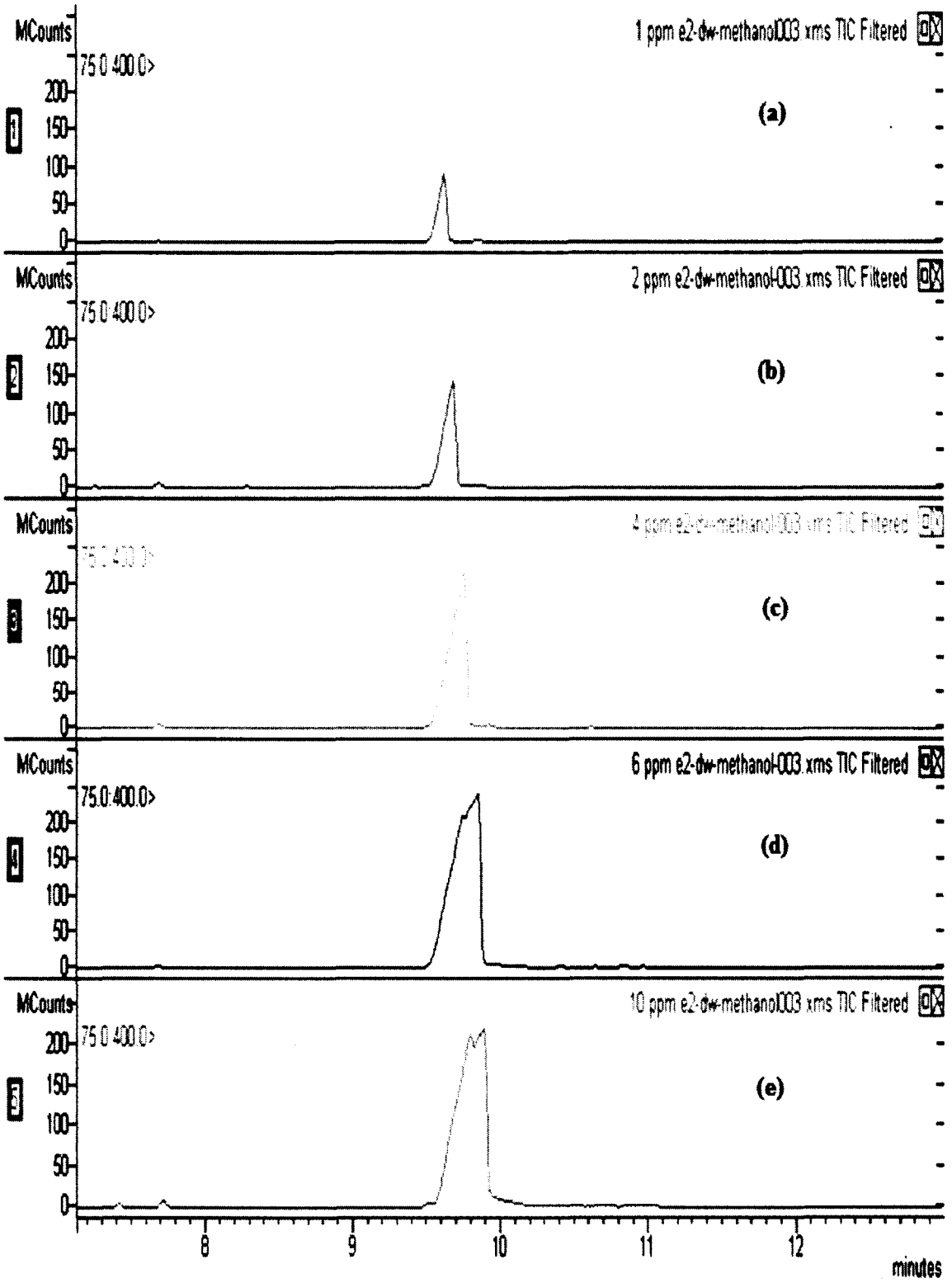
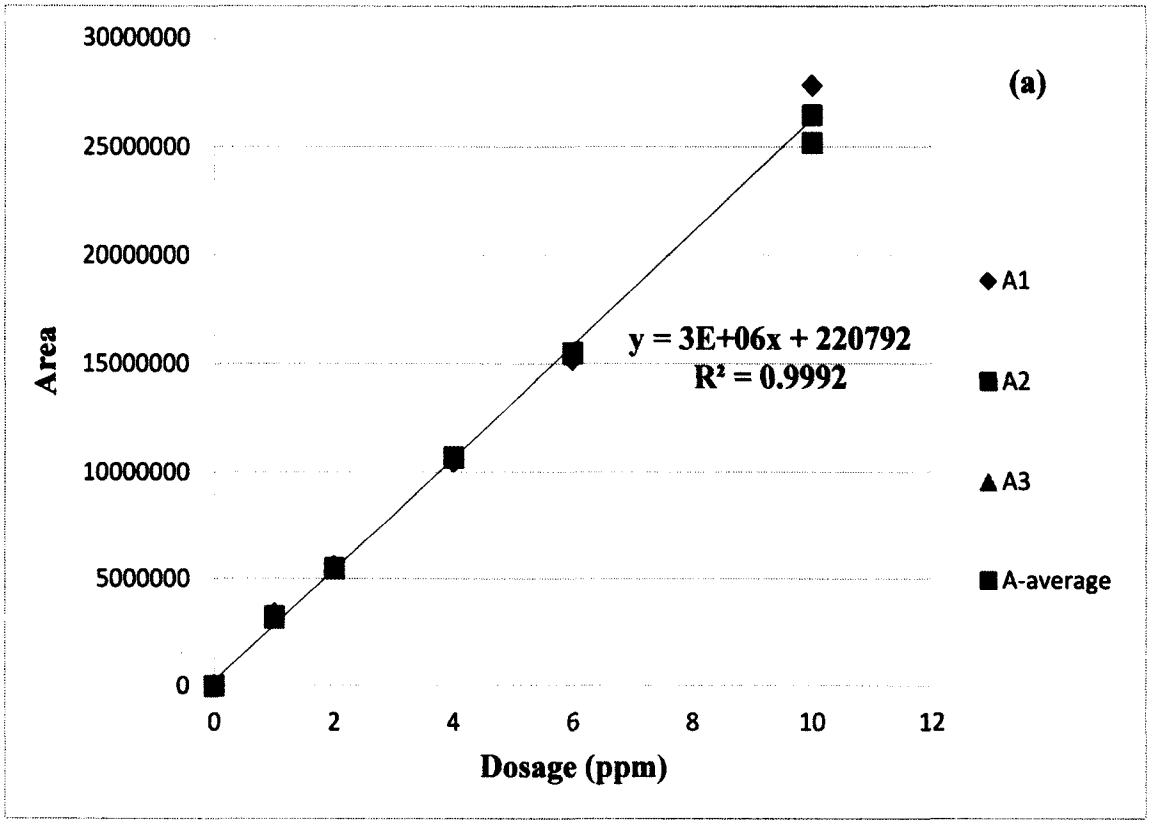


Figure 6.2: 17β-estradiol (E2) processed calibration- E2-DW-methanol: (a) 1 ppm, (b) 2 ppm, (c) 4 ppm, (d) 6 ppm, (e) 10 ppm



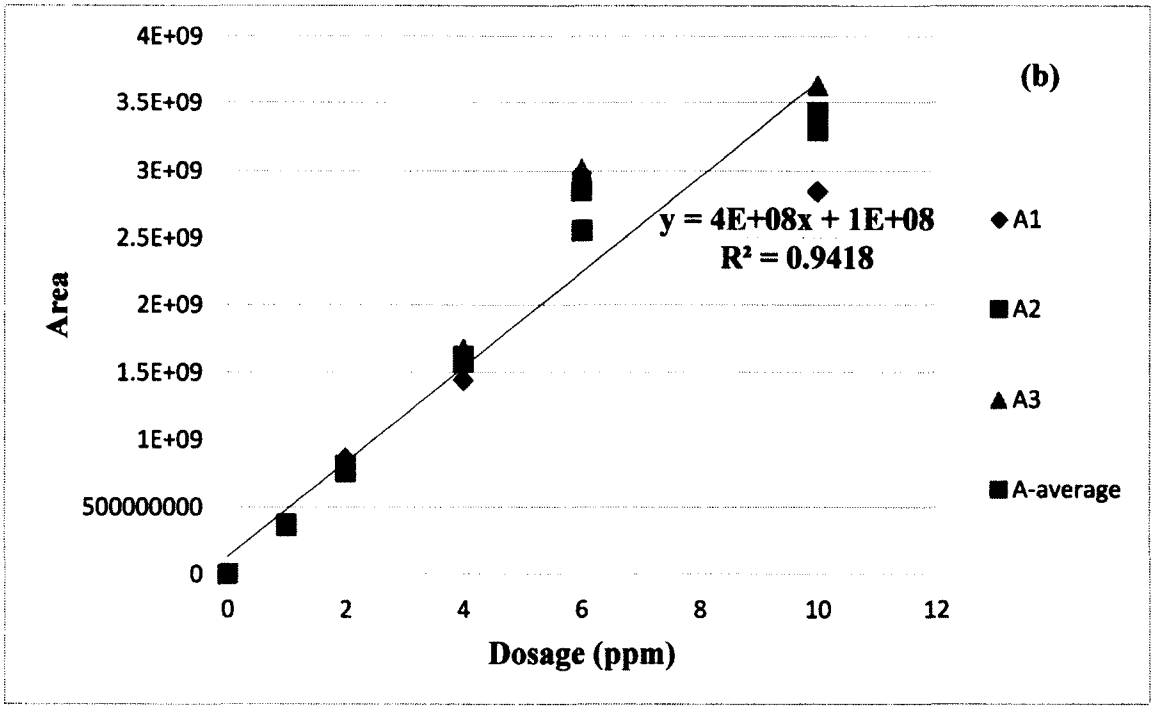


Figure 6.3: 17β-estradiol (E2) calibration curves for (a) direct analysis, (b) processed analysis

The calibration equations for both direct and processed calibration are as follows:

$$y = 3E+06x + 220792, R^2 = 0.9992 \quad \text{(Equation 6.1)}$$

$$y = 4E+08x + 1E+08, R^2 = 0.9418 \quad \text{(Equation 6.2)}$$

The calibration equations in Figure 6.3 demonstrated good linearity of the method. Comparison of Figure 6.1 and Figure 6.2, it was found that the processed calibration (Figure 6.2) increased the intensity of peaks significantly, while direct analysis (Figure 6.1) achieved a better linearity (a higher R^2) and better resolution (sharper peaks). This phenomenon illustrated that sample matrix, directly versus indirectly dissolved in methanol, played an important role in the calibration results. Given the processed calibration has the same matrix (the same treatment processes) as the samples, the processed calibration curve for 17β-estradiol (E2) was used.

6.1.2 Calibration for Clofibric Acid and Ketoprofen

As illustrated in Section 4.1.3, the peak responses of 10 ppm clofibric acid and ketoprofen could not be computed by direct analysis, so the processed analysis method was used for clofibic acid and ketoprofen calibration, shown in Figure 6.4 and Figure 6.5, respectively.

The processed calibration curves for both clofibric acid and ketoprofen are shown in Figure 6.6.

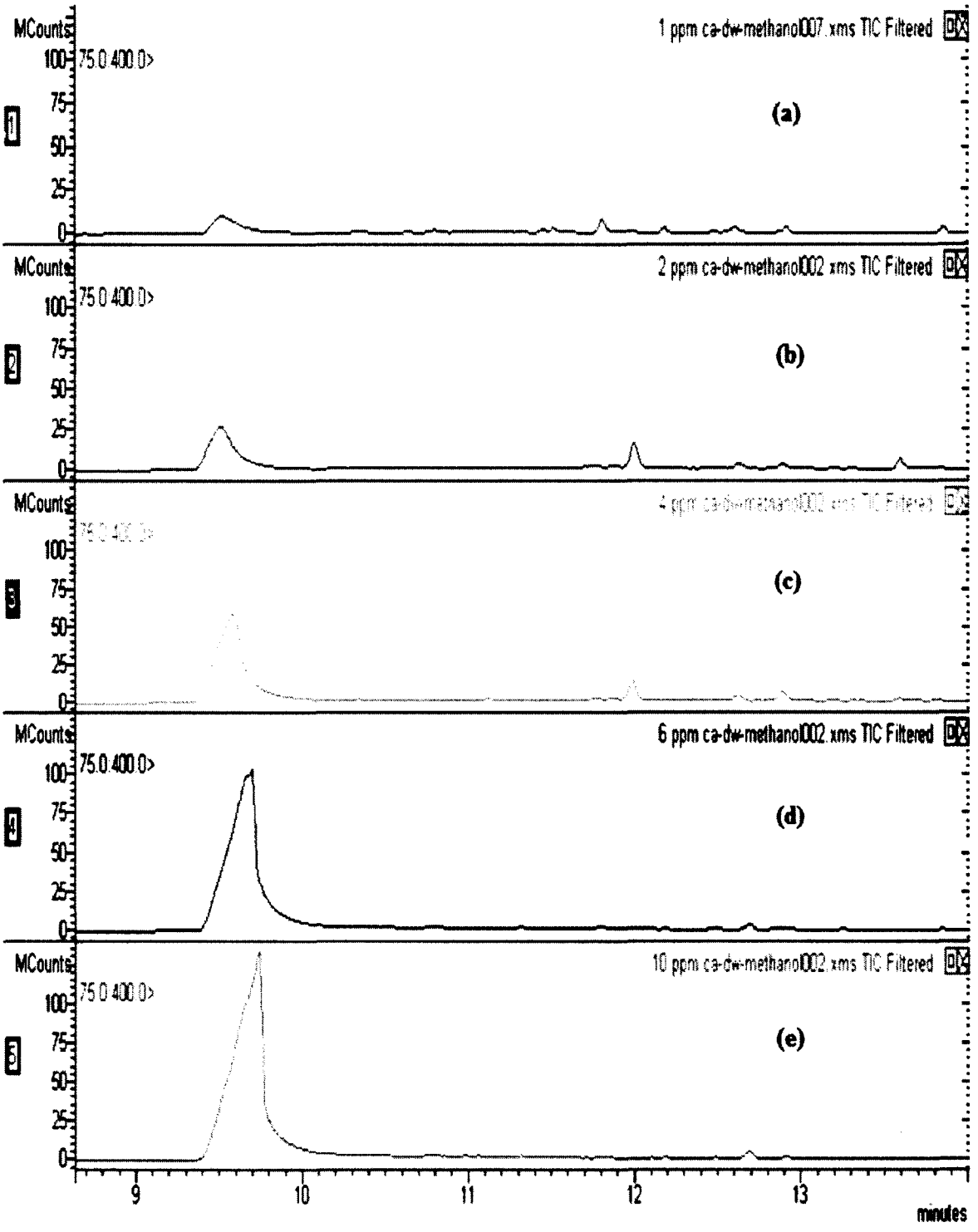


Figure 6.4: Clofibric acid (CA) processed calibration-CA-DW-methanol: (a) 1 ppm, (b) 2 ppm, (c) 4 ppm, (d) 6 ppm, (e) 10 ppm

