Dispersive liquid-liquid micro-extraction coupled with gas chromatography for the detection of trihalomethanes in different water sources in the Western Cape, South Africa

By
Marshalle Lane

Thesis submitted in part-fulfillment of the requirements for the degree of

Magister Technologiae: Chemistry

In the

Faculty of Applied Science

at the

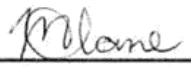
Cape Peninsula University of Technology

Supervisor: Dr. S. Le Roux

Bellville 2018

DECLARATION

I, **Marshalle Glenville Lane**, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.



Signed

28 September 2018

Date

ABSTRACT

Trihalomethanes (THMs) are a group of four compounds that are formed, along with other disinfected by-products. This happens when chloride or other disinfectants are used to control microbial contamination in drinking water, which then reacts with natural organic or inorganic substances in water. Trihalomethanes are better known by their common names such as chloroform, bromodichloromethane, chlorodibromomethane and bromoform. These four compounds are known to be classified as cancer group B carcinogens (shown to cause cancer in laboratory animals). Trihalomethane levels tend to increase with pH, temperature, time and the level of “precursors” present. Precursors are known to be organic substances which react with chloride to form THMs. One significant way of reducing the amount of THMs in water is to eliminate or reduce chlorination before filtrations and reduce precursors. There are guideline limits for THMs in the SANS 241:2015 document, but they are not continuously monitored and their levels in natural water are not known.

The aim of this study is to develop a rapid, fast and reliable liquid-liquid micro-extraction technique, to determine the presence of THMs in natural water sources. This study particularly focuses on different water sources e.g. river, underground, borehole and chlorinated water. Chlorinated water is the water that has been presumably treated for bacteria and fungus growth. The results that were obtained for chlorinated water are as follow, 10.120 µg/L – 11.654 µg/L for chloroform, 2.214 µg/L - 2.666 µg/L for bromodichloromethane, 0.819 µg/L – 0.895 µg/L chlorodibromomethane and 0.103 µg/L - 0.135 µg/L for bromoform from validation data. All these THMs concentrations have been found to be below the SANS 241:2015 limits. Natural water shows a very high affinity for chloroform. This is what is expected under normal conditions as chloroform is the most abundant THM of all THMs present in natural water. The liquid-liquid micro-extraction technique that was optimized and used for the determination of THMs in this study is a rapid, simple and inexpensive technique that provides low limits of detection (LOD) e.g. 0.1999 µg/L chlorodibromomethane and 0.2056 µg/L bromoform and wide dynamic range (LOQ) of 0.6664 µg/L chlorodibromomethane and 0.6854 µg/L bromoform for the determination of THMs.

ACKNOWLEDGEMENTS

I wish to thank:

My External Supervisor, Dr Shirley Theodora Rose Le Roux for all her values suggestions and advice during the final stages of writing up the thesis.

My Operational Manager, Hannes Esterhuizen for his excellent guidance, patience and willingness to always assist and share his vast amount of knowledge with me.

My Assistant Laboratory Manager, Salwa Banien for her input as well as guidance and support.

My Laboratory Supervisor, Sibusiso Tsewu for his significant input and vast amount of knowledge regarding method validation.

My Aunt, Charlotte De Villiers for her continued love and support.

My Manager Director Raymond Hartley, for giving me the opportunity and time to complete my studies and project at Microchem Laboratory Service.

LAYOUT OF THESIS

The aim of this study is to detect quantitatively THMs in natural drinking water, by development of rapid liquid-liquid micro-extraction technique. The method will be validated and applied to municipal and natural drinking water.

The thesis is subdivided into the following chapters:

- **Chapter 1**, the introduction provides a background on THMs. It lists the overall objectives, problem statements, sub-problems and hypothesis and clearly states topics that were not covered by the study. The literature review provides a summary of background studies performed on THMs, their origins, toxicity and their routes into animal and human bodies. The second part of the literature review covers the validation master plan, standard U.S EPA method 551.1 of analysis for THMs and the modification of the method into UMass protocol method.
- **Chapter 2,** the research methodology chapter summaries the optimization of the UMass protocol method for THMs. Validation criteria of analytical method and information regarding sampling, transportation and sampling handling.
- **Chapter 3,** summarizes the results which consists of limit of detection, limit of quantification, instrument linearity, instrument precision, matrix effect based on recovery, matrix effect based on interferences, precision based on reproducibility, accuracy based on recovery, selectivity based on recovery, selectivity based on interferences, inter-laboratory comparison, precision based on repeatability, robustness, measure of uncertainty and proficiency testing. The following sample matrices will be used to gather method validation data: river water, borehole water, municipal water, sea water, chlorinated water, tap water, underground water, tap water and medical water.
- **Chapter 4,** overall discussion and conclusion, while also listing recommendations for future research.
- **Chapter 5,** the reference in accordance with Harvard method of referencing.

DEDICATION

This is optional, and may be omitted.

For (whomever)

Glossary of Terms

BDS - Blue Drop System

BDCM - Bromodichloromethane

CCT - City of Cape Town

CDBM - Chlorodibromomethane

CRM - Certified Reference Material

COT- Committee on Toxicity Chemicals in Food

DWA - Department of Water Affairs

DBP - Disinfection By-Products

DAI - Direct Aqueous Injection

DWDS - Drinking Water Distribution Stations

DWTP - Drinking Water Treatment Plants

EPA - Environmental Protection Agency

GC-ECD - Gas Chromatography Electron Captive Detector

GC-MS - Gas Chromatography Mass Spectroscopy

HSE - Health Service Executive

HT- Headspace Technique

HP - 5MS UI- HP Coloumn-5MS Ultra Inlet Column

IARC - International Agency for Research on Cancer

LLE - Liquid-Liquid Extraction

LIMS - Laboratory Information Management Systems

LOD - Limit of Detection

LOQ - Limit of Quantification

MoU - Measure of Uncertainty

MSDS - Material data Safety Sheet

MTBE - Methyl tert-butyl ether

Na_2SO_4 - Sodium Sulphate

NH_4Cl - Ammonium Chloride

SANS 241- South African National Standards

SRI - Solidarity Research Institute

SHT - Static Headspace Technique

THMs - Trihalomethanes

TDS - Total Dissolve Solids

TOC - Total Organic Carbon

TTHMs - Total Trihalomethanes

VOC-Volatile Organic Carbons

WHO- World Health Organization

Table of Content

5. Chapter FIVE: References

CHAPTER 1

INTRODUCTION

1.1 Background

The amount of water on earth is constant and cannot increase or decrease, but it is unevenly distributed across the earth. The earth receives 985 millimeters annual rainfall whereas South Africa receives annual rainfall of 492 millimeters of rain (Rand Water & Water Wise, 2011). This amount contributes to almost half of the amount of the earth's average. Therefore, South Africa is considered a water stressed country. The Department of Water and Environmental Affairs stated that the demand for water will outstrip the supply in Gauteng by 2013 and the rest of South Africa by 2025. Thus, demand for water in South Africa is very high, and as the human population increases, with its increasing needs for survival, the greater the demand for water in South Africa becomes.

A further problem that adds to the supply for water is water quality. Water quality is defined as water that is safe, drinkable and appealing to all life on earth. Drinking water treatment is a fundamental step and using disinfectant chemicals, prevents the spreading of waterborne infectious diseases. The use of disinfectant chemicals normally results in the formation of disinfectant byproducts (DBP) (HSE & EPA, 2011). In South Africa the most common disinfectant method used is chlorination. Chloride is regulated primarily to minimize the formation of DBPs, the most common of which are trihalomethanes (THMs). Trihalomethanes are a group of organic chemicals often present in drinking water and normally forms when chlorine reacts with naturally occurring organic matter in raw water. Chlorine is a powerful oxidizing agent and normally breaks down complex organic compounds, which are considered the coloring agents of water. This then leads to the formation of small reactive entities. These small entities then react with chlorine to form what is better known as THMs. Trihalomethanes are a group of four chemicals known as chloroform, bromoform, dibromochloromethane and dichlorobromomethane. Trihalomethanes are formed when there is either inadequate pre-treatment of the water or poor control over

the disinfection process itself according to the World Health Organization (WHO, 2004).

In South Africa, drinking water quality is governed by policies and regulations based on international standards. Municipalities or district municipalities, governed by Water Service Authorities are required to submit information regarding water quality and management thereof regularly to the national Blue Drop System (BDS). Since 2009, a trend has emerged in which under-resourced municipalities are failing to improve their water quality management systems, while urban municipalities have shown to consistently improve their water quality systems. A major concern has emerged in which rural municipalities are significantly failing to report the necessary required information and are regularly not complying with the regulated requirement that speaks to the overall management of the water quality monitoring rather than water quality itself (Rivett et al., 2012). A recent study that was done in February 2016 (Solidarity Research Institute, 2016) (SRI) showed that eighteen Free State municipalities have failed to attain BDS status for which ageing infrastructure and lack of expertise are to blame. The BDS usually gives an indication that water is safe to drink. To obtain blue drop status a municipality must obtain a score of at least 95% on adherence to BDS status. The only two municipalities that obtained BDS status in the Free State is Mangaung & Metsimaholo. Chlorine is used as a disinfectant agent by most municipal water supplying systems, as they are extremely efficient and cost effective. Although the chlorination of drinking water provides many advantages, THMs remain a human health concern. Water supplied by municipalities that contains DBP's may lead to potential human health risks and many of the DBP's have been classified as probable or possible carcinogens (Jamaleddin et al., 2016). A study carried out to assess THM levels in drinking water system from several areas in South Africa for the year 2013 reported variation of THM level with some reported to have relatively high levels of THMs (Booi, 2013). No study has since been carried out in municipalities in South Africa.

1.2.1 Problem Statement

Chlorination is the most common disinfected method used in South Africa, but this method produced the toxic, carcinogens THMs which can seriously affect

human health. The detection and monitoring of THM levels in drinking water is of vital importance. The aim of this study is to quantitatively determine THMs in natural drinking water, by development of rapid a liquid-liquid micro-extraction technique. The method will be validated and applied to municipal and natural drinking water.

1.2.2 Sub-Problems

1. Optimizing a rapid and simple method for analysis of THMs in municipality and natural water by using dispersive liquid-liquid micro-extraction (DLLME) coupled with gas chromatography-electron capture detection (GC-ECD).

2. Comparing the newly developed method with other conventional methods in terms of their sensitivity, simplicity and rapid determination of THMs.

3. Validation of the method will be done in compliance with SANS 241: 2015 and SANAS TR 28-01 method validation guidelines. This method validation guideline is only applicable to agriculture, mining, petroleum and food & beverage industries.

4. Applying the developed technique for monitoring of THMs in municipal water for three different municipalities and natural water systems

1.2.3 Hypothesis

Tap water supplied by municipalities to local communities is not polluted with trihalomethanes. This statement is made on the basis that government has a responsibility to ensure that the water is sterile and harmless for consumption.

1.2.4 Assumptions

1. That drinking water does not exceed the SANS 241-1:2015 limits set out by SANS, if it does, South Africa has an action plan in place in the event of a significant increase of THMs in drinking water.

2. All drinking water is safe (without pathogenic microorganisms and toxic compounds), attractive (free from colour, taste and odour) to avoid accumulation

of solids, corrosion and after-growth of bacteria in the distribution and transport pipeline.

3. That municipalities test their drinking water on a regular basis

1.2.5 Delimitations

1. All investigations regarding this study will be carried out on gas chromatography electron capture detector. There will be no studies carried out on any alternative instruments e.g. high performance liquid chromatography, ion-exchange chromatography, thin-layer chromatography, gas chromatography-mass spectroscopy or liquid chromatography-mass spectroscopy.

2. The study will be limited to liquid-liquid micro-extraction technique.

3. For this method validation, the following sample matrices will be used to gather method validation data: river water, borehole water, municipal water, sea water, chlorinated water, tap water, underground water, tap water and medical water.

1.3 Literature Review

1.3.1 Introduction

Water is life. For millions of years and generations still to come life on earth has been dependent on water for survival. Today, water holds the key to our future survival. When Neil Armstrong landed on the moon in 1969 he described Planet Earth as “a shining blue pearl spinning in space”. This blue color that Neil Armstrong was referring to is in fact the amount of water that is present on the surface. A 70% of the earth surface is covered with water but of this, approximately 97% is salt water, with the remaining 3% being fresh water. From this 3% less than 1% is available for life on earth (Rand Water & Water Wise, 2011), while the rest is in the form of ice at the poles. For complete control of microbial contamination in drinking water chlorine or other disinfectants are used. When chlorine reacts with natural occurring organic or inorganic materials in water, it results in the formation of disinfectant by-product. Trihalomethanes are one of these disinfectant byproducts (WHO, 2004). The main aim of treating drinking water is to produce water that is safe, these are water that contains no

pathogenic microorganisms and toxic compounds, attractive (free from colour, taste and odor) and to avoid any accumulation of solids, corrosion and after-growth of bacteria in the distribution and transportation through pipelines (Mamba et al., 2008).

The water quality and management systems are monitored by different standards across the world. In most parts of the world the monitoring is done by water suppliers while the data is audited by public health authorities. However there are international guidelines that all drinking water regulations need to adhere to. This is to ensure that every country follows the exact same procedure regarding protocol when it comes to treatment of drinking water. This body is known as the World Health Organization (WHO). However, every country has its own public health authority that needs to ensure that their country is following the WHO protocol. The main reason why every country has its own public health authorities is because the water quality differs from country to country.

1.3.2 Toxicity of Trihalomethanes

Trihalomethanes are rarely present in significant concentrations in raw water. Chloroform is the THM that is highest in concentration. Information regarding bromoform, chlorodibromomethane and bromodichloromethane are very limited. In industry bromoform is produced in small quantities and is often used as a chemical intermediate during chemical processing. Chlorodibromomethane and bromodichloromethane are produced in even smaller quantities than bromoform and are used for laboratory use only (EFS, 2006). These three THMs are largely released into the environment through air during water chlorination. Chronic oral exposure of humans (Christina et al., 2017) to chloroform at high doses results in adverse effects on the central nervous, liver, kidneys and heart. Animal studies (Jamaleddin et al., 2016) have shown a decrease in body weight in rats and mice given chloroform at high oral doses, and an increased incidence of respiratory disease at higher doses. At even higher oral doses, liver abnormalities and decreased size of the reproductive organs were observed in rats (Jamaleddin et al., 2016). Investigating studies (Jamaleddin et al., 2016) regarding the other THMs has led to observation of chronic exposure and liver toxicity. Bromodichloromethane also caused kidney toxicity. Evidence from an animal

study (Jamaleddin et al., 2016) now strongly indicated that exposure to chloroform causes cancer in animals, after first producing toxic cells.