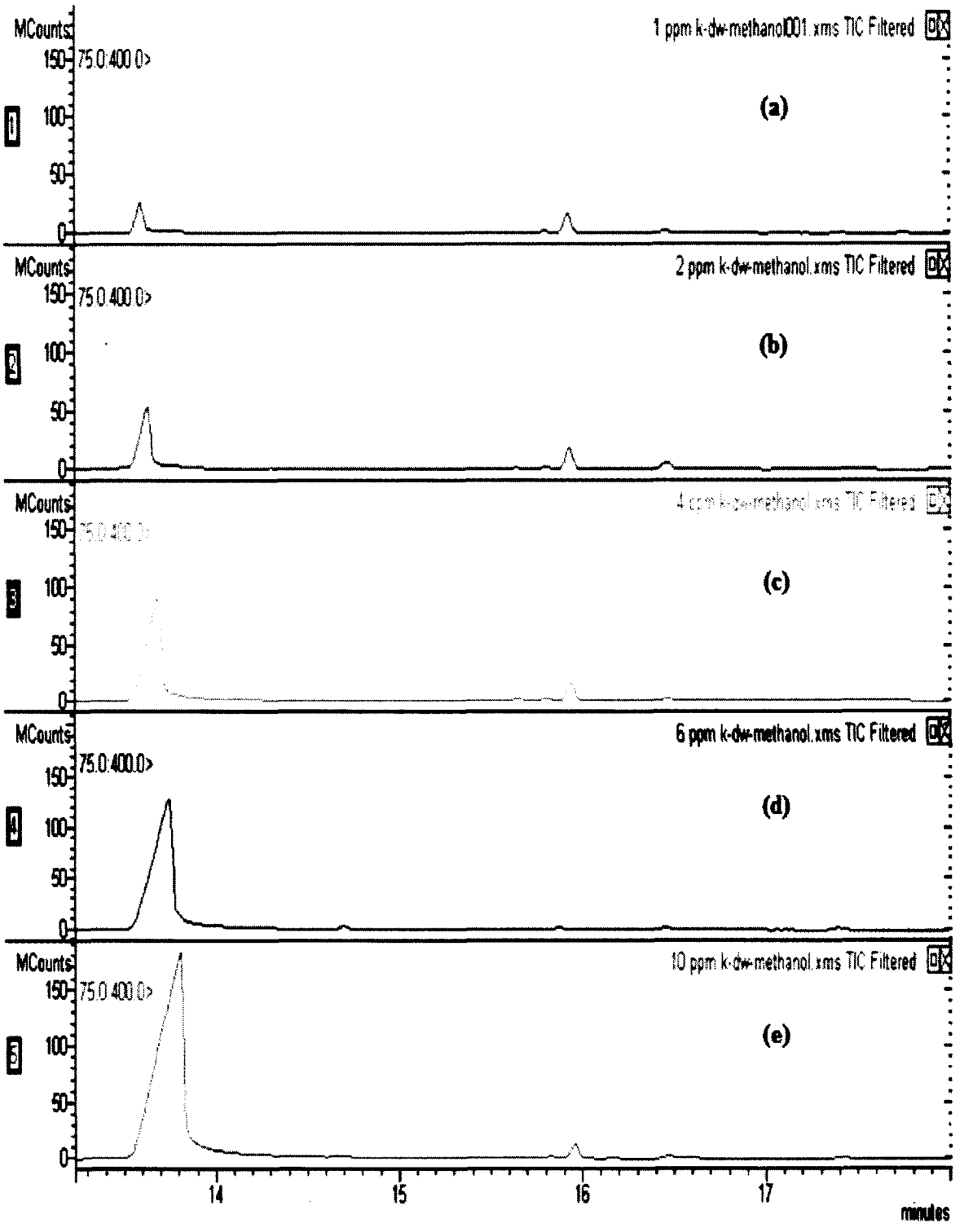
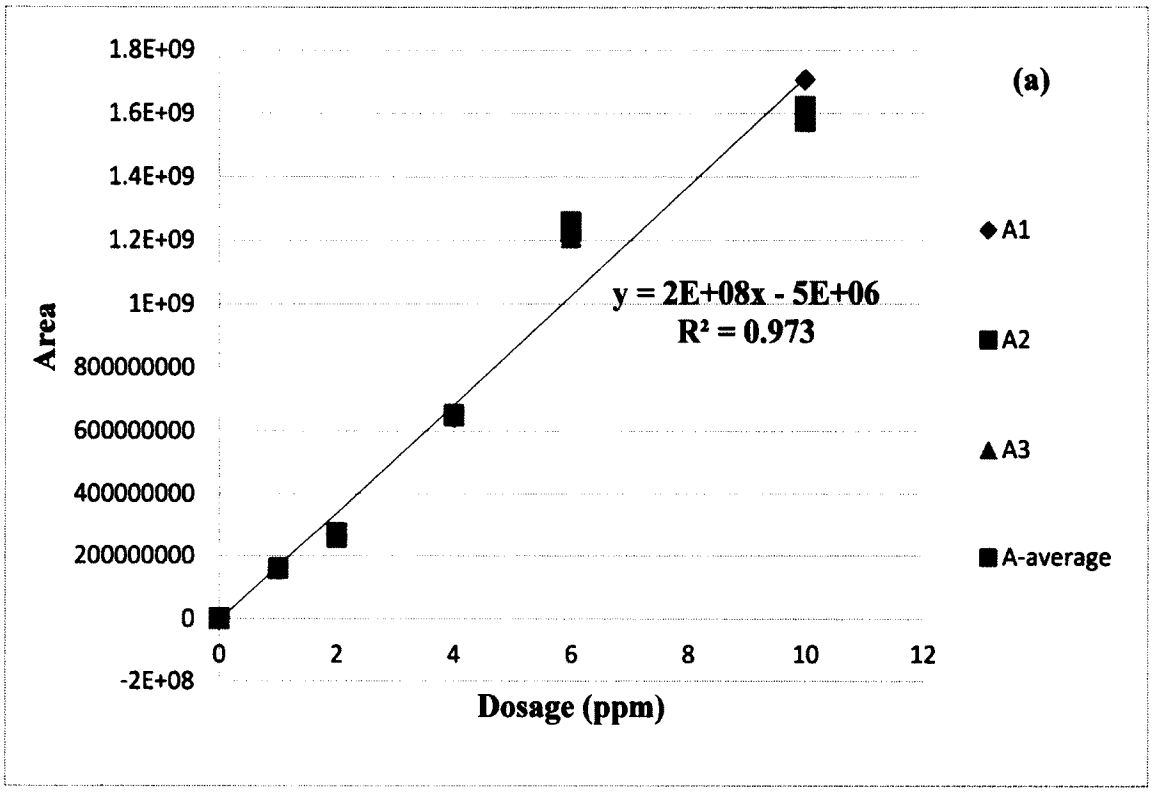


Figure 6.5: Ketoprofen (K) processed calibration-K-DW-methanol: (a) 1 ppm, (b) 2 ppm, (c) 4 ppm, (d) 6 ppm, (e) 10 ppm



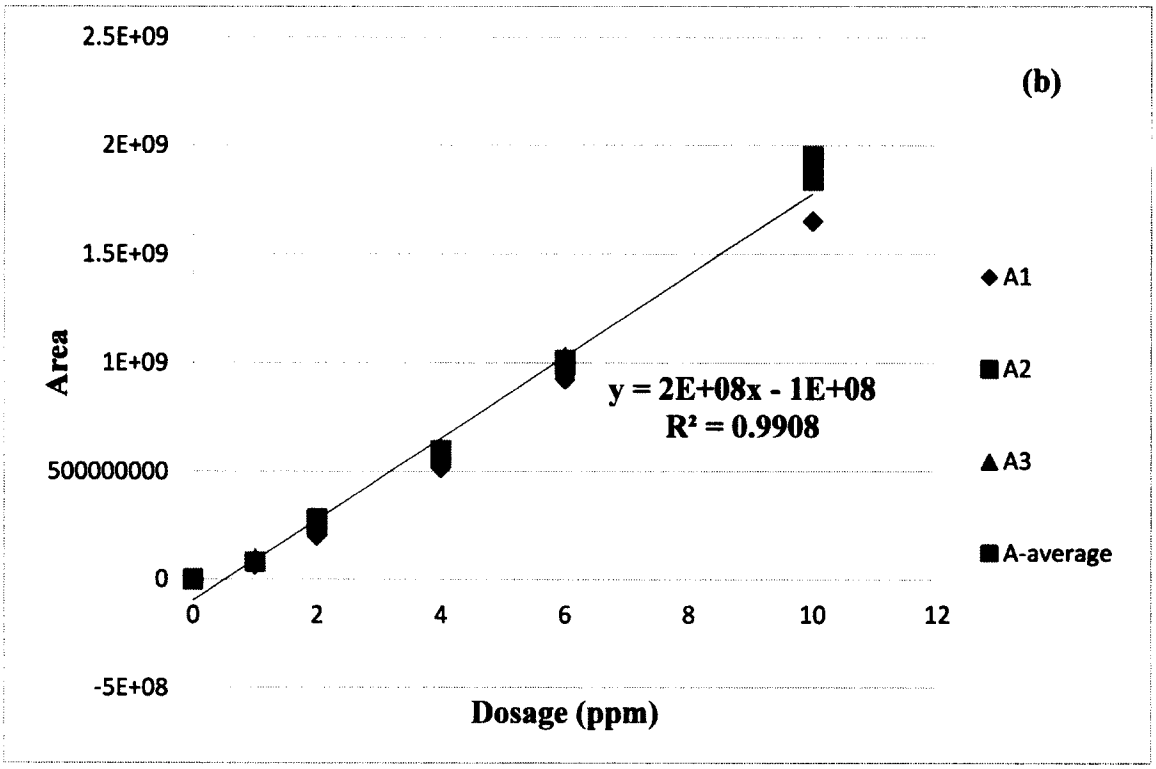


Figure 6.6: Processed analysis calibration curves for (a) clofibric acid, (b) ketoprofen

The calibration equations for clofibric acid and ketoprofen are:

$$y = 2E+08x - 5E+06, R^2 = 0.973 \quad \text{(Equation 6.3)}$$

$$y = 2E+08x - 1E+08, R^2 = 0.9908 \quad \text{(Equation 6.4)}$$

The calibration equations showed good linearity of the methods of both clofibric acid and ketoprofen.

6.1.3 Method Detection Limit (MDL) for 17β-estradiol (E2), Clofibric Acid, and Ketoprofen

The calibration curves constructed above were evaluated by the method detection limit (MDL) calculations. For each standard, MDL was evaluated at the spike level of 1 ppm in 1000 mL distilled water. The equation for calculating MDL was as follows [72]:

$$\text{MDL} = \text{Standard deviation} * t_{(n-1,99)} \quad \text{(Equation 6.5)}$$

$t_{(n-1,99)}$ refers to the student's t-value with 99% confidence and n-1 degrees of freedom. The average recovery rate was also estimated according to the following equation [83]:

$$\text{Average recovery rate} = \text{Actual Average Concentration} / \text{spike level} * 100 \quad \text{(Equation 6.6)}$$

Results of actual concentration, recovery rate, MDL for 17β-estradiol (E2), clofibric acid, and ketoprofen are illustrated in Table 6.1.

From Table 6.1, the standard deviation and the average recovery rate values showed that for the methods developed for the three standards, the order of precision was: clofibric acid >

ketoprofen > 17β-estradiol (E2), and the order of accuracy was: ketoprofen > clofibric acid > 17β-estradiol (E2). The overall low recovery rate (< 99%) calculated for the three standards, especially 17β-estradiol (E2, 72.2 %), illustrated that biases were very notable in the processed calibration curves due to matrix interferences [72]. These biases might cause a certain inaccuracy when quantifying a specific compound in the samples.

Table 6.1: MDL for 17β-estradiol (E2), Clofibric Acid, and Ketoprofen

Standards	17β-estradiol (E2)	Clofibric Acid	Ketoprofen
Method (Section 4.1)	Table 4.7	Table 4.8	
Spike Level	1 ppm in 1000 mL distilled water		
Sample Number (#)	8	7	
$t_{(n-1,99)}$	2.998	3.143	
Actual Average Concentration (ppm)	0.722	0.839	0.954
Average Recovery Rate (%)	72.2%	83.9%	95.4%
Standard Deviation	0.060	0.033	0.058
MDL (ppm)	0.181	0.102	0.182

The feasibility of these calibration curves were evaluated by the MDLs calculated for 17β-estradiol (E2), clofibric acid, and ketoprofen according to the following criteria [72]:

$$\text{Calculated MDL} < \text{Spike Level} < 10 \times \text{Calculated MDL} \qquad \text{(Equation 6.7)}$$

By checking MDLs of 17β-estradiol (E2), 0.181 ppm < 1 ppm < 1.81 ppm, clofibric acid, 0.102 ppm < 1 ppm < 1.02 ppm, and ketoprofen, 0.182 ppm < 1 ppm < 1.82 ppm, it was found that the calculated MDLs for these three standards were valid, which means the standard calibration curves constructed for 17β-estradiol (E2), clofibric acid, and ketoprofen were feasible.

6.2 Oxidation of 17β-estradiol (E2), Clofibric Acid, and Ketoprofen by PAA/NaClO

After the calibration curves for the three standards were constructed and validated, 1 ppm of 17β-estradiol (E2), clofibric acid, and ketoprofen was separately oxidized by 10 ppm PAA/NaClO in 1000 mL distilled water. After a 60-min mixing period, 250 mL samples were collected, treated, and finally dissolved in methanol for GC/MS analysis.

6.2.1 Oxidation of 17β-estradiol (E2) by PAA/NaClO

As illustrated in Chapter 3, experimental runs for the oxidation of 17β-estradiol (E2) by PAA/NaClO included blank (treated distilled water in methanol), PAA/NaClO + blank (treated mixture of PAA/NaClO and distilled water in methanol), 17β-estradiol (E2) + blank (treated mixture of E2 and distilled water in methanol), and PAA/NaClO + 17β-estradiol (E2) + blank (treated mixture of PAA/NaClO, E2, and distilled water in methanol). Results for PAA/NaClO are shown in Figure 6.7 and Figure 6.8, respectively.

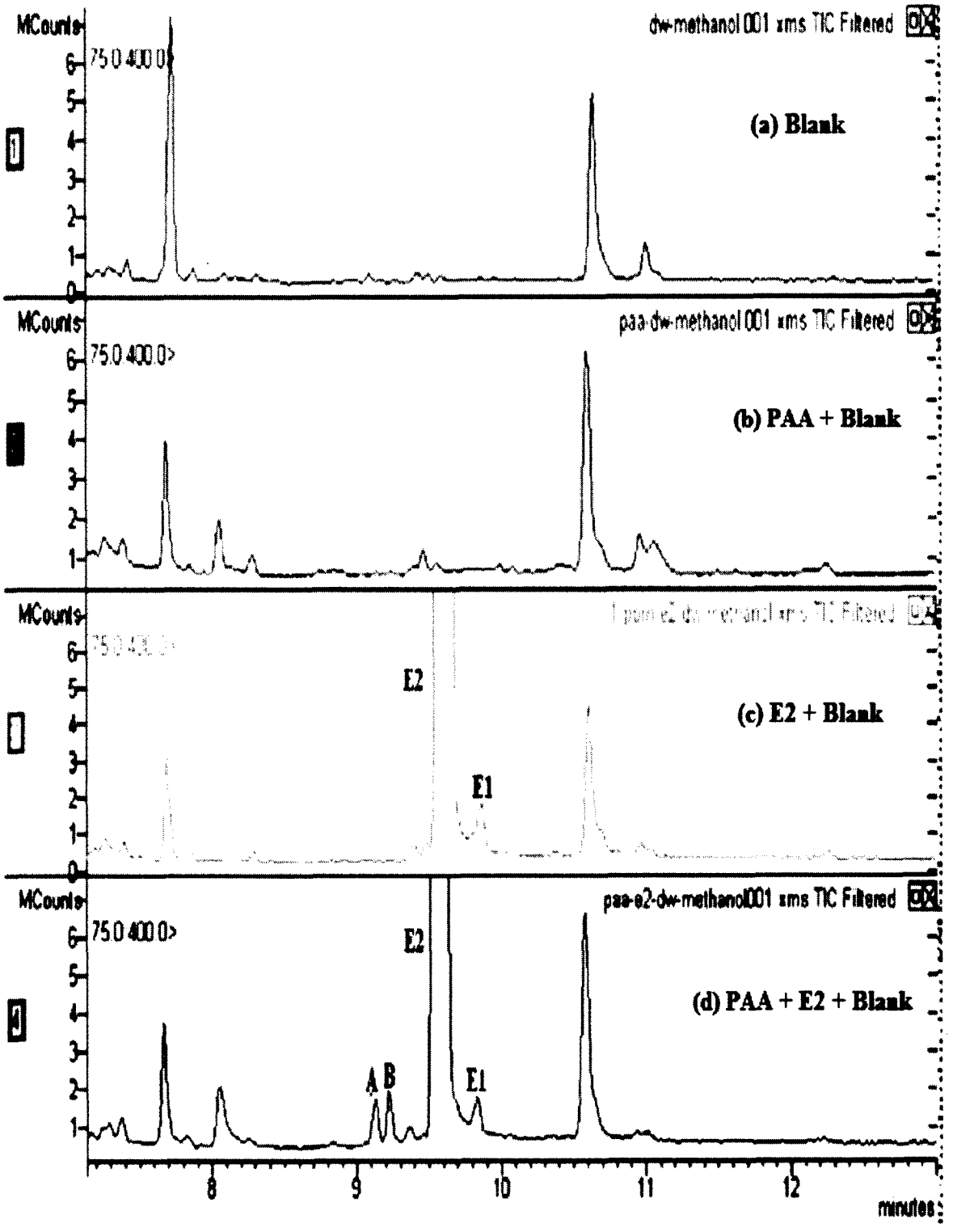


Figure 6.7: MS analysis for oxidation products of 17β-estradiol (E2) by PAA: (a) blank (distilled water-methanol), (b) PAA + blank, (c) E2 + blank, (d) PAA + E2 + blank

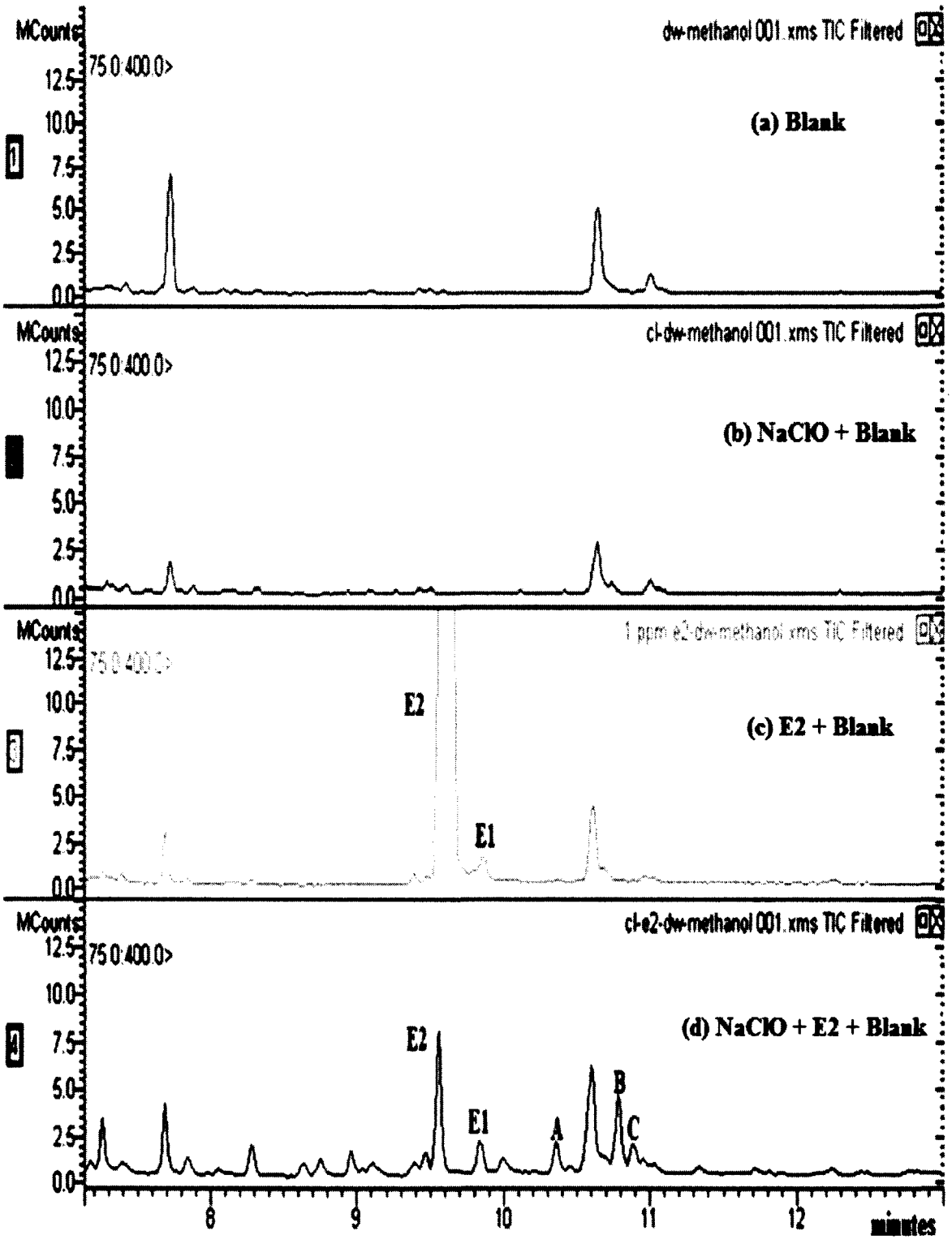


Figure 6.8: MS analysis for oxidation products of 17β-estradiol (E2) by NaClO: (a) blank (distilled water-methanol), (b) NaClO + blank, (c) E2 + blank, (d) NaClO + E2 + blank

Comparison of the first two chromatograms (blank and PAA/NaClO + blank) in both Figure 6.7 and Figure 6.8, the three peaks consistently appearing in both chromatograms illustrated that these peaks were from the treatment processes of distilled water. In the second chromatograms (Figure 6.7b and Figure 6.8b), the intensity of the three peaks was decreased by the addition of PAA/NaClO, but neither PAA/NaClO nor their degradation products were found. This was probably because the masses of PAA/NaClO or degradation products (<75 m/z) were not in the measured mass range (75-400 m/z).