Studies were done by Jamaleddin et al., (2016) in human population using chlorinated drinking water where chloroform is the most present THM. Small increases in the incidence of rectal, colon and bladder cancer have been consistently observed, with evidence strongest for bladder cancer. Chloroform has been classified as Group B2 or “probable human carcinogen” by the U.S Environmental Protective Agency (EPA), this is due to sufficient animal evidence (Jamaleddin et al., 2016) and inadequate human evidence of carcinogenicity. For cell toxicity to occur a certain threshold level of exposure needs to occur, cancer can only form from chloroform if that threshold is exceeded. Based on the result of animal studies, it has shown that bromodichloromethane increased tumors of the large intestine, kidney and liver, and bromoform increased the tumor of the large intestine. They are also classified as Group B2, wherease chlorodibromomethane is classified as Group C “possible human carcinogen”. Results have been inconclusive regarding exposure of THMs and adverse developments or reproductive effects in humans. However, the results of a recent study (Sharp et al., 2013) suggest an increased risk of early-term miscarriage from high levels of THMs in tap water, particularly bromodichloromethane.

According to the Health Service Executive and Environmental Protective Agency, chlorine’s primary function in drinking water is to act as disinfectant, however it also provides a stable disinfectant residual to preserve the quality of the water throughout the distribution network. Acute effects of THMs in drinking water are rare.

The International Agency for Research on Cancer (IARC) reviewed studies and updated its findings from 1991 to 1997 in 1997. It founded that chlorinated drinking water was not classified as carcinogenic to humans (HSE & EPA, 2011). In 2008 a study done by the Committee on Toxicity Chemicals in Food (COT) (UK) found that there is association between cancer and THMs in drinking water and others showed no association. The main responsibility of the COT is to assess the quality and totality and draw a conclusion based on the evidence present. They concluded:

"Problems remain in the interpretation of published studies. These include the small relative risks recorded, the possibility of residual confounding, and the problems with exposure assessment'. They conclude 'the evidence for a causal association between cancer and exposure to chlorination by-products is limited and any such association is unlikely to be strong. Efforts to minimise chlorination by-products in drinking water should continue but must be balanced against the need for effective disinfection of drinking water".

A study by (Booi, 2013) was conducted to determine THMs, in seven drinking Water Treatment Plants, (DWTP) and one Drinking Water Distribution Station (DWDS) in the Western Cape. The seven DWTP that were studied were Atlantis, Blackheath, Brooklands, Faure, Steenbras, Wemmershoek and Voelvlei. The one DWDS that was studied was Plattekloof. The study was performed using liquid-liquid gas chromatography-electron capture detector.

The average total THMs (TTHMs) concentrations detected from the DWTPs and DWDS was found to range from 32.82 µg/L for Brooklands to 26.52 µg/L for Plattekloof, with the observed total concentrations being comparable. The average chloroform concentration was found to be the highest for DWTP and DWDS, it ranged from 22.29 µg/L for Voelvlei to 11.74 µg/L for Plattekloof. Dichlorobromomethane had the lowest concentrations for all seven DWTP and DWDS. Atlantis was the only one of the seven DWTP in which the average TTHMs concentration was highest and found to be 83.48 µg/L. The chloroform concentration of 46.06 µg/L for Atlantis was found to be significant higher than any of the other chloroform concentrations for the DWTPs. Tap water samples was collected from 14 Western Cape suburbs. The average TTHMs concentration ranged from 5.30 µg/L for Mandalay to 13.12 µg/L for Brown Farm, Phillipi. All the TTHMs concentrations for the 14 Western Cape suburbs were lower than the TTHMs concentrations for the DWTPs. Overall the TTHMs and individual THMs concentration from the 14 Western Cape suburbs and the seven DWTP concentrations were well below the levels recommended by SANS 241:2011

A country-wide study conducted in South Africa over a period of two years on THMs levels in drinking water, indicated that the average TTHMs levels in water

was found to be 45 µg/L, with 10 % of the samples exceeding guideline levels (van Steenderen et al., 1991). The TTHMs study was based on a South African survey. The average THMs levels of 45 µg/L was found to be well below the guidelines limits of SANS 241 which specifies a maximum of 300 µg/L chloroform or 100 µg/L bromoform THM's and the WHO guideline of 200 µg/L chloroform or 100 µg/L bromoform. The WHO guidelines were reviewed and increased from 30 µg/L to 200 µg/L as the toxicological data was considered to be inconclusive (US-EPA, 2004; WHO, 2004).

In 2008 in Zimbabwe, a very interesting observation was made when a study was carried out regarding THMs in drinking water (Guyo et al., 2008). Water samples were collected from dams during June-September 2008, 3 water samples were collected once a month from dams which supply water to the city of Gweru. The THMs found were chloroform, chlorodibromomethane, dibromochloromethane and bromoform chloroform, being predominantly present, with concentration levels ranging from 3.70 µg/L to 45.89 µg/L. The concentration levels of THMs increased with increasing distance from the chlorination point according to the study. Total THMs concentration in raw water ranged from non-detectable levels to 18.13 µg/L and in treated water from 6.83 µg/L to 145.50 µg/L. A slight increase in concentration levels of THMs were present in warmer months with the highest concentration level of 145.80 µg/L being recorded in September. Generally, the concentration levels obtained were lower than the maximum permissible limits set by the World Health Organization (WHO).

A study done by Rahanama et al., (2007) that was conducted in Tehran and Iran founded that the total concentration of THMs in drinking water from the two areas were 10.9 and 14.1 µg/L. The conditions were optimized and the enrichment factor ranges from 116 to 335. The limit of detection ranged from 0.0005 to 0.040 µg/L, while the linear range was 0.01-50 µg/L. Relative standard deviations (RSD) for 2.00 µg/L with internal standard were in the range 1,3-5,9 % (n=5), without internal standard they were in the range 3,7-8,6 % (n=5).

A similar study was conducted in Spain by Rodrıguez-Cabo et al., (2012) of THMs in tap and swimming pool water and reported the following results i.e. limits of quantification (LOQs) between 0.05 and 1.3 ng/ mL^{-1} and an excellent

linearity was noticed up to 100 ng mL^{-1}. Relative recoveries, measured for spiked aliquots of tap and swimming pool water samples, remained between 79% and 113%, with associated standard deviations below 12%. The applicability of the developed methodology was demonstrated with chlorinated water samples analysis. As regards tap water samples, the sum of THMs concentrations remained under the limit fixed by the European Union 100 ng mL^{-1}, however, some samples contained levels close to 80 ng mL^{-1}, the maximum allowable concentration established by the United States Environmental Protection Agency (EPA).