Identification of 17β-estradiol (E2) and estrone (E1)

Comparison of the first chromatogram (blank) and the third chromatogram (E2 + blank) in both Figure 6.7 and Figure 6.8, two new peaks were found in the third chromatogram at 9.615 min and 9.830 min. By searching a library of spectrum, they were identified as 17β-estradiol (E2) and estrone (E1), respectively. Estrone (E1) is another estrogen that has high estrogenic potency. It has been reported to react with NaClO to produce chlorinated estrones [48]. The detection of estrone (E1) in the E2-treated distilled water illustrated that 17β-estradiol (E2) could degrade to estrone (E1) in an aqueous solution [47]. The spectrum of estrone (E1) is shown in Figure 6.9.

E2 and E1 peaks were also detected in both Figure 6.7d and Figure 6.8d. Comparison of the fourth chromatograms with the third ones, it was found that the addition of PAA had almost no influence on the intensity of E2/E1 peaks (Figure 6.7), while NaClO significantly reduced the intensity of E2 peak and slightly increased the E1 peak (Figure 6.8). The increase of E1 peak indicated that E2 could further transform to E1 by the addition of NaClO.

Estrone (E1, $C_{18}H_{22}O_2$, 270.366 g/mol, 9.830 min)

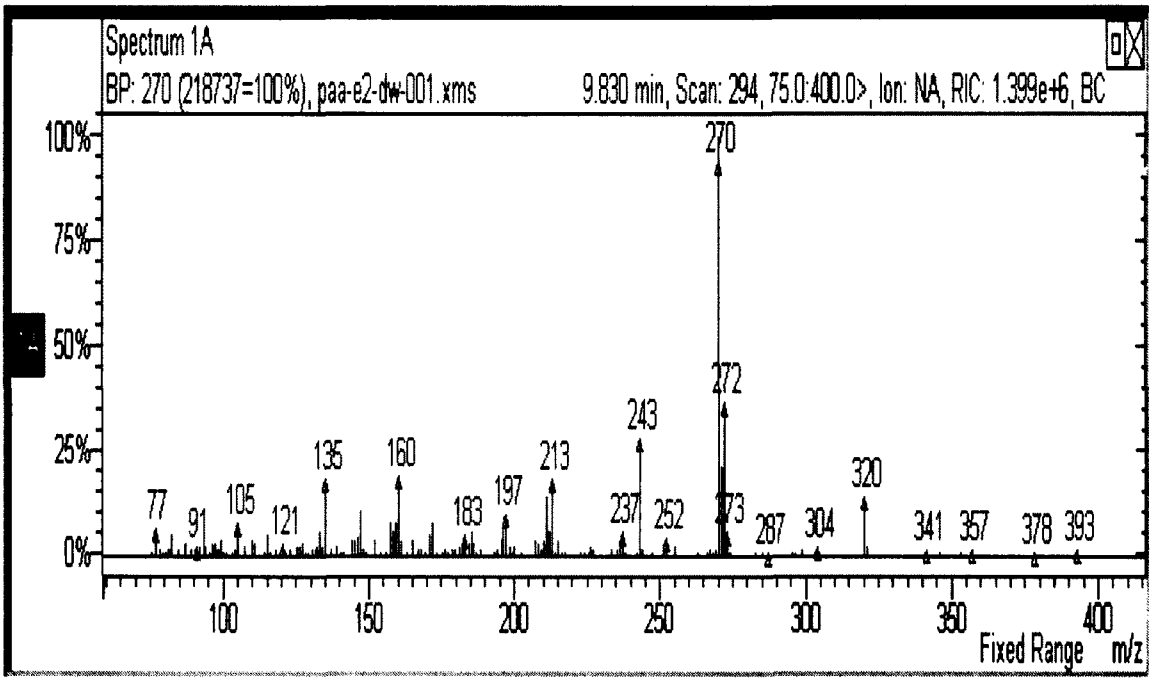


Figure 6.9: Mass spectrum of estrone (E1)

To obtain more accurate conclusion, the concentrations of 17β-estradiol (E2) after oxidation by PAA/NaClO were quantified, shown in Table 6.2.

Table 6.2: Concentration of 17β-estradiol (E2) after oxidation by PAA/NaClO

1 ppm (E2)	E2-DW-methanol	PAA-E2-DW-methanol	NaClO-E2-DW-methanol
Actual Concentration of E2 (8 samples)	0.722 ± 0.181 ppm	0.587 ± 0.276 ppm	-0.201 ± 0.002 ppm

From Table 6.2, the average calculated concentration of 17β-estradiol (E2) after the oxidation of PAA and NaClO (PAA/NaClO-E2-DW-methanol) was 0.587 ppm and -0.201 ppm, respectively. Compared to the calculated concentration (0.722 ppm) of E2 from the processed standard, the corresponding reduction concentrations of E2 by PAA and NaClO were 0.135 ppm and 0.923 ppm.

The reduction of E2 by PAA (0.135 ppm) did not show in the fourth chromatograms (PAA/NaClO + E2 + blank) by the peak intensity, probably because of the increased intensity of the other small peaks (peaks at t = 8.05 min, 9.122 min, 9.218 min, and 10.6 min).

The negative concentration (-0.201 ppm) of E2 calculated for NaClO indicated that an error might be involved. The most possible error was from the E2 processed calibration. Since only 72.2% of 1 ppm E2 was recovered through the processed treatment, a 0.278 ppm error initially existed when building the calibration curve. This means if 100% were recovered, 0.278 + 0.587 = 0.865 ppm of 17β-estradiol (E2) and 0.278 + (-0.201) = 0.077 ppm would be calculated after oxidation of PAA and NaClO, respectively. The corresponding reduction concentration of E2 would be 1-0.865 = 0.135 ppm and 1-0.077 = 0.923 ppm for PAA and NaClO. Compassion of these two values calculated in the assumption of 100% recovery with the previously ones at 72.2% recovery rate, it was found that the same values (0.135 ppm for PAA and 0.923 ppm for NaClO) were calculated. The same values obtained proved that it was just the error of 0.278 ppm unrecovered E2 that resulted in a negative value calculated.

Based on the analysis above, it could be concluded that around 13.5% and 92.3% reductions of 1 ppm 17β-estradiol (E2) were achieved by the addition of 10 ppm PAA and NaClO in 1000 mL distilled water, respectively. This reduction indicated that the estrogenic effect of 17β-estradiol (E2) might be reduced significantly by NaClO, and slightly by PAA.

Identification of oxidation products of 17β-estradiol (E2) by PAA/NaClO

Combined the first three chromatograms (blank, PAA/NaClO + blank, E2 + blank) and compared to the fourth one (PAA/NaClO + E2 + blank) in both Figure 6.7 and Figure 6.8, two new peaks were found in PAA-oxidized samples at 9.122 min and 9.218 min (peaks PAA-A and PAA-B in Figure 6.7) and three new peaks were found at in NaClO-oxidized samples 10.359 min, 10.780, 10.885 min (peaks NaClO-A, NaClO-B, and NaClO-C in Figure 6.8). The spectra of these peaks are shown in Figure 6.10 and Figure 6.11. These peaks were the oxidation products of 17β-estradiol (E2) by PAA/NaClO.

To identify these peaks, the spectra of these peaks were first compared to a library of spectrum, but none of them were identified. Therefore, structure analysis was conducted. The principal of structure analysis was based on an integral analysis of the molecular ions in the spectrum, as well as structures of reactants and solvent-methanol (PAA/NaClO, E2, E1 produced by E2, and CH_4O).

As mentioned before, although the concentration of estrone (E1) was very small compared to 17β-estradiol (E2), it could still react with PAA/NaClO due to its high reactivity, caused by C=O bond in its structure, shown in Figure 6.12. For this reason, both E2 and E1 were considered for the oxidation products analysis.

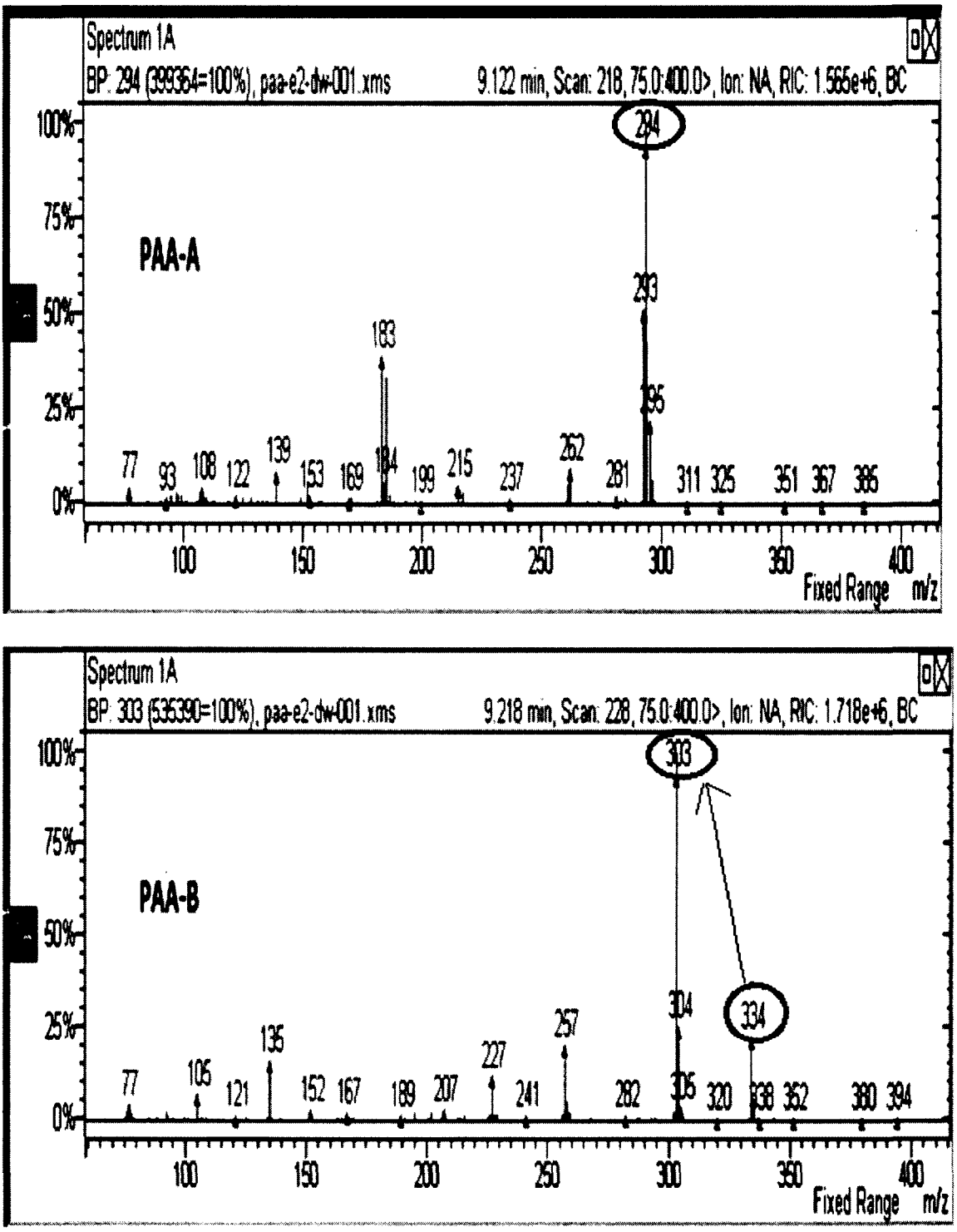


Figure 6.10: Mass spectra of the oxidation products of 17β-estradiol (E2) by PAA: PAA-A and PAA-B

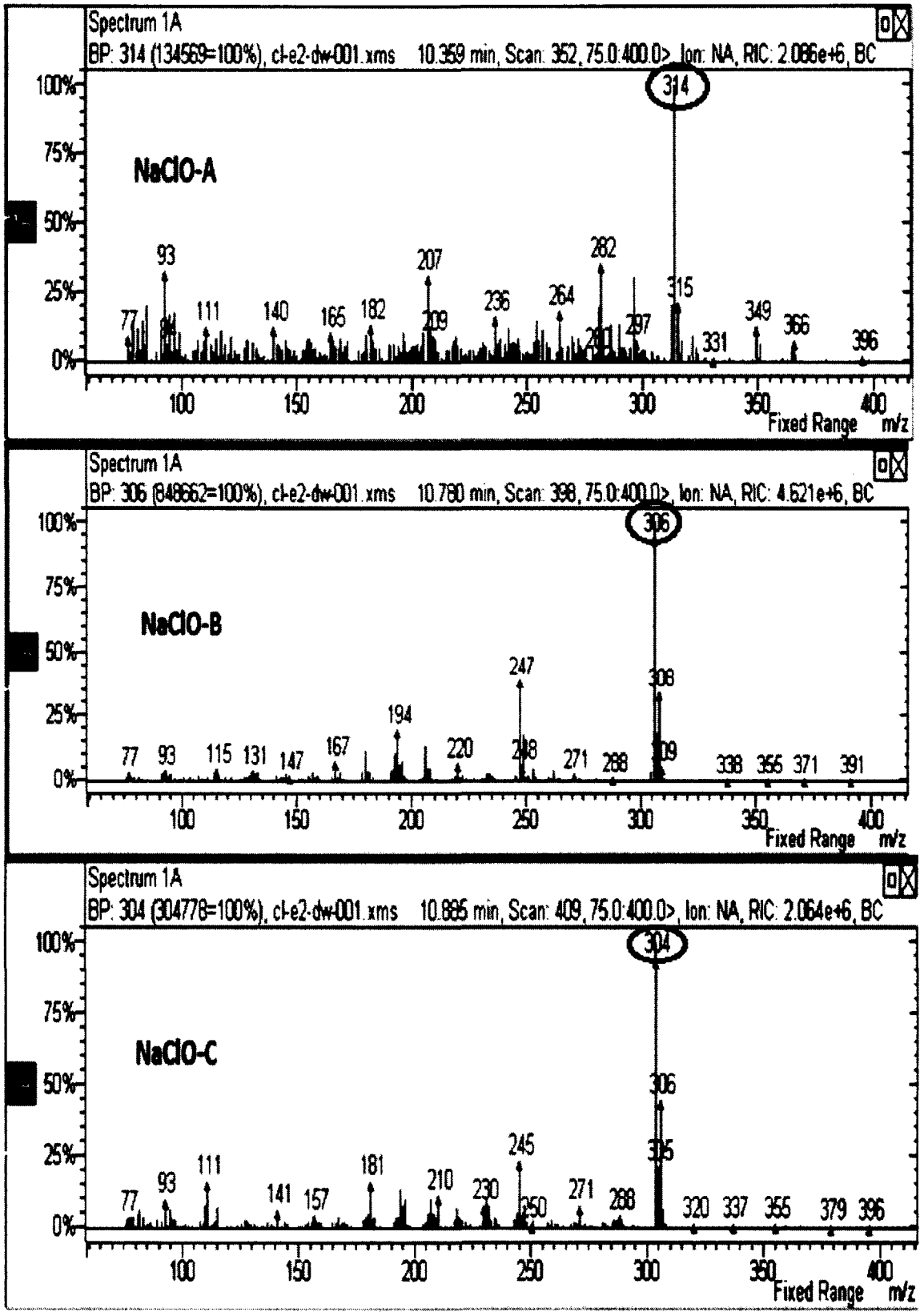
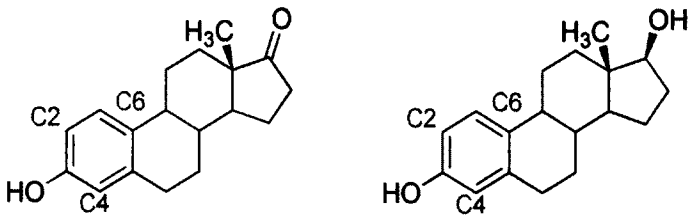


Figure 6.11: Mass spectra of the oxidation products of 17β-estradiol (E2) by NaClO: NaClO-A, B, and C



E1 **E2**

Figure 6.12: Structures of 17β-estradiol (E2) and estrone (E1)

E2 and E1 can be oxidized at three positions [23, 25, 47, 48]: C2 and C4 next to the hydroxyl group (-OH) in the six-cyclic, as well as C6 (Figure 6.12). However, C2 is much more active than C4 and C6. The possible fragments from PAA/NaClO were able to replace the hydrogen at C2, C4 and/or C6 positions to form oxidation products. The mass of a fragment from PAA/NaClO could be estimated by deducting mass of E2 or E1 from the mass of its corresponding oxidation product.