1.3.3 Validation Master Plan

As part of GMPs (Good Manufacturer Practices) for pharma, biotech, beverage, and medical device companies, a guide validation master plan is documented that outlines and defines which processes and equipment needs to be validated (FDA, 2013) and the priority and order in which it will be done. The validation master plan will overcome challenges faced in heavily regulated sectors, since FDA (Food and Drug Administration) may request documentation summarizing an organization's process. Validation master plan is part of industry GMPs and not a formal requirement, but can help reduce the chances of receiving an FDA warning letter. In South Africa, the national body that oversees the drinking water regulations is South African National Standards (SANS) 241. However it is of critical importance to look at the function of SANS 241 and its validation master plan.

1.3.4 Importance of South African National Standards 241

The South Africa Bureau of Standards (SABS) is a South African regulatory body established in terms of the Standard Act, 1945 (Act No. 24 of 1945) (DWA, 2013). It continues to operate in terms of the newest edition of the Standard Act, 2008 (Act No. 29 of 2008) as the national institute that promotes and maintains standardization and quality in connection with commodities and rendering of services (DWA, 2013). Safe drinking water is one of the basic requirements for human rights and vital to human health (WHO, 2014). The South African National Standards (SANS) 241 drinking water specification states the minimum

requirements (SANS, 241:2015) for potable drinking water to be considered safe for human consumption. The requirements include microbiological, chemical and physical properties of the water. Water that complies with SANS 241 regulations does not pose a risk to health over a lifetime of consumption (DWA, 2013). SANS 241 shows various properties of water that need to be checked to determine whether drinking water is safe for human consumption. SANS 241 categorizes the properties of drinking water into 4 main risk categories and states the minimum values that drinking water must conform to before considered safe to drink.

Table 1.1 Guideline values by World Health Organization 2004

Analytes of Interest	WHO guideline value µg/L	WHO tolerable daily intake (TDI) µg/kg/day
Trihalomethanes (total)	Ratio of 1	
Chloroform	300	15.0
Bromoform	100	21.4
Dibromochloromethane	100	17.9
Bromodichloromethane	60	-

Table 1.2 Standards Limits for THMs

Determination	Risk	Standard limit µg/L
Chloroform	Chronic Risk	≤300
Bromoform	Chronic Risk	≤100
Dibromochloromethane	Chronic Risk	≤100
Bromodichloromethane	Chronic Risk	≤60
Combined Trihalomethanes'	Chronic Risk	≤1

Seven municipalities in South Africa failed the quality standards for drinking water in tests conducted in 2016 (BDS, 2016). The water and sewage quality in 132 towns was tested by civil rights group AfriForum. It was reported that seven municipalities did not meet the quality standards for drinking water in 2016 (BDS, 2016). This was a slight decrease from the previous years. A significant study (BDS, 2016) performed in 2014 showed that the drinking water system of 11 towns did not meet the standards. This figure decreased to 5 in 2015, but increased to 7 for 2016 (BDS, 2016). The municipalities that did not meet the standards are as outlined in table 1.3 below.

Table 1.3 Municipalities that failed the drinking water test in South Africa

Municipality in Pretoria West & Tshwane	Areas of Non-Compliance
Rayton, Tshwane Metropolitan Municipality	High concentration of Phenol and Chromium
Schweizer-Reneke, Naledi Local Municipality	E. coli.
Witbank, Emalahleni Local Municipality	Nitrates above allowable limit
Belfast, Emakhazeni Local Municipality	E. coli and faecal coliforms
Piet Retief Municipality	E. coli and faecal coliforms
Stella, Naledi Local Municipality	E. coli.
Mkhondo Local Municipality	E. coli and faecal coliforms

Important to note is that AfriForum reported their findings to the municipalities. Follow-up samples indicted that only five out of the seven municipalities took immediate action while two of the municipalities considered taking any action regarding the situation (BDS, 2016). AfriForum communicated the non-compliance of the water quality to the municipality of Belfast & Piet Retief, these municipalities did not resolve the crisis (BDS, 2016). For its green drop report, which focuses on sewage systems in South Africa, twenty seven of 72 towns across the country did not meet the quality standards (BDS, 2016). It must be noted that the City of Cape Town scored a magnificent 98% during the study for their water quality.

1.4 Analytical methods for Trihalomethanes

Over the years many different techniques for the determination of THMs and other VOCs (Volatile Organic Carbons) in water have been developed, e.g. liquid-liquid extraction, direct aqueous injection or headspace technique to mention a few (Pavon et al., 2008). These developments and optimization of sensitive, rapid and simple analytical methods are essential for THMs concentration determination, providing continuous comprehensive understanding of their formation and removal in distribution systems (Pavon et al., 2008). With this information, the human exposure to THMs can be estimated. Most research regarding THMs in drinking water has been carried out in the form of gas chromatography electron capture detector (GC-ECD) or mass spectrometry detection (MSD) (Pavon et al., 2008). The concentration of these compounds is

generally in the range of ng/L to µg/L, such that it is normal to consider a pre-concentration step of the analytes to achieve a level that can be measured by the chosen analytical method. Generally there are four major techniques for the determination of THMs: direct aqueous injection (DAI), headspace technique, membrane - based sampling technique, liquid-liquid extraction (LLE). These methods differ significantly one from each other, whether it is sample preparation, matrix match or instrumentation. Therefore it is important that the best method must be chosen for instrumentation availability, accredited quality systems and company protocol quality controls (Pavon et al., 2008).

1.4.1 Direct Aqueous Injection (DAI)

Direct Aqueous Injection (DAI) of water samples into a GC system is considered the fastest and simplest “first step” in the analysis of aqueous samples by means of gas chromatography injection. This technique requires no isolation or pre-concentration of the sample to be performed. This is done to such an extent that the loss of volatile analytes and the possibility of sample pollution during manipulation are minimized. However, it is considered as a significant alternative because it avoids the use of solvents which are quite expensive and toxic (Pavon et al., 2008). The introduction of water as a solvent into the GC system is not considered as a desired step, because it commonly degrades the coatings of the analytical column. The analytical column is covered with a thick film of polar liquid phase that allows the water to elute before the analytes of interest. An on column injector is used, so that the sample is introduced into the chromatographic system with no prior vaporization. The disadvantage that this technique provides is the deterioration of the initial segment of the column due to the presence of non-volatile organic compounds or inorganic salts in aqueous samples. Another drawback is that the sensitivity of the technique is limited to the volume of the sample that can be introduced onto the analytical column. Over the years, this technique has become quite common for the analysis of volatile halocarbons.

1.4.2 Headspace Technique (HT)

Headspace techniques (HT) have been widely used over the last decade for the determination of THMs and other volatiles in water samples. However it is important to note that this technique itself is split into two techniques known as static headspace and headspace-solid dynamic headspace. These two techniques will be individually assessed to determine how they can be utilized in the best way possible for our needs.

1.4.3 Static Headspace Technique (SHT)

The static headspace technique (SHT) is the simplest and fastest headspace technique and permits a high degree of automation. In the static headspace mode, an aliquot of the gas phase from the vial, in equilibrium with the sample, is introduced into the carrier gas stream, which then carries it to the column. The main disadvantage with this technique is its low sensitivity. In most cases the concentration of the analyte in the headspace is lower than the limit of detection of the technique itself (Pavon et al., 2008). The sensitivity can be increased by increasing the sample volume onto the analytical column. However the drawback of this is band broading effect and a loss of resolution. Therefore, it is worth noting that the resulting sensitivity depends, apart from on detector sensitivity, on the capacity of the column for a gas sample. The sensitivity levels obtained with this pre-concentrated technique tend to be lower than those obtained with two-step headspace techniques (Dynamic Headspace), which include a prior analyte pre-concentration step.