The masses of oxidation products were assumed to be the molecular ions (M^+), $M^+ + 1$, or $M^+ - 1$ in their spectra [73]. The molecular ions were determined based on analyzing the spectra of the reactants (E2 and E1) that had been identified by MS. From the spectra of E2 (Figure 4.13) and E1 (Figure 6.9), it was found that the base peaks (ions that have 100% intensity) were just their molecular ions: 272 m/z and 270 m/z. Therefore, the molecular ions (M^+) of oxidation products, PAA-A, PAA-B, NaClO-A, NaClO-B, and NaClO-C, were assumed to be the base peaks in their spectra in Figure 6.10 and Figure 6.11: 294 m/z, 303 m/z, 314 m/z, 306 m/z, and 304 m/z, respectively. Their corresponding mass ranges (M^+ or $M^+ + 1$ or $M^+ - 1$) would be 293-295 m/z, 302-304 m/z, 313-315 m/z, 305-307 m/z, and 303-305 m/z.

According to "Nitrogen Rule" [73], odd mass/charge (m/z) can only arises if the molecule contains an odd number of nitrogen atoms. Since no nitrogen exists either in E2/E1 or PAA/NaClO or solvent-methanol (CH_4O), the odd numbers in the above mass ranges are impossible. Therefore, the corresponding masses of PAA-A, PAA-B, NaClO-A, NaClO-B, and NaClO-C should be 294 m/z, 302 or 304 m/z, 314 m/z, 306 m/z, and 304 m/z. By deducting 272 m/z and 270 m/z (mass of E2 and E1) from these masses, the mass ranges of the fragments from PAA-A, PAA-B, NaClO-A, NaClO-B, and NaClO-C are 22 and 24 m/z, 30-32 and 32-34 m/z, 42 and 44 m/z, 34 and 36 m/z, 32 and 34 m/z, respectively.

Since the fragment ions in the range from (M-21) to (M-25) are not reasonable losses [73], PAA-A (22 and 24 m/z) was unable to be identified. However, for PAA-B (30-32 m/z for E2, 32-34 m/z for E1), fragment -OCH_3 (31 m/z) is possible to attach at C2 or C4 position of E2 (272 m/z) to form H_3CO-E2 (304 m/z), and attach at C6 position of E2 to form H_3CO-E2 (302 m/z). Therefore, PAA-B should be 2-H_3CO-E2 or 4-H_3CO-E2. The proposed pathways for PAA-B (H_3CO-E2) are shown in Figure 6.13. By analyzing the structure of PAA, it is impossible for -OCH_3 to be fragmented from PAA. However, PAA as a strong oxidant can activate E2 to lose electrons and facilitate methanol-groups (-CH_3O) being attached at both C6 and C2 or C4 position to form H_3CO-E2-OCH_3 (334 m/z in the spectrum of PAA-B, Figure 6.10). Since C2 position of E2 and C4 is more active compared to C6 and C6 is easier to be fragmented [23, 25, 47, 48], H_3CO-E2-OCH_3 could further lose one methanol group (H_3CO-) to form H_3CO-E2.

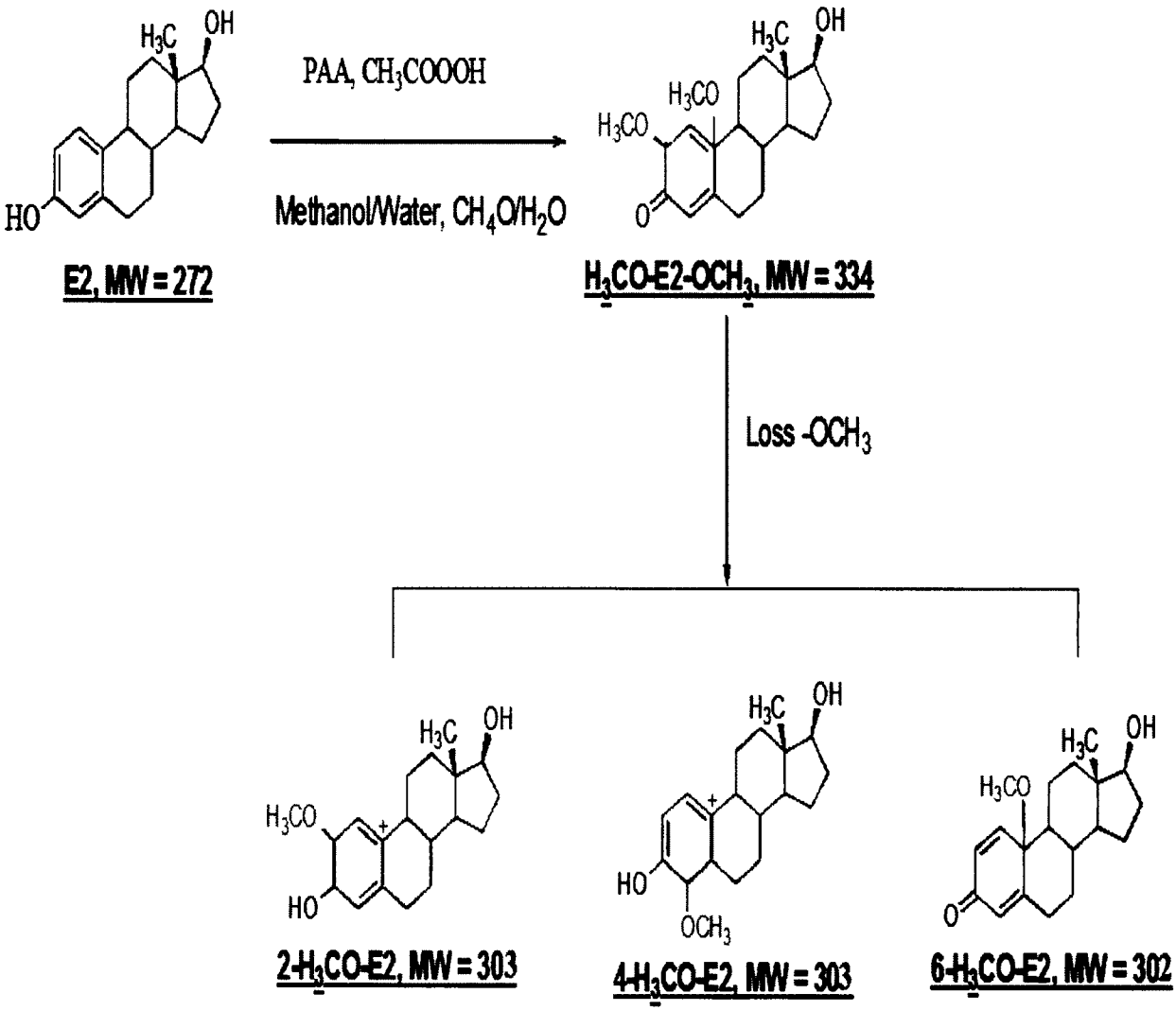


Figure 6.13: Proposed pathways for the oxidation product of 17β-estradiol (E2) by PAA: PAA-B (2- or 4-H_3CO-E2)

The possible fragment from NaClO is the chlorine ion (Cl^-), so the masses of fragments from NaClO were evaluated based on the mass of chlorine atoms (35 m/z) and isotopes of Cl (^{35}Cl and ^{37}Cl). The fragment mass (42 for E2, 44 m/z for E1) of NaClO-A shows that NaClO-A is not a chlorinated product. Since no other identification methods were available so far, NaClO-A is still unknown.

The masses of fragment from NaClO-B (34 m/z for E2) and NaClO-C (34 m/z for E1) showed that NaClO-B and NaClO-C were probably mono-chloro-E2 and mono-chloro-E1, respectively. To further confirm NaClO-B and NaClO-C, the intensity ratios of isotope peaks that correspond to molecular ions of NaClO-B and NaClO-C (Figure 6.11) were observed. Three isotope peaks were found for both NaClO-B (306 m/z, 308 m/z, and 309 m/z, Figure 6.11) and NaClO-C (304 m/z, 305 m/z, and 306 m/z, Figure 6.11). It was found that the intensity ratios of M and M+2 peaks for NaClO-B (306 m/z and 308 m/z) and NaClO-C (304 m/z and 306 m/z) were both 3:1, corresponds to the ratio (3:1) of ^{35}Cl and ^{37}Cl [73]. Therefore, NaClO-B and NaClO-C could be confirmed as mono-chloro-E2 and mono-chloro-E1.

Another isotope peaks that correspond to the molecular ion of NaClO-B (309 m/z, Figure 6.11) and NaClO-C (305 m/z, Figure 6.11) illustrated that ^{13}C exists in the formula of mono-chloro-E2 and mono-chloro-E1. The relative abundance of ^{13}C exists in a compound that contains one carbon is 1.08 % of the ^{12}C atoms [73], so for 18 carbons-containing compounds E2 and E1, (18 *1.08) % ^{13}C exists. Therefore, isotope peak 309 m/z for NaClO-B could be explained by one ^{37}Cl and one ^{13}C in the formula of mono-chloro-E2, and isotope peak 305 m/z for NaClO-A could be explained by one ^{35}Cl and one ^{13}C in the formula of mono-chloro-E1.

Three positions at C2, C4, and C6 positions of E2/E1 could be attached by Cl to form mono-chloro-E2 and mono-chloro-E1, respectively. The possible pathways for NaClO-B (mono-chloro-E2) and NaClO-C (mono-chloro-E1) are shown in Figure 6.14. Similar results for 2- or 4-chloro-E2/E1 were confirmed in a previous study [47].

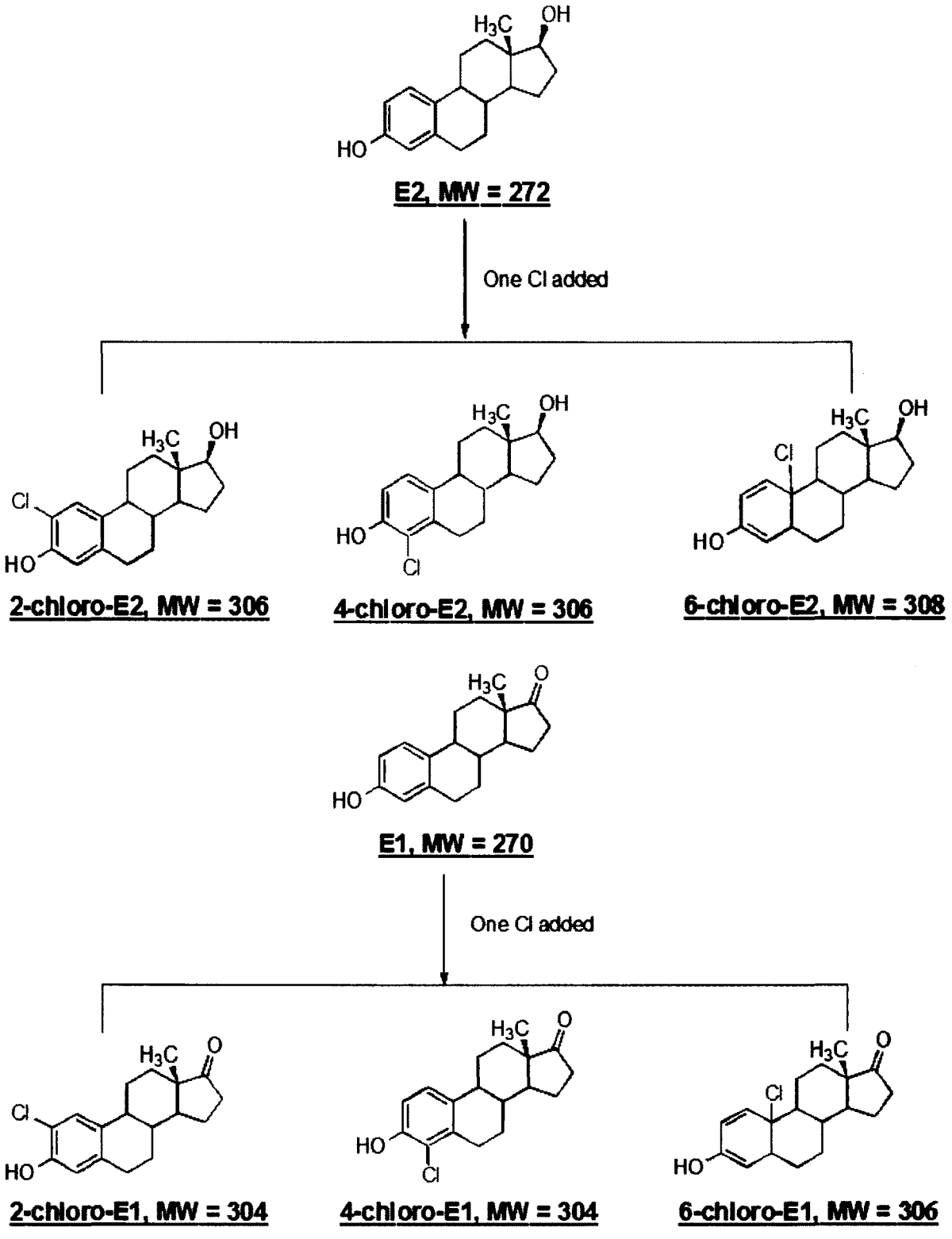


Figure 6.14: Proposed pathways for the oxidation products of 17β-estradiol (E2) by NaClO: NaClO-B (mono-chloro-E2) and NaClO-C (mono-chloro-E1) [47]

Comparison the oxidation efficiency and products of 17β-estradiol (E2) by PAA/NaClO

Based on the above analysis, NaClO significantly reduced E2 by 92.3%, and three oxidation products, including NaClO-A (unknown), NaClO-B (2- or 4-chloro-E2), and NaClO-C (2- or 4-chloro-E1), were formed. PAA slightly reduced E2 by 13.5%, and two oxidation products, including PAA-A (unknown) and PAA-B (2- or 4-H_3CO-E2) were formed.

The higher oxidation efficiency of NaClO compared to PAA indicates that NaClO could reduce the estrogenic effects of E2 more significantly than PAA. However, the mono-chloro-E2 or E1 formed by NaClO might have more toxic effects than H_3CO-E2 formed by PAA.

6.2.2 Oxidation of Clofibric Acid by PAA/NaClO

For both clofibric acid and ketoprofen, experimental runs included blank (treated mixture of clofibric acid/ketoprofen and distilled water in methanol), and PAA/NaClO + blank (treated mixture of PAA/NaClO, clofibric acid/ketoprofen, and distilled water in methanol). Three samples were prepared for both clofibric acid and ketoprofen experiments and three runs were conducted for each sample: one for identification of the possible oxidation products and the other two for reproducibility tests. Results of MS analysis for oxdiation products of clofibric acid by PAA/NaClO are shown in Figure 6.15 and Figure 6.16, respectively.

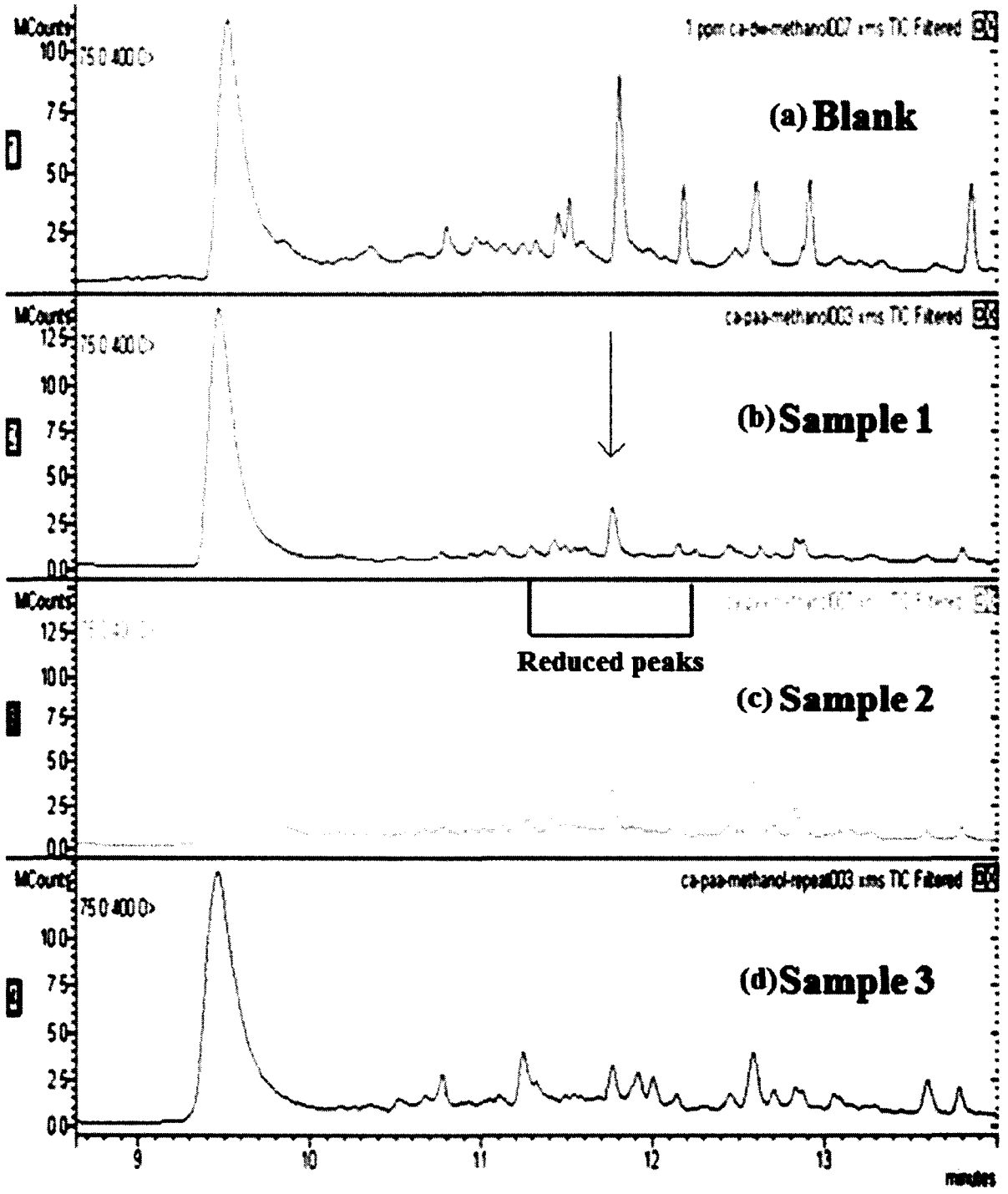


Figure 6.15: MS analysis for oxdiation products of clofibric acid by PAA: (a) blank (CA-DW-methanol), (b) PAA + blank (sample 1), (c) PAA + blank (sample 2), (d) PAA + blank (sample 3)