1.4.4 Dynamic Headspace (DH)

The techniques discussed so far have a similar disadvantage in common, which is known as the sensitivity to the technique. New techniques were developed to address this situation. This technique is known as dynamic headspace purge and trap-gas chromatography, developed by Swinnerton and Linnebom in 1962. This technique has become very popular, valuable and a widely acceptable method for the determination of VOCs in water (Pavon et al., 2008). In the extraction gas, the purge volatiles are diluted before being focused in the trap. The sample is then introduced into the analytical column. This can normally be performed in a

cold trap. This technique is exceptional with regard to sensitivity. However, the one major disadvantage with regard to methodology is the excessive water vapor that is purged with the volatiles by a stream of inert gas (Pavon et al., 2008). This would give rise to significant peak distortion especially in the early part of the chromatogram. It is worth nothing that all these headspace techniques require the use of a gas chromatography head space instrument. It should be noted that for the purpose of this project no headspace techniques will be used, as there is no gas chromatography headspace instruments available at site.

1.4.5 Membrane based sampling technique

The membrane based techniques are techniques that have been develop in the laboratory by analyst. Several membrane based techniques have been developed for the analysis of VOCs in water. The advantage that the membrane based method offers is that it allows the THMs concentration to be monitored on line (Pavon et al., 2008). Additionally, in these systems no solvents are used because introduction of the analysis into the system is done directly through the membrane by means of a process called pre-vaporation. Due to limited available research, this technique will not be suitable for the testing purposes.

1.4.6 Liquid-Liquid Extraction (LLE)

Liquid-Liquid Extraction (LLE) is the most commonly used sample preparation techniques in water analysis. In contrast with classical DAI techniques, which use large amounts of solvent in order to deplete the sample out of the analytes, the process is normally completed with a much lower solvent volume. The sample volume varies between 5 and 100 mL. A study (Nikolaou et al., 2008) performed in which pre-concentration techniques was used for the determination of THMs in water. The study was a modification of the EPA method 551.1, which includes liquid-liquid extraction with methyl tertiary butyl ether (MTBE), after the addition of anhydrous sulphate. The addition of the sodium sulphate was to increase the ionic strength of the solution, enhancing the extraction of the compounds by the salting-out effect. The studies found that the LLE-GC-ECD method was the most sensitive one for the determination of THMs in water samples. A lot of alternative LLE methods have been recently develop over the years but the LLE-GC-ECD method is still considered the best method for the determination of THMs.

Advantages of this method include low cost. The most popular of this alternative method up until today, is still the analysis of trihalomethanes and relative pentane- extractable organic halides.

For this study, due to instrument availability a decision had to be made between DAI and LLE. It was decided that the LLE is a safer option and meets instrument availability in the laboratory.

1.5 The Standard method of analysis for THMs was US EPA method 551.1

The US EPA method was developed in 1990. Due to the significant development in technology over the past decade a lot of alterations have been made to the method, to keep up to date with more advanced state of the art instrumentation equipment e.g. better column conditions, more sensitive detectors and different carrier gasses. Reckhow et al., (2012) optimized the US EPA method in 2012 at University of Massachusetts Environmental Engineering Research Laboratory. This method is currently the latest method that has been optimized for THMs. Table 1.4 shows the changes that have been implemented to suite a more state of the art instrumentation and more suitable chemicals for extraction processes.

Table 1.4 Instrument parameters for trihalomethanes analysis

Methods	551.1 US EPA Method	Study by Reckhow et al.,(2012)
Parameters	Hewlett-Packard 6890 GC-MS Electron Capture Detector HP 7963 auto-sampler series injector	Hewlett-Packard 6890 GC-Electron Capture Detector HP 7673 auto-sampler series injector
Analytical Column	DB-1	DB-5
Length (m)	30	30
Internal Diameter (mm)	0.2	0.25
Film Thickness (µm)	1.0	1.0
Injection volume (µL)	2	2
Injection Type		Splitless
Split Flow		none
Carrier Gas	Helium	Zero-grade Nitrogen
Carrier Flow	Sufficient for 25 cm/sec linear	1.5 mL/min
Make-up Flow (mL/min)		30
Injector Temp (°C)	200	175
Detector Temp (°C)	290	275
Oven Program	Hold at 35 °C for 22 min Ramp to 145 °C at 10°C/min (11 min) Hold at 145 °C for 2 min Ramp to 225 °C at 20°C/min (4 min) Hold at 225 °C for 15 min Ramp to 260 °C at 10 °C/min (3.5 min) Hold at 260 °C for 30 min	Hold at 27 °C for 10 min Ramp to 41 °C at 3°C/min (4.67 min) Hold at 41 °C for 6 min Ramp to 81°C at 5 °C/min (8 min) No hold Ramp to 180 °C at 25 °C/min (3.96 min) Hold at 180 °C for 6 min

Table 1.4 shows the optimized parameters by Reckhow et al., (2012) compared to standard 551.1 US EPA Method. The parameters that were optimized by Reckhow et al., (2012) were the analytical column, carrier gas, injection temperature, detector temperature and oven program.

CHAPTER 2

METHODOLOGY

2.1 Introduction

For the present study, the method of Reckhow et al., (2012) will be optimized and validated in the study. The method was developed on a 6890 N gas chromatography electron capture detector instrument HP 7683 auto-sampler series injector. Due to significant changes regarding instrumentation, some of the parameters were modified to suite instrument needs and availabilities. The method developed by Reckhow et al., (2012) was developed on a Hewlett Packard 6890 GC-ECD. The method for the present study was developed on a 6890 N GC-ECD. The instruments make use of different PTV inlets, septums and liners. Below is a table of comparison that shows the modifications that were done regarding instrument parameters.

Table 2.1 Optimization of GC-ECD method for determination of THMs

Parameters	Reckhow et al., (2012)	Present Study
Analytical Column	DB-5	HP-5MS UI
Length (m)	30	30
Internal Diameter (mm)	0.25	0.25
Film Thickness (µm)	1.0	0.25
njection Volume (µL)	2	2
Injection Type	Splitless	Splitless
Split Flow	none	none
Carrier Gas	Zero-Grade Nitrogen	Helium
Carrier Flow (mL/min)	1.5	1.5
Make up Flow (mL/min)	30 mL/min	30 mL/min
Injection Temperature (ºC)	175 ºC	175 ºC
Detector Temperature (ºC)	275 ºC	275 ºC
Oven Program	Hold at 27 ºC for 10 min Ramp to 41 ºC at 10 ºC/min (4.67 min) Hold at 41 ºC for 6 min Ramp to 81 ºC at 5 ºC/min (8 min) No Hold Ramp to 180 ºC at 25 ºC/min (3.96 min) Hold at 180 ºC for 6 min	Hold at 40 ºC for 10 min Ramp to 45 ºC at 3 ºC/min (4.67 min) Hold at 45 ºC for 6 min Ramp to 81 ºC at 5 ºC/min (8 min) No Hold Ramp to 90 ºC at 5.5 ºC/min No Hold

Table 2.1 shows the optimized parameters of the present study compared to Reckhow et al., (2012). Some of the parameters that were optimized were the analytical column, carrier gas, film thickness and oven program.