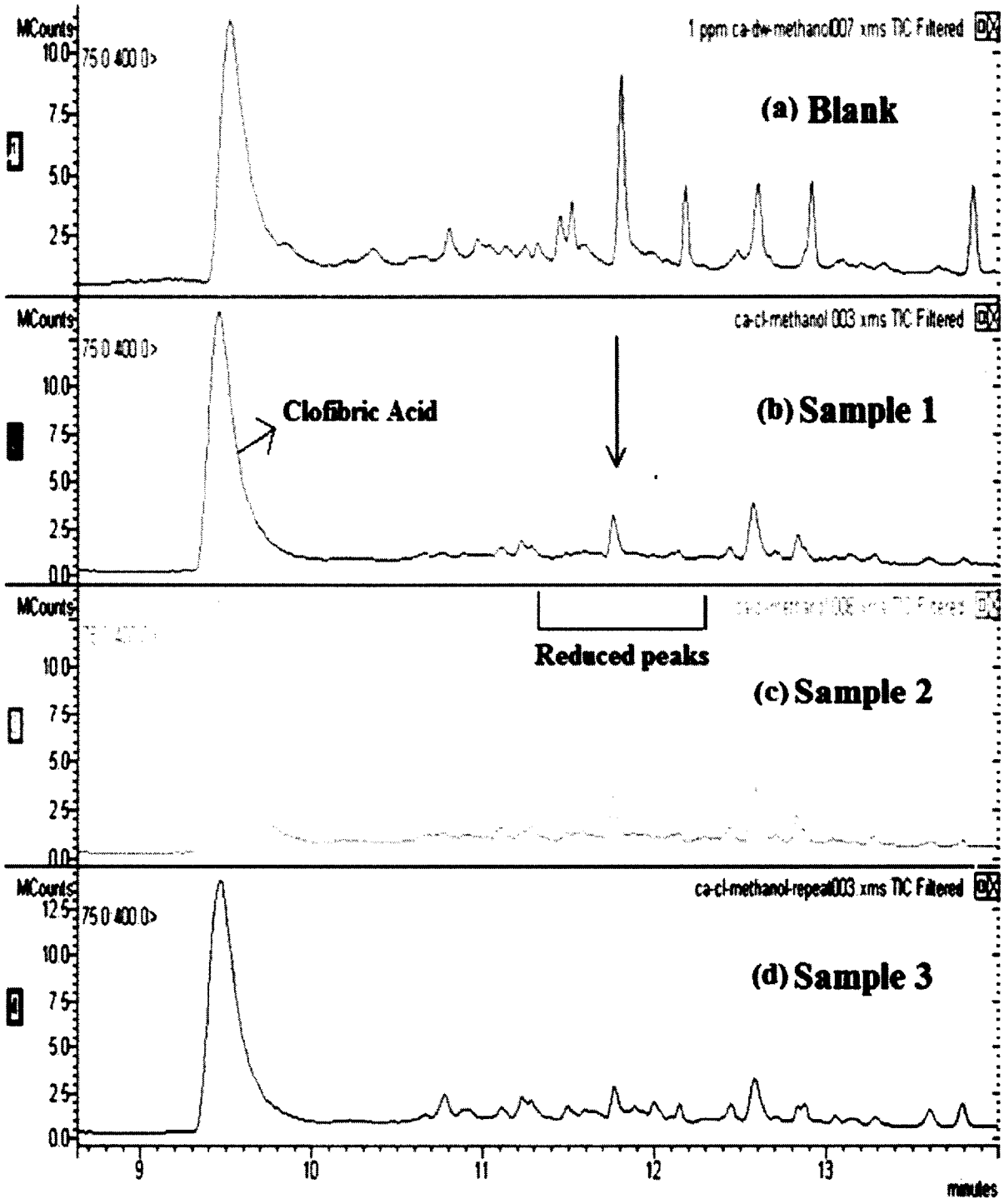


Figure 6.16: MS analysis for oxdiation products of clofibric acid by NaClO: (a) blank (CA-DW-methanol), (b) NaClO + blank (sample 1), (c) NaClO + blank (sample 2), (d) NaClO + blank (sample 3)

As shown in both Figure 6.15 and Figure 6.16, good reproducibility was found. Comparison of the last three chromatograms (PAA/NaClO + blank) with the first one (Blank) in both figures, it was found that the peak intensity of clofibric acid was not obviously reduced by either PAA or NaClO, and no new peaks were found. Only the intensity of peaks at 11.3 min to 12.3 min was reduced.

The actual concentrations of clofibric acid after oxidation by PAA/NaClO were calculated according to the results of nine runs for sample 1, 2, and 3, shown in Table 6.3. It was found that both PAA and NaClO reduced the average concentrations of clofibric acid by 0.265 ppm.

Table 6.3: Concentrations of clofibric acid (CA) after oxidation by PAA/NaClO

1 ppm CA	CA -DW-Methanol	PAA-CA -DW-Methanol	NaClO-CA -DW-Methanol
Actual concentration of clofibric acid	0.839 ± 0.033 ppm (7 samples)	0.574 ± 0.138 ppm (9 samples)	0.574 ± 0.160 ppm (9 samples)

6.2.3 Oxidation of Ketoprofen by PAA/NaClO

Results of MS analysis for oxdiation products of ketoprofen by PAA/NaClO are shown in Figure 6.17 and Figure 6.18, respectively. Although the intensity of peaks in the third chromatogram (NaClO + Blank-2) of Figure 6.18 was smaller than the other parallel chromatograms (some misoperation might be involved in preparing the samples), it was

found that for all the nine runs, no any oxidation products were found. Only the reduction of peaks at 15.5-16 min happened.

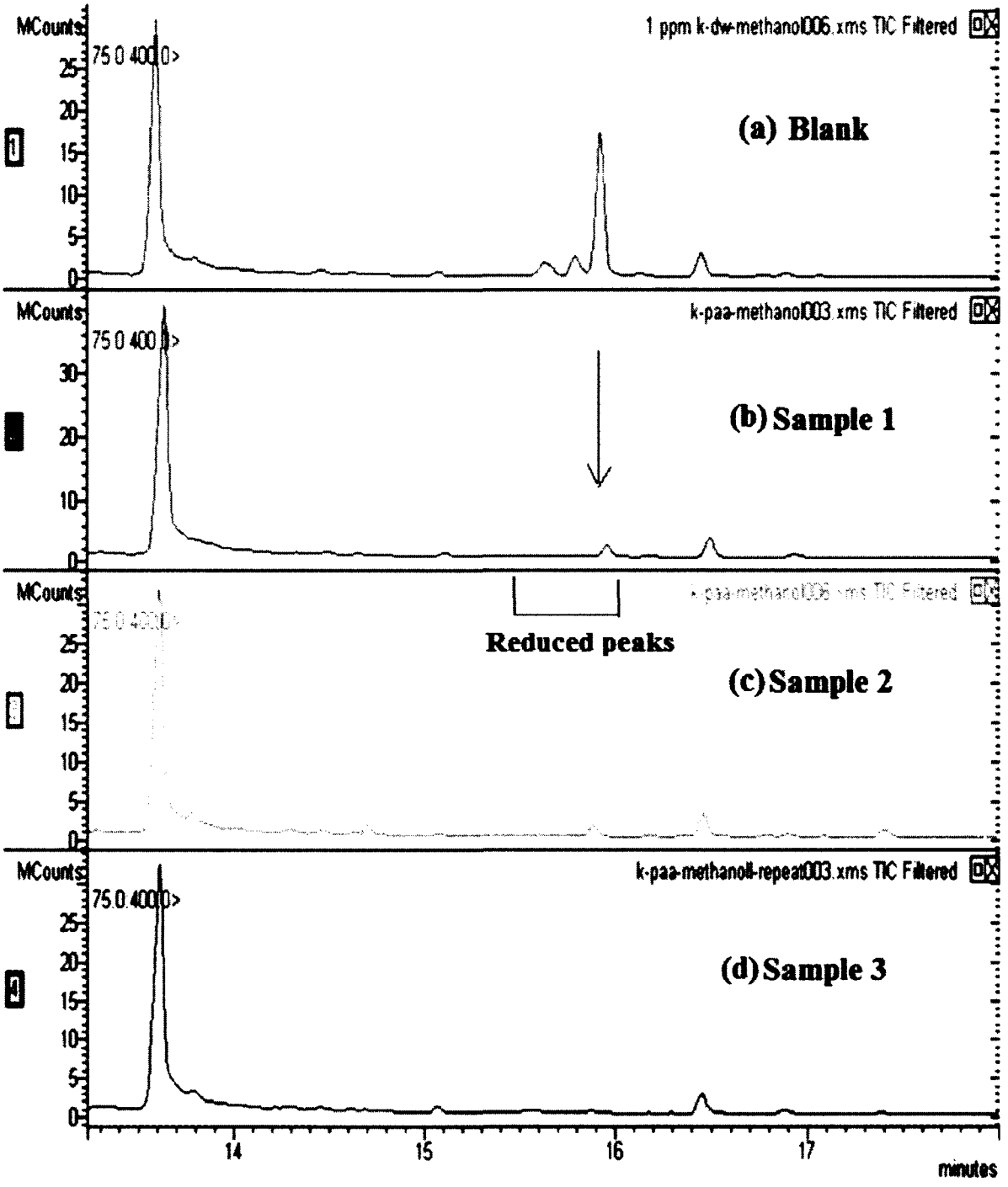


Figure 6.17: MS analysis for oxdiation products of ketoprofen by PAA: (a) blank (ketoprofen-DW-methanol), (b) PAA + blank (sample 1), (c) PAA + blank (sample 2), (d) PAA + blank (sample 3)

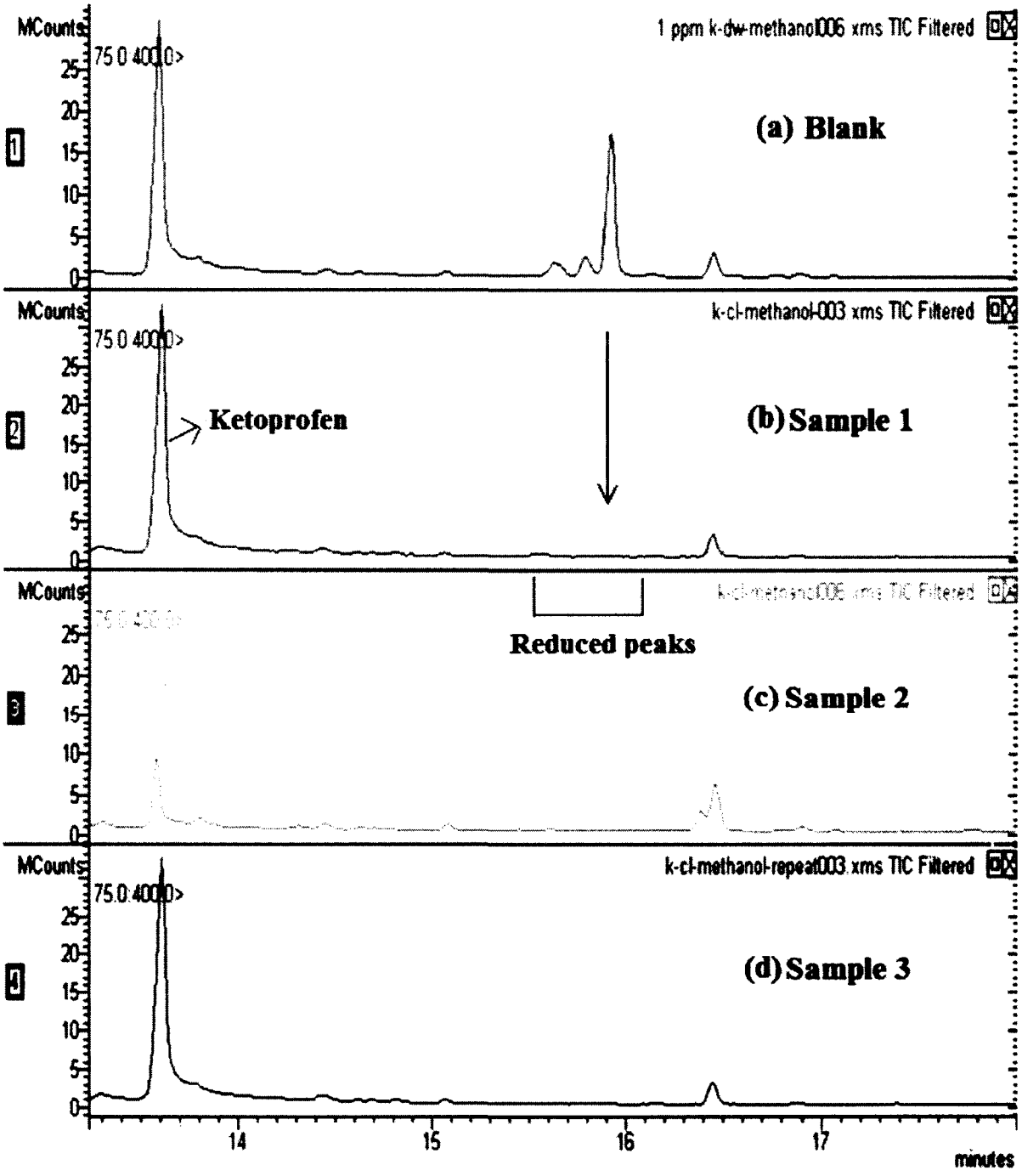


Figure 6.18: MS analysis for oxdiation products of ketoprofen by NaClO: (a) blank (ketoprofen-DW-methanol), (b) NaClO + blank (sample 1), (c) NaClO + blank (sample 2), (d) NaClO + blank (sample 3)

The actual concentrations of ketoprofen after oxidation by PAA/NaClO were calculated, shown in Table 6.4. It was found that both PAA and NaClO had almost no influence on the reduction of ketoprofen.

Table 6.4: Concentrations of ketoprofen (K) after oxidation by PAA/NaClO

1 ppm K	K -DW-methanol	PAA-K-DW-methanol	NaClO-K-DW-Methanol
Actual Concentration of Ketoprofen	0.954 ± 0.058 ppm (7 samples)	1.062 ± 0.101 ppm (9 samples)	0.943 ± 0.096 ppm (9 samples)

6.3 Summary

The selected EDCs/pharmaceuticals including 17β-estradiol (E2), clofibric acid, and ketoprofen showed different reactivity with PAA/NaClO. 17β-Estradiol (E2) was reduced by PAA slightly (13.5%) but reduced by NaClO significantly (92.3%). This indicated that NaClO could reduce the estrogenic effects of E2 significantly. However, NaClO also produced three oxidation products, one unknown and 2- or 4-chloro-E2/E1, which might have more toxic effects than E2/E1 itself. The estrogenic effect of H_3CO-E2 produced by PAA might be less than that of 2- or 4-chloro-E2/E1. Nevertheless, an evaluation of estrogenic activities of E2, E1, 2-/4- chloro-E2/E1, and H_3CO-E2 is needed. The unknown peaks produced by PAA and NaClO also need to be further identified.

Compared to 17β-Estradiol (E2), 26.5% reduction of clofibric acid by both PAA and NaClO was found; ketoprofen showed almost no reactivity with both PAA and NaClO. The different removal efficiencies of the three compounds by PAA/NaClO were probably caused by their different structures. Generally, the compounds that have aromatic rings with functional groups like –OH and $–OCH_3$ in their structures are more reactive because the electron rich compounds in these groups could substitute in organic molecules to increase reactivity with PAA/NaClO [46]. Therefore, PAA/NaClO could easily oxidize 17β-estradiol (E2, two –OH) than clofibric acid and ketoprofen, which mainly contains carboxylic groups in their structures.

In addition to the different structures of these compounds, pH also plays an important role in their reactivity with PAA/NaClO. Since both clofibric acid and ketoprofen are acid, it would be very hard for them to react with other acids like PAA and hypochlorous acid formed during NaClO hydrolysis.

For the above two reasons, 17β-Estradiol (E2) could be removed by oxidation of PAA/NaClO more easily compared to clofibric acid and ketoprofen. These results corresponded to the previous studies by others [47, 48].

Chapter 7

CONCLUSIONS AND RECOMMENDATIONS

In this study, methods for identifying and quantifying the target compounds-THMs, 17β-estradiol, clofibric acid and ketoprofen were developed, their calibration curves were constructed, and the reduction and DBP formation of these compounds by PAA/NaClO oxidation were found. GC/MS is a promising technology for identification and quantification of DBPs from PAA and NaClO oxidation if an appropriate method (optimal GC/MS conditions) for the target compound is developed. Some key findings from this research were:

- Method development as the first step of GC/MS analysis is found to be a very challenging and time-consuming step because different compounds need different methods, and each method needs a series of trial and error processes.

- Focusing on the main GC conditions (column, oven temperature, split ratio, solvent) and controlling the minor GC conditions (injector temperature, inject volume, detector temperature, gas selection and flow rate) can help build a method more rapidly.