2.2.1 Optimization Parameters

The parameters that were optimized for the study were the carrier gas, analytical column, film thickness and oven program. All these parameters were optimized to make the method more efficient towards, instrument suitability and capability.

2.2.2 Carrier Gas

Most laboratories instruments have moved over from nitrogen to helium as a carrier gas, as it offers significant improvements compared to nitrogen. Helium provides high column efficiency at moderate linear velocity flow rate, shorter retention time and less carrier gas being consumed during analysis. However, it does have the disadvantage of limited number of peaks, as peaks start to co-eluent. This can be minimized by using a longer column.

2.2.3 Analytical Column

The DB-5 column (figure 2.1) shows severe baseline noise, which led to an increase in signal to noise ratio on the chromatogram, which led to significant increase in limit of detection and limit of quantification values.

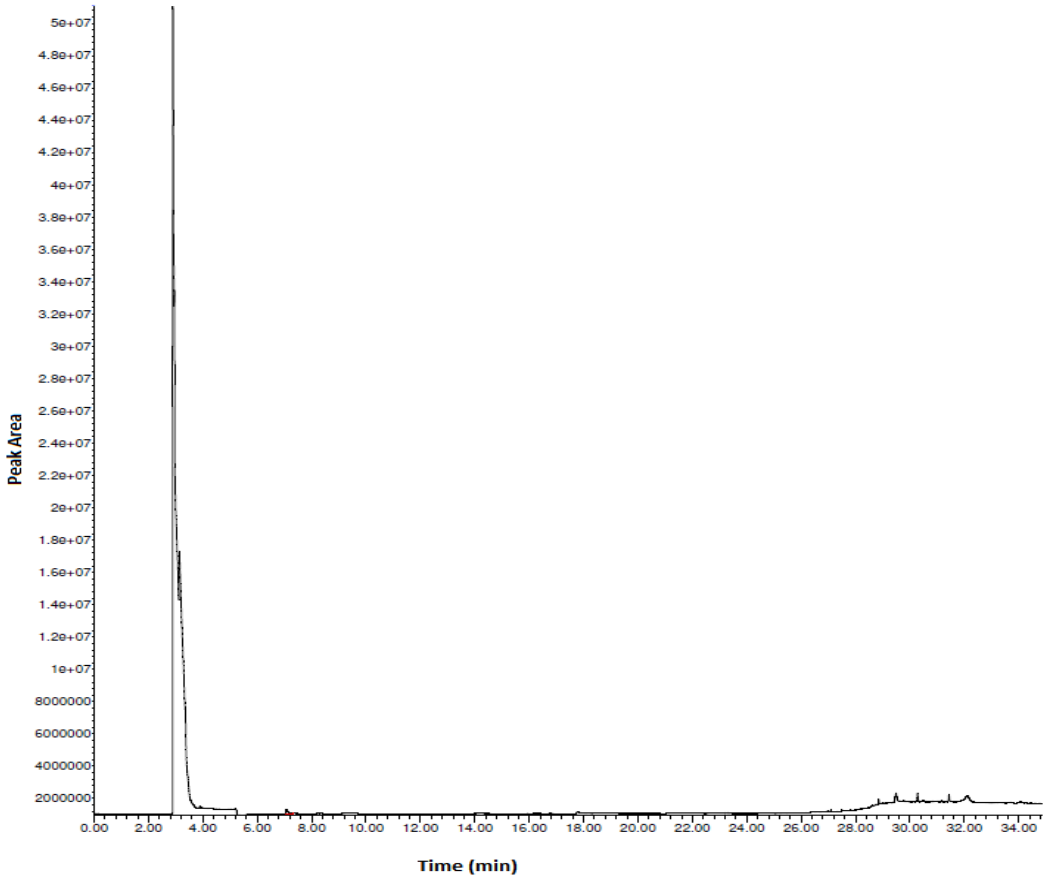


Figure 2.1 Chromatogram of baseline noise from using DB-5 coloumn which was inserted into GC-ECD HP 7683 auto-sampler series injector.

The chromatogram in figure 2.1 shows severe baseline noise using the DB-5 column that was experienced from 28-36 min during the chromatographic run.

The HP-5 MS UI column was selected, due to the fact that the ultra-inlet column is much more selective than DB-5 column. Another advantage is that the HP-5 MS UI column is much more robust when running chromatography water through it than the DB-5 column.

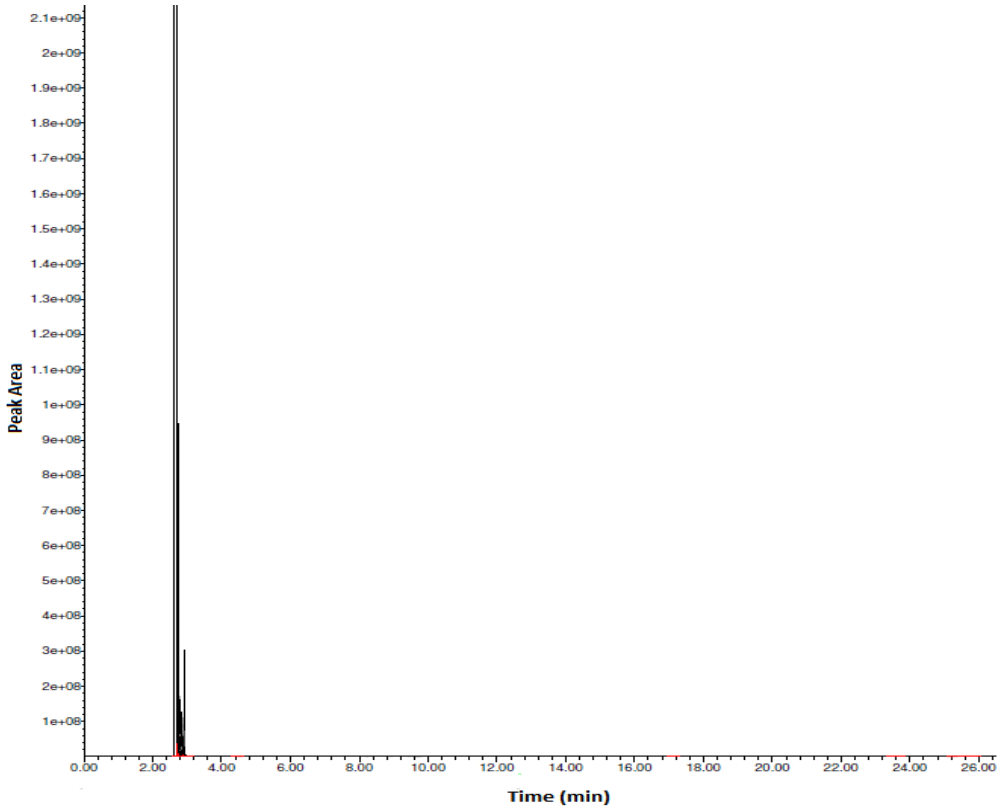


Figure 2.2 Chromatography of baseline noise in GC-ECD HP 7683 auto sampler injection series injection.