- In combination with the physical properties (polarity, volatility, and boiling point) of the target compounds, an initial method can be developed by consulting with the GC operating manuals, experienced GC users, and previous analysis information for the target compounds.

- A proposed method for detecting a target compound cannot be finalized until the standard peak preliminarily identified by GC/FID is confirmed and quantified by an appropriate MS analysis.

- Two MS modes, full-scan mode and selected ion monitoring (SIM) mode can be used for standards analysis. Full-scan could achieve most analyses, and SIM should only be used when the areas under the target peaks in full-scan analysis are too small to be computed.

- Both direct analysis (diluted standard solutions were directly analyzed by MS) and processed standards (target concentrations of standards in distilled water were acidified, extracted, evaporated and derived before being analyzed by MS) can accomplish good linearity of calibration for a target compound. Processed standards calibration is recommended since the sample handling processes are used for samples.

- Processed standards calibration is not suitable for THMs since they will volatize during the evaporation process, resulting in no detection of THMs in both PAA/NaClO disinfected wastewater samples.

- The changing characteristics of wastewater influenced the detection and reproducibility of DBPs formed by PAA, as a result it was impossible to quantify or actually identify any of the DBPs formed.

- The structures of the target compounds including 17β-estradiol (E2), clofibric acid and ketoprofen, determine their reactivity with PAA/NaClO. E2 which has

aromatic rings with electron rich bonds like hydroxyl groups was more easily oxidized by PAA/NaClO than clofibic acid and ketoprofen which contain carboxylic acid groups.

- 17β-estradiol (E2) concentration can be reduced by both PAA and NaClO oxidation, but the reduction by NaClO is more significantly than PAA. For 1 ppm E2 oxidized by 10 ppm PAA/NaClO, 92.3% of E2 was reduced by NaClO and 13.5% was reduced by PAA.

- Three DBPs were detected during the oxidation of 17β-estradiol (E2) by NaClO: one unknown compound, 2- or 4-chloro-E2, and 2- or 4-chloro-E1; Two DBPs were detected during PAA oxidation: one unknown compound, and 2- or 4-CH3O-E2.

- No DBPs were detected from oxidation of clofibric acid and ketoprofen. 26.5% reduction of clofibric acid concentration was found by both PAA and NaClO; while no change of ketoprofen concentration by the addition of both PAA and NaClO.

Based on the results and discussions presently previously, the following recommendations are made.

- The processed standard calibration for THMs should be adjusted by using pentane to directly extract THMs and then analyzed by GC/MS. This adjustment would avoid THMs volatizing, helping the detection of THMs in PAA/NaClO disinfected wastewater. Further quantification of THMs could be also achieved,

and the different THMs formation potential by PAA and NaClO could be found.

- Liquid chromatography-mass spectrometry (LC/MS) should be used for further identification of the unknown peaks detected from the oxidation of 17β-estradiol (E2) by PAA/NaClO. LC/MS would more easily detect these compounds and provide more information for their structure. In addition, MS-MS technique could also be used to provide further structural details.

- Quantifications and toxicity tests for the identified DBPs such as 2- or 4-chloro-E2, 2- or 4-chloro-E1, and CH3O-E2 should be conducted to evaluate the risks of these DBPs to human health and to aquatic wildlife. By comparing of the estrogenic effects of these DBPs with that of E2 or E1, the impact of PAA/NaClO oxidation on the estrogenic effects of the water samples and treatment processes could be known.

- Various wastewater samples should be tested for the DBP formation by PAA/NaClO. The quantification and toxicity tests should be followed.

- The reduction of ammonia in raw wastewater by PAA should be further studied to evaluate the toxicity reduction by PAA.

Chapter 8

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APPENDIX A: PRELIMINARY RESULTS

Table A-1: Final volumes of 2g/L $Na_2S_2O_3$ decided for quenching PAA/NaClO in 100 mL distilled water (DW)

Evaluation	Step 1: 100 mL of 10 ppm PAA/NaClO in 1000 mL DW					
	PAA					NaClO
Trials	Initial (# 1)	# 2	# 3	# 4	# 5	Initial (# 1)
Volume (mL) of $Na_2S_2O_3$	1.0	10	20	15	**12**	**1.0**
Total Cl_2 Residual (mg/L)	Over measuring range	0.1	0.0	0.0	0.0	0.0

Target	Samples	# 1	# 2	# 3	# 4	# 5	# 6	Average
Raw DW	Total Cl_2 Residual (mg/L)	1.2	1.2	1.2	1.2	1.2	1.2	**1.2**

Dosage (ppm)	Initial Volume (mL)	Step 2: Checking Total Cl_2 Residual (mg/L) in DW by DPD method									
		PAA					NaClO				
		Final Volume (mL)	10 min	15 min	30 min	60 min	Final Volume (mL)	10 min	15 min	30 min	60 min
1	**0.1**	**1.2**	0.0	0.0	0.0	0.0	**0.1**	0.0	0.0	0.0	0.0
2	**0.2**	**2.4**	0.0	0.0	0.0	0.0	**0.2**	0.1	0.0	0.0	0.0
4	**0.4**	**4.8**	0.1	0.0	0.0	0.0	**0.4**	0.0	0.0	0.0	0.0
6	**0.8**	**7.2**	0.0	0.0	0.0	0.0	**0.8**	0.0	0.0	0.0	0.1
8	**1.2**	**9.6**	0.0	0.0	0.0	0.0	**1.2**	0.0	0.0	0.0	0.0
10	**1.6**	**12.0**	0.0	0.0	0.0	0.0	**1.6**	0.0	0.0	0.1	0.0

Table A-2: Final volumes of $Na_2S_2O_3$ decided for quenching PAA/NaClO in 100 mL wastewater samples (WW)

Target	Step 1: 100 mL of 10 ppm PAA/NaClO in 1000 mL WW			
	PAA	NaClO		
Trials	Initial (# 1)	Initial (# 1)	# 2	# 3
Volume (mL) of $Na_2S_2O_3$	12	2.0	10	12
Total Cl_2 Residual (mg/L)	0.0 (After adding 0.01 mL catalase)	4.4	0.1	0.0

Target	Samples	# 1	# 2	# 3	# 4	# 5	# 6	Average
Raw WW	Total Cl_2 Residual (mg/L)	0.0	0.1	0.1	0.1	0.1	0.1	0.1

Dosage (ppm)	Initial Volume (mL)	Checking Total Cl_2 Residual (mg/L) in WW by DPD method									
		PAA					NaClO				
		Adjusted Volume (mL)	10 min	15 min	30 min	60 min	Adjusted Volume (mL)	10 min	15 min	30 min	60 min
1	0.1	1.2	0.0	0.1	0.1	0.1	1.2	0.0	0.0	0.0	0.0
2	0.2	2.4	0.1	0.1	0.1	0.1	2.4	0.0	0.0	0.0	0.0
4	0.4	4.8	0.1	0.0	0.1	0.0	4.8	0.0	0.0	0.0	0.0
6	0.8	7.2	0.0	0.0	0.2	0.1	7.2	0.0	0.0	0.0	0.0
8	1.2	9.6	0.0	0.1	0.1	0.0	9.6	0.0	0.0	0.0	0.0
10	1.6	12.0	0.0	0.0	0.0	0.0	12.0	0.0	0.0	0.0	0.0

Table A-3: pH results for PAA/NaClO in distilled water (DW)

Contact time (min)	Sample #	DW-PAA-pH							DW-NaClO-pH						
		Dosage (ppm)													
		0	1	2	4	6	8	10	0	1	2	4	6	8	10
60	1	7.43	6.38	5.02	5.21	4.23	4.35	3.80	7.44	7.67	7.40	7.20	7.51	7.64	8.05
	2	7.14	6.60	5.04	5.12	4.18	4.08	3.80	7.25	7.64	7.08	7.17	7.45	7.64	8.08
	3	6.97	6.36	4.98	5.05	4.17	4.03	3.79	7.18	7.62	7.04	6.96	7.42	7.61	8.04
	4	7.00	6.18	4.94	5.03	4.19	4.01	3.77	7.17	7.52	7.01	6.99	7.46	7.65	8.08
	5	7.03	6.03	4.89	4.91	4.18	3.95	3.72	7.13	7.52	6.98	7.05	7.44	7.62	8.10
	6	7.01	5.84	4.89	4.87	4.19	3.88	3.75	7.11	7.56	6.94	7.08	7.47	7.64	8.09
	7	6.97	5.78	4.89	4.85	4.15	3.86	3.76	7.07	7.58	6.85	7.12	7.41	7.62	8.08
	8	6.94	5.65	4.89	4.83	4.17	3.91	3.77	7.11	7.56	6.81	7.00	7.40	7.62	8.10
	9	6.94	5.67	4.85	4.60	4.15	3.89	3.75	7.05	7.55	6.76	7.09	7.38	7.61	8.07
	10	6.74	5.60	4.83	4.52	4.16	3.88	3.76	7.05	7.45	6.74	7.12	7.34	7.65	8.04
	11	6.35	5.56	4.85	4.54	4.14	3.85	3.70	7.07	7.49	6.73	7.15	7.34	7.61	8.10
	12	6.17	5.49	4.81	4.56	4.16	3.86	3.74	7.06	7.48	6.67	7.17	7.35	7.64	8.11
	13	5.80	5.43	4.82	4.58	4.16	3.86	3.72	7.02	7.50	6.79	7.14	7.35	7.64	8.09
	14	5.85	5.45	4.79	4.54	4.15	3.78	3.76	7.04	7.47	6.80	7.16	7.29	7.64	8.11
	15	5.99	5.27	4.79	4.45	4.15	3.80	3.74	6.98	7.47	6.78	7.00	7.34	7.64	8.11
	16				4.44	4.14	3.82	3.73	6.96	7.47	6.64	7.03	7.33	7.65	8.11
	17				4.39	4.13	3.84	3.74	6.98	7.46	6.65	7.06	7.34	7.69	8.12
	18				4.41	4.15	3.80	3.76	6.98	7.46	6.78	7.10	7.31	7.67	8.11
	19				4.39	4.14	3.84	3.74	7.02	7.40	6.77	7.13	7.37	7.67	8.12
	20				4.40	4.14	3.85	3.74	6.95	7.40	6.73	7.10	7.33	7.71	8.14
	Average	**6.69**	**5.82**	**4.89**	**4.68**	**4.16**	**3.91**	**3.75**	**7.08**	**7.51**	**6.85**	**7.09**	**7.38**	**7.64**	**8.09**

Table A-4: pH results for PAA NaClO in wastewater (WW)

Contact time (min)	Sample #	WW-PAA-pH							WW-NaClO-pH					
		Dosage (ppm)												
		0	1	2	4	6	8	10	1	2	4	6	8	10
60	1	6.79	6.79	6.85	6.86	7.87	6.86	6.93	7.34	7.47	7.07	7.48	7.63	7.17
	2	6.78	6.81	6.82	6.88	6.87	6.86	6.95	7.34	7.45	7.09	7.46	7.60	7.19
	3	6.79	6.81	6.88	6.89	6.88	6.86	6.93	7.32	7.42	7.07	7.46	7.58	7.20
	4	6.81	6.79	6.84	6.90	6.99	6.87	6.93	7.32	7.40	7.06	7.48	7.57	7.20
	5	6.81	6.83	6.84	6.90	6.89	6.89	6.96	7.32	7.38	7.06	7.47	7.56	7.22
	6	6.83	6.83	6.87	6.92	6.90	6.91	6.95	7.32	7.36	7.08	7.45	7.44	7.22
	7	6.83	6.85	6.87	6.93	6.92	6.92	6.95	7.32	7.36	7.07	7.47	7.40	7.22
	8	6.83	6.87	6.89	6.93	6.94	6.94	6.95	7.31	7.34	7.09	7..46	7.40	7.21
	9	6.85	6.87	6.89	6.95	6.94	6.95	6.96	7.31	7.34	7.08	7.45	7.41	7.21
	10	6.85	6.87	6.91	6.97	6.96	6.96	6.99	7.31	7.32	7.08	7.45	7.40	7.18
	11	6.87	6.90	6.91	6.97	6.96	6.97	6.99	7.31	7.31	7.08	7.42	7.40	7.20
	12	6.87	6.90	6.94	6.99	6.98	6.99	7.01	7.29	7.31	7.09	7.42	7.40	7.20
	13	6.88	6.92	6.94	6.99	7.00	6.99	7.00	7.29	7.29	7.09	7.42	7.39	7.20
	14	6.88	6.94	6.96	7.00	7.02	7.01	7.01	7.29	7.29	7.09	7.42	7.38	7.21
	15	6.90	6.94	6.98	7.01	7.02	7.01	7.01	7.29	7.29	7.09	7.42	7.39	7.20
	16	6.90	6.96	7.00	7.03	7.03	7.03	7.02	7.29	7.28	7.11	7.41	7.38	7.21
	17	6.90	6.95	7.02	7.04	7.04	7.02	7.02	7.29	7.28	7.11	7.41	7.39	7.21
	18	6.90	6.95	7.04	7.05	7.04	7.02	7.04	7.29	7.28	7.10	7.40	7.40	7.21
	19	6.92	6.97	7.05	7.06	7.05	7.03	7.04	7.29	7.26	7.10	7.40	7.38	7.22
	20	6.95	6.99	7.07	7.07	7.06	7.03	7.05	7.28	7.26	7.10	7.40	7.38	7.21
	Average	**6.86**	**6.89**	**6.93**	**6.97**	**7.02**	**6.96**	**6.98**	**7.31**	**7.33**	**7.09**	**7.44**	**7.44**	**7.20**

Table A-5: TOC results for PAA/NaClO in distilled water (DW)

Dosage (ppm)	DW-PAA-TOC					DW-NaClO-TOC				
	Contact time (min)									
	10	15	30	60	Average	10	15	30	60	Average
0	0.5151	0.479	0.4663	0.7693	0.5574	0.545	0.6123	0.5813	0.5722	0.5777
1	2.024	2.003	1.961	2.101	2.0222	0.7025	0.6326	0.4814	0.3656	0.5455
2	4.077	4.109	3.807	3.786	3.944	0.6481	0.5989	0.4192	0.4731	0.5348
4	6.487	6.399	6.476	6.372	6.4335	0.6157	0.6247	0.6128	0.7054	0.6397
6	9.845	10.04	9.712	9.885	9.8705	0.4783	0.4447	0.3839	0.3808	0.4219
8	13.61	14.74	14.15	13.44	13.985	0.449	0.4191	0.4046	0.4197	0.4231
10	17.93	18.15	17.98	17.94	18	0.511	0.4555	0.4596	0.4602	0.4716

Table A-6: TOC results for PAA/NaClO in wastewater (WW)

Dosage (ppm)	WW-PAA-TOC				WW-NaClO-TOC			
	Contact time (min)							
	10	15	30	60	10	15	30	60
0	13.03	13.21	13.08	13.03	13.11	12.64	13.19	13.25
1	9.353	9.759	9.605	11.260	8.279	7.605	8.145	7.759
2	9.579	9.919	11.830	11.880	7.617	7.407	7.647	7.530
4	10.730	9.842	11.830	10.290	7.969	8.150	7.257	7.939
6	11.170	11.590	11.770	12.120	7.051	7.617	7.783	7.786
8	10.020	10.400	11.950	12.320	7.283	7.158	7.031	7.363
10	11.580	11.840	12.670	12.050	7.480	7.256	7.064	7.191

Table A-7: COD results for PAA/NaClO in distilled water (DW)

Dosage (ppm)	DW-PAA-COD					DW-NaClO-COD				
	Contact time (min)									
	10	15	30	60	Average	10	15	30	60	Average
0	-5.1	3.4	-2.3	2.5	-0.38	2.5	2.7	1.3	2.2	2.2
1	14.0	13.1	13.4	13.3	13.45	3.0	3.4	2.1	-1.2	1.8
2	18.1	17.2	17.7	15.0	17.00	2.4	4.2	1.0	-0.2	1.9
4	30.3	29.4	26.4	29.5	28.90	3.7	2.6	1.0	3.7	2.8
6	43.0	45.0	42.0	39.0	42.25	2.4	0.7	2.8	2.2	2.0
8	55.0	62.0	54.0	51.0	55.50	0.3	-0.6	-1.0	1.5	0.1
10	83.0	78.0	43.0	61.0	66.25	-0.5	-2.9	-1.3	-0.7	-1.6

Table A-8: COD results for PAA/NaClO in wastewater (WW)

Dosage (ppm)	WW-PAA-COD					WW-NaClO-COD				
	Contact time (min)									
	10	15	30	60	Average	10	15	30	60	Average
0	16.8	17.1	18.9	21	18.45	15.9	19.5	19.2	20.7	18.83
1	48.1	50.0	51.2	53.3	50.65	21.7	22.9	24.6	26.3	23.88
2	50.7	55.7	56.4	51.0	53.45	24.6	28.3	28.1	23.5	26.13
4	54.2	56.1	53.8	57.8	55.48	25.8	27.6	23.8	29.7	26.73
6	56.9	62.4	59.3	65.1	60.93	25.4	36.0	29.8	27.1	29.58
8	57.6	66.1	68.6	61.4	63.43	31.3	31.3	30.7	32.6	31.48
10	73.9	73.6	73.4	74.0	73.73	32.7	28.0	35.8	31.9	32.10

Table A-9: UV_{254} absorbance results for PAA NaClO in distilled water (DW)

Contact time (min)	Sample #	Dosage (ppm)												
		DW-PAA-UV_{254}							DW-NaClO-UV_{254}					
		0	1	2	4	6	8	10	1	2	4	6	8	10
10	1	-0.0773	0.0081	0.0140	0.0408	0.0491	0.0739	0.0914	0.0087	0.0100	0.0142	0.0139	0.0159	0.0189
	2	-0.0775	0.0067	0.0134	0.0406	0.0491	0.0709	0.0882	0.0087	0.0095	0.0138	0.0135	0.0162	0.0164
	3	-0.0781	0.0074	0.0138	0.0403	0.0494	0.0709	0.0880	0.0079	0.0094	0.0123	0.0170	0.0148	0.0163
	4	-0.0780	0.0067	0.0178	0.0393	0.0486	0.0699	0.0882	0.0080	0.0095	0.0120	0.0146	0.0146	0.0187
	5	-0.0787	0.0099	0.0179	0.0403	0.0483	0.0673	0.0877	0.0074	0.0094	0.0125	0.0137	0.0148	0.0184
	6	-0.0784	0.0079	0.0174	0.0404	0.0486	0.0680	0.0878	0.0081	0.0095	0.0128	0.0135	0.0140	0.0160
	Average	-0.0669	0.1495	0.2992	0.6060	0.8990	1.2030	1.5045	0.1498	0.2939	0.5825	0.8695	1.1558	1.4435
60	1	-0.0019	0.0098	0.0104	0.0292	0.0465	0.0700	0.0770	0.0066	0.0085	0.0087	0.0093	0.0098	0.0146
	2	-0.0027	0.0071	0.0103	0.0293	0.0463	0.0709	0.0776	0.0075	0.0085	0.0081	0.0088	0.0084	0.0146
	3	-0.0026	0.0075	0.0100	0.0298	0.0458	0.0697	0.0770	0.0063	0.0090	0.0073	0.0088	0.0084	0.0141
	4	-0.0028	0.0064	0.0098	0.0280	0.0450	0.0691	0.0772	0.0069	0.0092	0.0078	0.0092	0.0095	0.0138
	5	-0.0033	0.0063	0.0094	0.0302	0.0464	0.0718	0.0785	0.0060	0.0083	0.0074	0.0093	0.0115	0.0160
	6	-0.0034	0.0058	0.0093	0.0281	0.0450	0.0691	0.0777	0.0062	0.0081	0.0091	0.0096	0.0095	0.0163
	Average	-0.0202	0.0250	0.0470	0.1026	0.1528	0.2114	0.2572	0.0247	0.0444	0.0805	0.1172	0.1534	0.1936

Table A-10: UV_{254} absorbance results for PAA NaClO in wastewater (WW)

Contact time (min)	Sample #	Dosage (ppm)												
		WW-PAA-UV_{254}							WW-NaClO-UV_{254}					
		0	1	2	4	6	8	10	1	2	4	6	8	10
10	1	0.2617	0.2994	0.3036	0.2967	0.3110	0.3130	0.3158	0.2225	0.2173	0.2381	0.2374	0.2473	0.2467
	2	0.2689	0.2859	0.3029	0.3106	0.3117	0.3193	0.3173	0.2184	0.2169	0.2392	0.2370	0.2452	0.2466
	3	0.2600	0.2879	0.2995	0.3035	0.3100	0.3160	0.3200	0.2260	0.2154	0.2384	0.2338	0.2439	0.2439
	4	0.2616	0.2935	0.2990	0.3004	0.3120	0.3208	0.3244	0.2178	0.2141	0.2382	0.2325	0.2439	0.2451
	5	0.2543	0.2907	0.3004	0.3186	0.3119	0.3244	0.3222	0.2216	0.2144	0.2366	0.2333	0.2440	0.2452
	6	0.2584	0.2888	0.3124	0.2988	0.3133	0.3257	0.3290	0.2187	0.2142	0.2381	0.2350	0.2447	0.2422
	7	0.2574	0.2912	0.3053	0.3008	0.3137	0.3248	0.3215						
	8	0.2550	0.2874	0.3011	0.3015	0.3144	0.3222	0.3198						
	Average	0.259	0.2906	0.3038	0.3039	0.3123	0.3208	0.3213	0.2208	0.2154	0.2381	0.2348	0.2448	0.2450
60	1	0.2579	0.2982	0.3189	0.3019	0.3170	0.3265	0.3328	0.2039	0.2159	0.2195	0.2409	0.2412	0.2506
	2	0.2583	0.2918	0.3022	0.3081	0.3160	0.3297	0.3299	0.2105	0.2170	0.2492	0.2376	0.2416	0.2514
	3	0.2637	0.2960	0.3171	0.3159	0.3182	0.3289	0.3318	0.2093	0.2159	0.2507	0.2384	0.2440	0.2530
	4	0.2607	0.2894	0.3076	0.3198	0.3164	0.3306	0.3340	0.2096	0.2167	0.2494	0.2373	0.2403	0.2512
	5	0.2607	0.2974	0.3037	0.3140	0.3175	0.3306	0.3442	0.2090	0.2167	0.2495	0.2372	0.2408	0.2506
	6	0.2597	0.2938	0.3145	0.3188	0.3172	0.3339	0.3343	0.2091	0.2169	0.2503	0.2388	0.2421	0.2531
	7	0.2593	0.3035	0.3124	0.3168	0.3183	0.3298	0.3424						
	8	0.2650	0.2931	0.3074	0.3158	0.3154	0.3354	0.3361						
	Average	0.260	0.2954	0.3105	0.3139	0.317	0.330	0.335	0.2086	0.2165	0.2448	0.2384	0.241	0.251

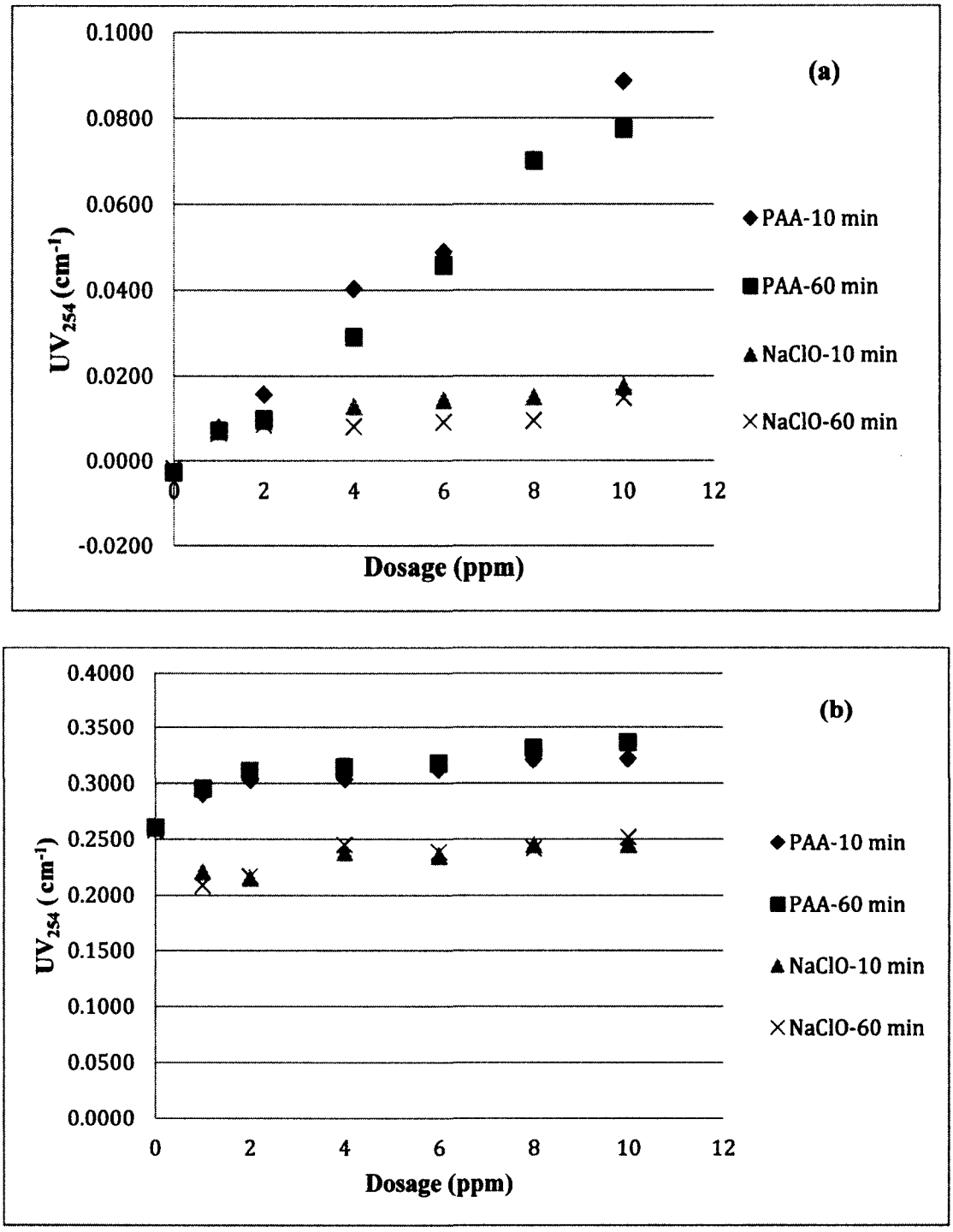

Figure A-1: UV_{254} absorbance for (a) PAA/NaClO in distilled water (DW), (b) PAA/NaClO in wastewater (WW)

Table A-11: SUVA results for PAA/NaClO in distilled water (DW)/wastewater (WW)

Dosage (ppm)	PAA-UV_{254}		NaClO-UV_{254}		Dosage (ppm)	PAA-TOC		NaClO-TOC	
	DW	WW	DW	WW		DW	WW	DW	WW
0	-0.0404	0.2602	-0.0396	0.2598	0	0.5574	13.09	0.5777	13.05
1	0.0075	0.2930	0.0074	0.2147	1	2.0222	9.99	0.5455	7.95
2	0.0128	0.3068	0.0091	0.2160	2	3.944	10.80	0.5348	7.55
4	0.0347	0.3089	0.0105	0.2415	4	6.4335	10.67	0.6397	7.83
6	0.0474	0.3147	0.0118	0.2366	6	9.8705	11.66	0.4219	7.56
8	0.0702	0.3258	0.0123	0.2433	8	13.985	11.17	0.4231	7.21
10	0.0831	0.3285	0.0162	0.2484	10	18	12.04	0.4716	7.25

Dosage (ppm)	DW--SUVA		WW--SUVA	
	PAA	NaClO	PAA	NaClO
0	-7.2479	-6.8548	1.9882	1.9912
1	0.3709	1.3474	2.9317	2.7016
2	0.3245	1.7016	2.8398	2.8602
4	0.5394	1.6414	2.8942	3.0841
6	0.4797	2.7969	2.6980	3.1299
8	0.5016	2.9071	2.9156	3.3744
10	0.4614	3.4351	2.7295	3.4266

Table A-12: NH_3-N results for PAA/NaClO in distilled water (DW)

Dosage (ppm)	DW-PAA-NH_3-N					DW-NaClO-NH_3-N				
	Contact time (min)									
	10	15	30	60	Average	10	15	30	60	Average
0	1.3	0.6	0.9	0.9	0.9	1.0	0.7	0.6	0.7	0.8
1	1.4	0.8	0.8	0.8	1.0	0.7	0.9	0.3	0.5	0.6
2	1.3	0.7	1.0	0.5	0.9	0.0	-0.6	-0.2	0.5	-0.1
4	0.9	0.9	0.8	1.0	0.9	0.3	0.1	-0.4	-0.4	-0.1
6	0.8	0.8	0.8	1.0	0.9	-0.3	-0.3	-0.5	-0.3	-0.4
8	0.8	0.7	0.4	0.5	0.6	-0.5	-0.3	-0.6	-0.5	-0.5
10	0.5	1.1	0.3	0.6	0.6	-0.1	0.5	-0.3	-0.1	0.0

Table A-13: NH_3-N results for PAA/NaClO in wastewater (WW)

Dosage (ppm)	WW-PAA-NH_3-N					WW-NaClO-NH_3-N				
	Contact time (min)									
	10	15	30	60	Average	10	15	30	60	Average
0	30.1	27.4	27.2	26.0	27.7	28.8	27.0	26.7	25.6	27.0
1	11.3	10.9	10.0	10.9	10.8	1.8	2.0	2.2	2.4	2.1
2	12.1	10.3	15.2	10.9	12.1	3.0	2.3	1.7	2.0	2.3
4	10.6	11.2	10.3	9.9	10.5	1.3	1.1	1.8	1.4	1.4
6	9.3	9.5	9.2	9.0	9.3	1.4	1.3	1.6	1.5	1.5
8	9.5	8.5	8.8	8.7	8.9	1.5	2.0	1.5	1.6	1.7
10	9.6	8.2	9.1	7.9	8.7	2.1	1.5	1.9	1.7	1.8

Validation of TOC and COD results for PAA: Comparison of measured values with theoretically calculated values

The theoretical oxygen demand (THOD), COD and TOC values can be calculated according to the chemical reaction form of 15% PAA (CH_3COOOH), 33% acetic acid (AA, CH_3COOH) with O_2, shown as follows (According to [12], at pH=5.5-8.2, the main reaction is PAA spontaneously decomposition, and complete transformation of PAA to AA is assumed) :

1) Decomposition of PAA to AA

$$2\ CH_3COOOH \longrightarrow 2\ CH_3COOH + O_2 \qquad \text{(Reaction A-1)}$$

2*76 2*60

1ppm 1.6667 ppm

2) AA in the mixture

$$CH_3COOOH \longrightarrow CH_3COOH \qquad \text{(Reaction A-2)}$$

15% 33%

1ppm 2.2 ppm

Total AA = 1.6667 + 2.2 = 3.8667 ppm

3) Oxygen Demand

$$CH_3COOH + 2\ O_2 \longrightarrow 2\ CO_2 + 2\ H_2O \qquad \text{(Reaction A-3)}$$

60 2 * 32

THOD = COD = 2 × 32 × 3.8667/60 = 4.1245 ppm O_2 / ppm PAA solution

Theoretical TOC = 3.8667 × 2 × 12/60 = 1.5467 ppm TOC / ppm PAA solution

The corresponding TOC and COD values for different dosages are shown as follows:

Table A-14: Comparison of the measured and theoretical TOC and COD values

PAA (ppm)	TOC (mg/L)		COD (mg/L)	
	Measured	Theoretical	Measured	Theoretical
0	0.5574	0	-0.38	0
1	2.0222	1.5467	13.45	4.1245
2	3.944	3.0934	17.00	8.2490
4	6.4335	6.1868	28.90	16.4980
6	9.8705	9.2802	42.25	24.747
8	13.985	12.3736	55.50	32.9960
10	18	15.4670	66.25	41.2450

APPENDIX B: THMs FULL-SCAN CALIBRATIONS

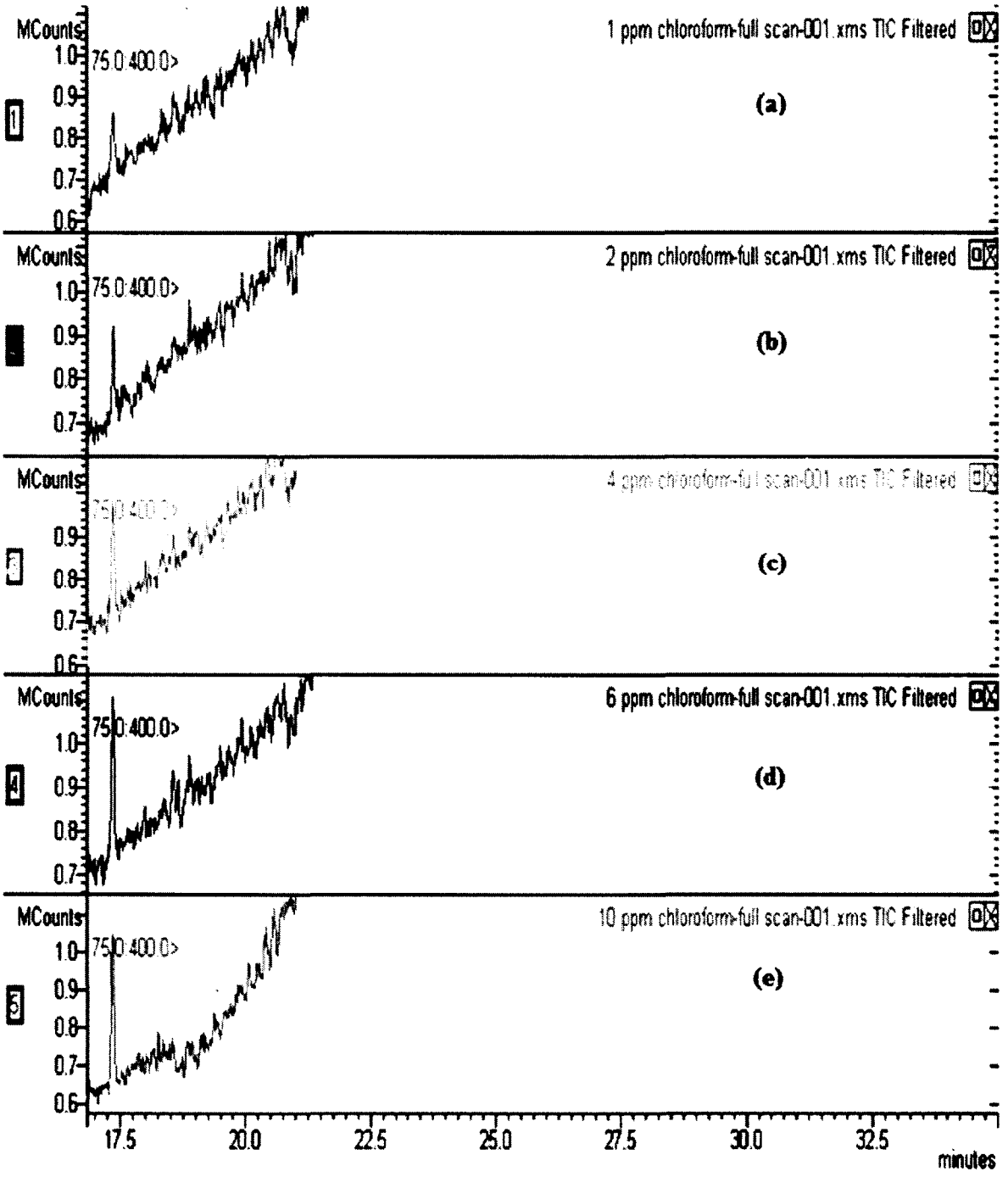

Figure B-1: Full-scan analysis of cloroform: (a) 1 ppm, (b) 2 ppm, (c) 4 ppm, (d) 6 ppm, (e) 10 ppm