Figure 2.2 is a much improved chromatograph baseline noise making use of HP-5 MS UI, it shows no baseline noise, which led to much better chromatogram.

2.2.4 Oven Program

The method developed by Reckhow et al., (2012), was for determination of trihalomethanes and related pentane-extractable organic halides. In the present study, analytes of interest were limited to THMs only, not the organic halides. Trihalomethanes elute in the order of chloroform, bromodichloromethane, chlorodibromomethane and bromoform. All peaks elute within 26.50 min. This instrument run time was reduced from 37.50 min to 26.50 min. This reduced retention time of analysis uses less gas. This decision was made because all THMs of interest has already eluted after 12.00 min. An additional 14.50 min was added to the time to discard all purities that remained trapped behind in the analytical column after each analytical run. The starting temperature was increased from 27 ºC to 40 ºC. In summer seasons, it becomes difficult to cool

the oven temperature to 27 ºC compared to winter seasons. These changes are cost effective and reduce amount of consumables used.

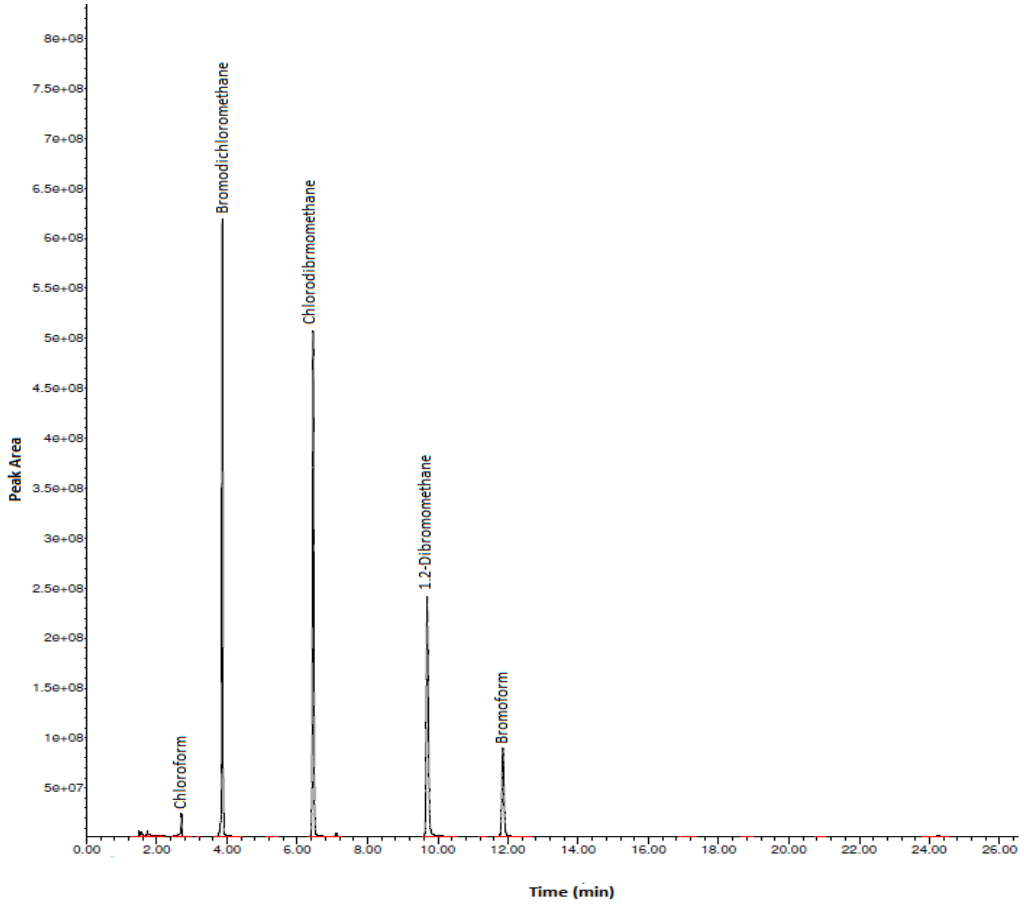


Figure 2.3 Chromatogram of 50 µg/L trihalomethanes calibration standard with eluent retention times. Chloroform (2.703 min), bromodichloromethane (3.875 min), chlorodibromomethane (6.452 min), 1,2 - dibromopropane (9.903 min) and bromoform (11.852 min).

Figure 2.3 shows all four trihalomethanes and internal standard peaks identified within 26.50 min.

2.2.5 Film Thickness

The benefits of decreasing film thickness are sharper peaks (which may increase resolution) and reduced column bleed. Analytical columns with smaller film thickness have higher maximum operating column temperature. Decreasing film thickness also allows analytes to elute with shorter retention time and at lower temperatures, depending on the application. A decrease in film thickness led to

improved chromatogram for analytes which are semi-volatile at trace levels. The drawbacks are increased analyte capacity. With such a small film thickness it's become problematic with high volume of sample injected into analytical column. This can be avoided by lower sample injection volume.

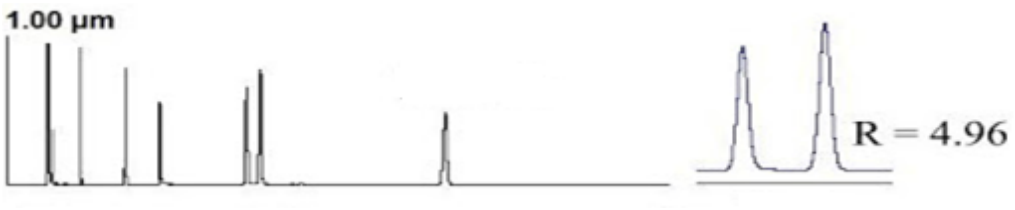


Figure 2.4 Chromatogram of 1.00 **µ**m film thickness shows an increase in retention run time

Figure 2.4 shows increase in retention time with better separated picks, which led to better analyte resolution. The peaks are thoroughly separated from one another which are expected from increased film thickness at longer retention time.

Figure 2.5 Chromatogram of 0.25 **µ**m film thickness shows a decrease in retention run time

Figure 2.5 shows a decrease in retention time which is expected at smaller film thickness, however peaks are much closer to one another, which led to cost saving and reduced amount of consumables used.

2.3 Validation

Validation of an analytical method is a process by which a laboratory establishes that all performance characteristics of the method meet all requirements for the intended analytical application. The limitations of the method and any other factors which may influence its characteristics are also established. The criteria which must be met to confirm the performance characteristics of a method are dependent on the origin and the amount of validation data backing up a particular method. Additionally, the scope in which it will be applied as well as the needs of

the customer must be taken into consideration. Certain methods may require only verification, while others demand full validation.

2.3.1 South African National Standards 241 Validation Master Plan

The purpose is to validate a method that will be used to quantify trihalomethanes in natural water sources. These THMs of interest are chloroform, bromodichloromethane, chlorodibromomethane and bromoform. Trihalomethanes need to be quantified to conform to the latest SANS 241:2015 regulations for water. The new update of the SANS 241:2015 regulation states that when chlorination is used to purify water, the relevant water treatment plants should implement immediate action to ensure that it does not exceed the SANS 241:2015 limits of trihalomethanes. If these trihalomethanes exceed the limits of 300 µg/L chloroform, 100 µg/L bromoform, chlorodibromomethane and 60 µg/L and bromodichloromethane, then water treatment needs to take place immediately. The following sample matrices will be used to gather method validation data: river water, borehole water, municipal water, sea water, chlorinated water, tap water, R.O water, Johnson-Johnson medical water and underground water.