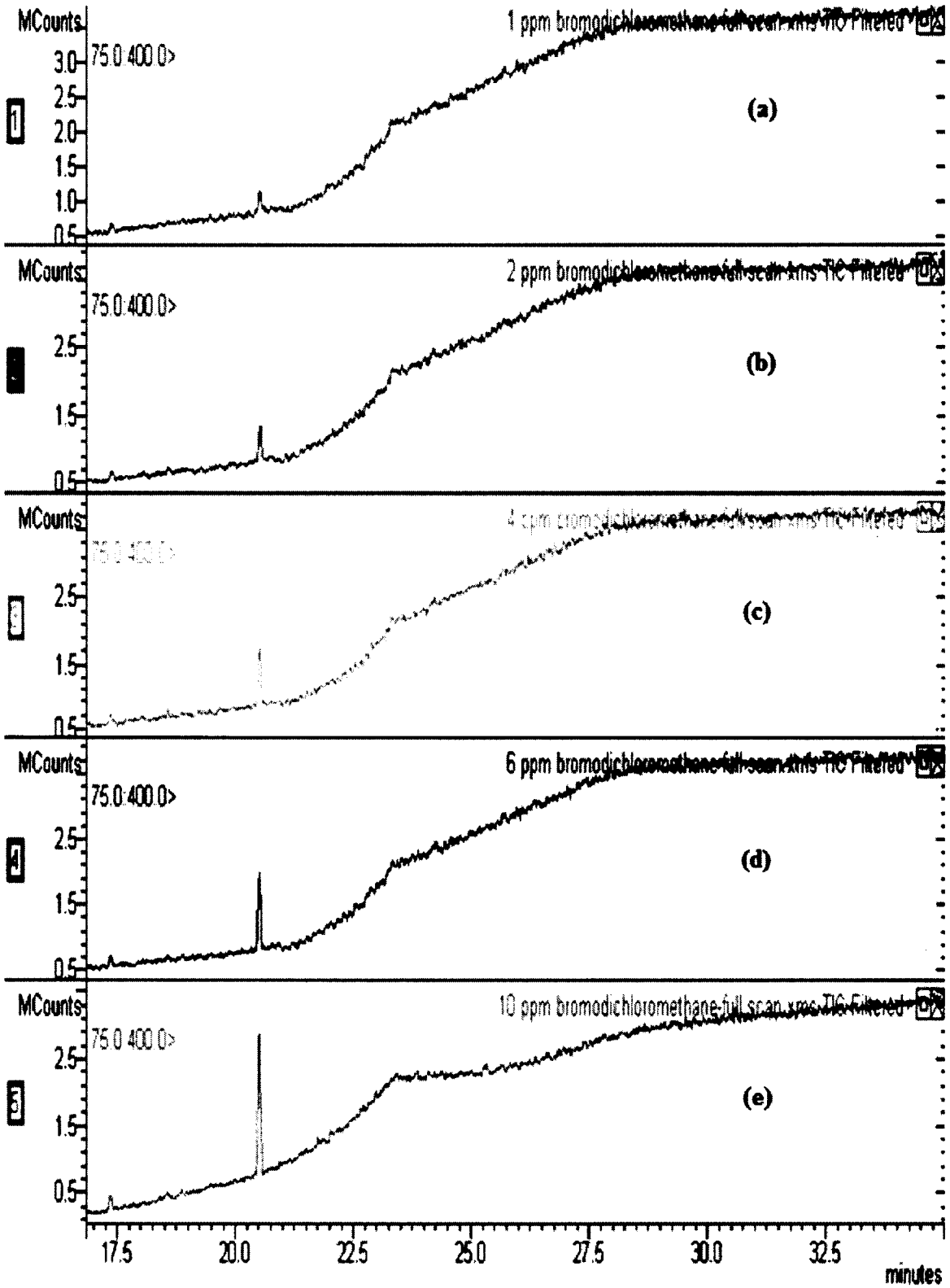

Figure B-2: Full-scan analysis of bromodichloromethane: (a) 1 ppm, (b) 2 ppm, (c) 4 ppm, (d) 6 ppm, (e) 10 ppm

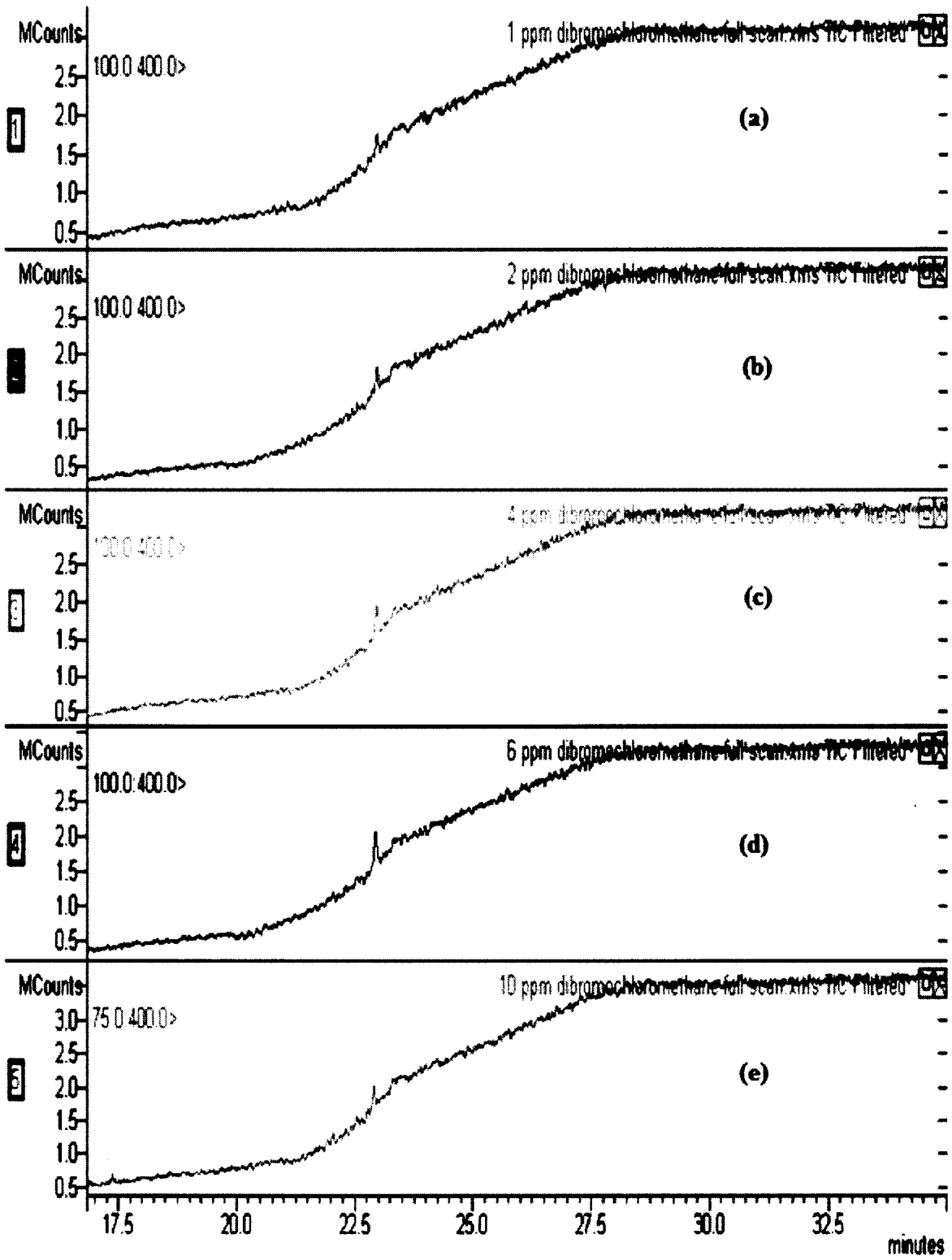

Figure B-3: Full-scan analysis of bromodichloromethane: (a) 1 ppm, (b) 2 ppm, (c) 4 ppm, (d) 6 ppm, (e) 10 ppm

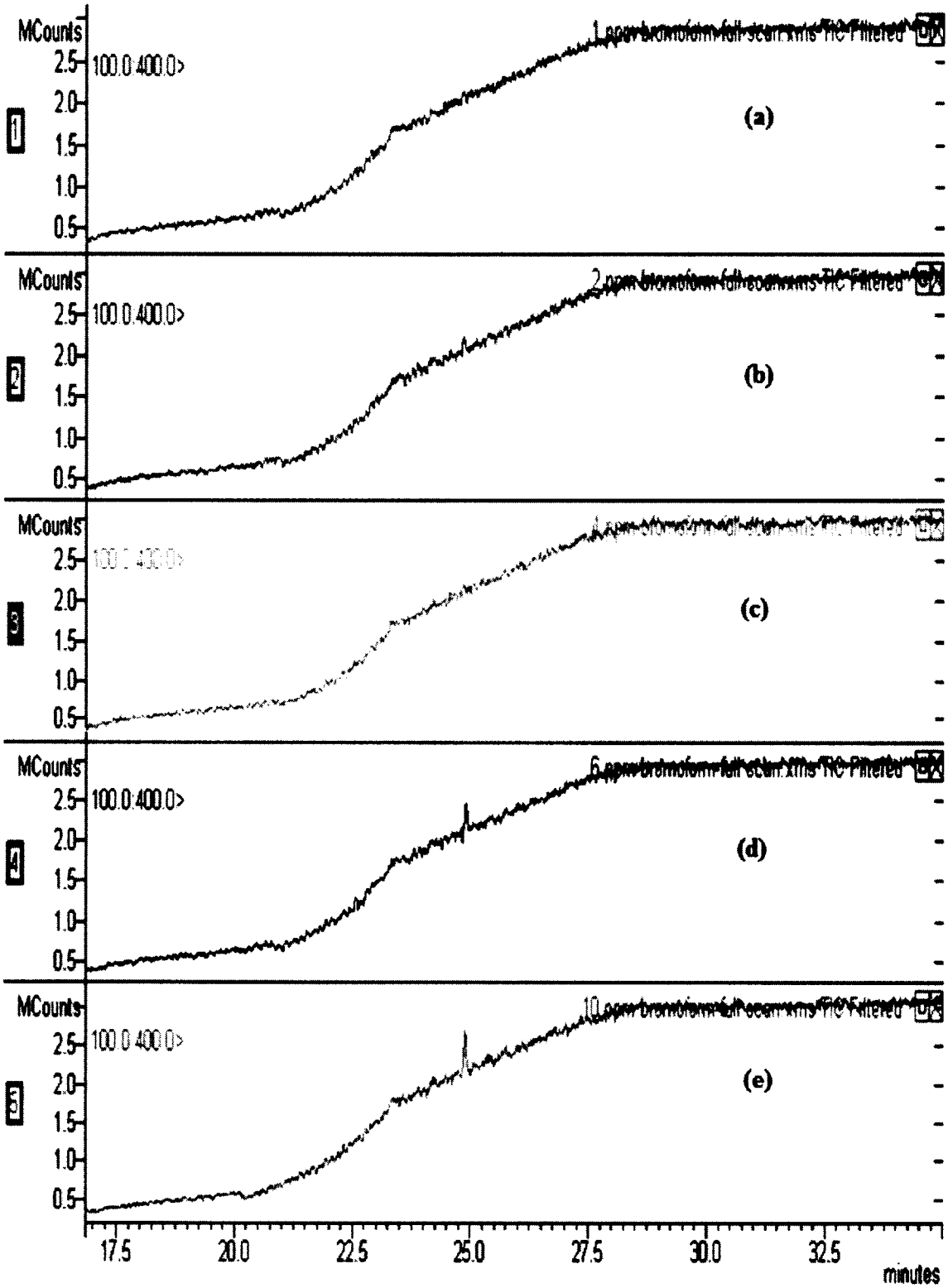

Figure B-4: Full-scan analysis of bromoform: (a) 1 ppm, (b) 2 ppm, (c) 4 ppm, (d) 6 ppm, (e) 10 ppm

APPENDIX C: OXIDATION OF 17β-ESTRADIOL (E2), CLOFIBRIC ACID AND KETOPROFEN BY PAA/NaClO

Table C-1: 17β-estradiol (E2) direct analyses

Concentration (ppm)	A1	A2	A3	A-average
0	0	0	0	0
1	3372000	3217000	2913000	3167333
2	5601000	5478000	5394000	5491000
4	10480000	10650000	10980000	10703333
6	15200000	15510000	15650000	15453333
10	27830000	25170000	26400000	26466667

Table C-2: 17β-estradiol (E2) processed calibration

Concentration (ppm)	A1	A2	A3	A-average
0	0	0	0	0
1	372700000	372100000	360900000	368566667
2	864100000	766900000	804400000	811800000
4	1444000000	1618000000	1674000000	1578666667
6	2982000000	2554000000	3009000000	2848333333
10	2843000000	3421000000	3631000000	3298333333

Table C-3: Clofibric acid processed calibration

Concentration (ppm)	A1	A2	A3	A-average
0	0	0	0	0
1	158600000	159900000	160100000	159533333
2	260900000	275300000	258500000	264900000
4	643300000	652100000	654000000	649800000
6	1220000000	1257000000	1211000000	1229333333
10	1707000000	1577000000	1578000000	1620666667

Table C-4: Ketoprofen processed calibration

Concentration (ppm)	A1	A2	A3	A-average
0	0	0	0	0
1	68160000	81840000	95930000	81976667
2	205000000	282100000	277300000	254800000
4	519100000	596800000	605800000	573900000
6	926300000	1012000000	1026000000	988100000
10	1653000000	1946000000	1940000000	1846333333

Table C-5: MDL for 17β-estradiol (E2), clofibric acid, and ketoprofen

Samples	Runs	1 ppm E2-DW-Methanol		1 ppm CA-DW-Methanol		1 ppm K-DW-Methanol	
		Area	Concentration (ppm)	Area	Concentration (ppm)	Area	Concentration (ppm)
#1	#1	372700000	0.682	158600000	0.818	68160000	0.841
	#2	372100000	0.680	159900000	0.825	81840000	0.909
	#3	360900000	0.652	160100000	0.826	95930000	0.980
	#4	361500000	0.654	168900000	0.870		
#2	#5	414100000	0.785	166700000	0.859	94790000	0.974
	#6	416200000	0.791	151600000	0.783	98380000	0.992
	#7	406800000	0.767	152400000	0.787	97280000	0.986
	#8	406100000	0.765			99190000	0.996
Average			0.722		0.839		0.954
Standard Deviation			0.060		0.033		0.058
$t_{(n-1,99)}$			2.998		3.143		3.143
MDL			0.181		0.102		0.182

Table C-6: Concentration of 17β-estradiol (E2) after oxidation by PAA/NaClO

Samples	Runs	1 ppm E2-DW-Methanol		NaClO-E2-DW-Methanol		PAA-E2-DW-Methanol	
		Area	Concentration (ppm)	Area	Concentration (ppm)	Area	Concentration (ppm)
#1	#1	372700000	0.682	18880000	-0.203	371500000	0.679
	#2	372100000	0.680	18620000	-0.203	379900000	0.700
	#3	360900000	0.652	19490000	-0.201	367200000	0.668
	#4	361500000	0.654	20370000	-0.199	364600000	0.662
#2	#5	414100000	0.785	20720000	-0.198	438600000	0.847
	#6	416200000	0.791	20250000	-0.199	430900000	0.827
	#7	406800000	0.767	18950000	-0.203	180100000	0.200
	#8	406100000	0.765	19200000	-0.202	145300000	0.113
Average			**0.722**		**-0.201**		**0.587**
Standard Deviation			**0.060**		**0.002**		**0.276**

Table C-7: Concentration of clofibric acid (CA) after oxidation by PAA/NaClO

Samples	Runs	1 ppm CA-DW-Methanol		NaClO-CA-DW-Methanol		PAA-CA-DW-Methanol	
		Area	Concentration (ppm)	Area	Concentration (ppm)	Area	Concentration (ppm)
#1	#1	158600000	0.818	109200000	0.571	61960000	0.335
	#2	159900000	0.825	60820000	0.329	73080000	0.390
	#3	160100000	0.826	65790000	0.354	82150000	0.436
#2	#4	157700000	0.814	119100000	0.621	138100000	0.716
	#5	157600000	0.813	122200000	0.636	135200000	0.701
	#6	159900000	0.825	117000000	0.610	143500000	0.743
#3	#7	163700000	0.844	133100000	0.691	140600000	0.728
	#8			128400000	0.667	118200000	0.616
	#9			133400000	0.692	95000000	0.500
Average			**0.839**		**0.574**		**0.574**
Standard Deviation			**0.033**		**0.160**		**0.138**

Table C-8: Concentration of ketoprofen (K) after oxidation by PAA/NaClO

Samples	Runs	1 ppm K-DW-Methanol		NaClO-K-DW-Methanol		PAA-K-DW-Methanol	
		Area	Concentration (ppm)	Area	Concentration (ppm)	Area	Concentration (ppm)
#1	#1	68160000	0.841	97020000	0.935	103100000	0.966
	#2	81840000	0.909	103600000	0.968	113400000	1.017
	#3	95930000	0.980	102900000	0.965	119500000	1.048
#2	#4	94790000	0.974	145800000	1.179	61340000	0.757
	#5	98380000	0.992	148200000	1.191	74890000	0.824
	#6	97280000	0.986	144100000	1.171	91570000	0.908
#3	#7			130900000	1.105	110700000	1.004
	#8			118100000	1.041	109700000	0.999
	#9			111200000	1.006	103800000	0.969
Average			**0.954**		**0.943**		**1.062**
Standard Deviation			**0.058**		**0.096**		**0.101**