The method must be able to quantify the trihalomethanes of interest under the specific conditions at any given time. This will be achieved by following the guideline of criteria set by the South African National Accreditation System for validation of methods used by chemical laboratories in the coal, oil petroleum, metals and minerals, food, water and related industries. These criteria are: limit of detection, limit of quantification, matrix effects, selectivity, linearity range, accuracy, precision (repeatability), recovery and reproducibility. A concentration linearity working range of 50 µg/L, 80µg/L, 100 µg/L, 150µg/L, 200 µg/L, 250 µg/L and 350 µg/L will be used. If there is a sample that has a peak area that is higher than that of the upper concentration 350 µg/L, then it must be reported that it falls outside of the SANS 241:2015 regulations requirements. There are no expected interfering analytes that might elute at the same time with the analytes of interest. If, during the validation process, it is found that there are possible interferences, alternate step(s) will be made to ensure that the interferences do not alter the quality of results. There are no specific conditions that need to be considered during the method validation. The overall experiment will be conducted at basic

laboratory conditions (ambient temperatures and a well-ventilated environment), and only the specific method conditions should be considered, from sample preservation, sample extraction, to instrument parameters. All research work was conducted on 6890 N gas chromatography and electron capture detector HP 7683 auto-sampler series injector.

Table 2.2 Performance characteristics for validation

Characteristics	**Procedures to be followed**	**Acceptance criteria**
Limit of Detection	Analysis of the lowest standard from the working range, at least 7 determinations.	LOD = 3 x SD SD = Standard Deviation.
Limit of Quantification	Analysis of the lowest standard from the working range, at least 7 determinations.	LOQ = 10 x SD SD = Standard Deviation.
Matrix effects	Analysis of sample matrix blanks, sample matrix spiked with standards, and standards. Once unspiked and once spiked to a representative level in each sample matrix.	A constant matrix effect.
Selectivity	Analysis of sample matrix blanks, sample matrix spiked with the lowest spiking standard representative of the analyte and possible interferences within the sample matrix.	Selective response to the analyte of interest in the presence of possible interferences, based on recovery of the analyte of interest.
Linearity Range	Analysis of a working range of standards. At least 7 at each of 7 concentrations over this working range.	Accepted based on performing linear regression on each of the original area data points, by using the calculated best fit line, y= mx + c. (least squares method) *c = y-intercept of best*

Characteristics	Procedures to be followed	Acceptance criteria
		line fit *m = the slope of best line fit.* The Correlation Coefficient should be $R^2 \geq 0.998$
Precision (repeatability)	Replicate analysis of sample matrix and calculate the RSD. At least 5 analyses of each sample matrix, unspiked and 5 analyses of each sample matrix spiked once to a representative level.	For precision to be accepted, the RSD must be ≤ 10%
Accuracy (can be expressed as the percent recovery of analyte)	Analysis of sample matrix blanks and sample matrix spiked with standards. At least 5 replicate analyses of the sample matrix.	A comparison of each mixture's theoretical value versus the obtained result for samples of known concentration should have less than the acceptable % error for the particular method and application. $\text{Accuracy} = \frac{x}{\mu} \times 100$ where **x** = obtained value and **μ**= theoretical value Acceptance range is 70 – 120 %
Recovery	Analysis of sample matrix blanks and sample matrix spiked with standards. At least 5 replicate analyses of the sample matrix.	Recovery of standards. $\text{Recovery} = \frac{x}{\mu} \times 100$ where **x** = obtained value and **μ**= theoretical value Acceptance range is 70 – 120 %
Reproducibility/ Ruggedness	Replicate analysis of sample matrix	For reproducibility to be

Characteristics	Procedures to be followed	Acceptance criteria
(precision between different runs)	over a longer set time period and calculate the RSD. At least 5 analyses of each sample matrix, unspiked and 5 analyses of each sample matrix spiked once to a representative level.	accepted, the RSD must be ≤ 10%

Listed in Table 2.2 are the requirement that needs to be met for method validation accreditation through SANAS for SANS 241:2015 drinking water.

2.3.2 Extended Validation Criteria

For method validation the characteristics tabled in Table 2.2 needs to be achieved for accreditation. However, method validation does not have to stop there. For this study a significant need to cover some characteristics that needed to be addressed to improve the reliability of the method as a newly established method that is not documented as Official Method of Analysis of AOAC International. The characteristics tabulated below are the additional validation criteria that needed to be included.

Table 2.3 Extended performance characteristics for validation

Characteristics	Procedure to be followed	Acceptable criteria
Measure of Uncertainty	A laboratory quality control are run 6 times in duplicate for the uncertainty to be calculated	The uncertainty is the experimenter's best estimate of how far an experimental quantity might be from the true value. the uncertainty must be ≤ 10%
FAPAS	Provided an independent assessment of laboratory performance and compares the results to that of laboratories worldwide	Must pass the FAPAS proficiency test
Inter-Laboratory	Comparing laboratory results against SANAS accredited	For inter-laboratory to be accepted, the RSD

	laboratories results by outsourcing samples	must be ≤ 10%
Robustness	Making changes on instrument parameters and comparing results to original method	Robustness of standards. $\text{Recovery} = \frac{x}{\mu} \times 100$ where **x** = obtained value and **μ**= theoretical value Acceptance range is 70 – 120 %

2.4 Preparation of Standards and Samples

All method validation and sampling protocol will be done at Microchem Laboratory Service. Microchem Laboratory Service is an independent, SANAS accredited testing laboratory that provides a comprehensive range of chemical and microbiological analyses and related services to the food and non-food industries

2.4.1 Reagent water

Standards and blanks were prepared from chromatography water, which was purchased from Merck (1.15333.2500) and stored at 20 º C - 25 º C temperatures.

2.4.2 Standard Solutions

The working solution was prepared from a standard stock solution of 2000 mg/mL purchased from Sigma-Aldrich (CRM48140), 100 µL of the stock solution was pipetted into 10 mL volumetric flask and filled up to the mark with analytical grade acetone purchased from Sigma (34480-2.5L). The working solution is stable for one month. A series of calibration standards was prepared from the working solution by pipetting 0, 50, 80, 100, 150, 200, 250, 350 µL into 40 mL amber vials. The calibration standards must be prepared fresh with each batch of samples.

2.4.3 Real life water samples

Before analysis 20 mL of analytical chromatography water was added to calibration standards and 20 ml of real life water samples to 40 mL amber vials. 1

g of pre-mixed buffer of 97.5% KH_2PO_4 purchased from Merck (SAAR5043600EM) and 2.5% Na_2HPO_4 purchased from Merck (SAAR5822880EM) was added to the vials. A mass of NH_4Cl crystals (40 mg) purchased from Merck (SAAR1122720EM) crystals was added to adjust the pH to 4.5-5.5. The vials were then centrifuge at 2450 rpm for 2 min. To the vial 4 mL of pre-mixed pentane purchased from Merck (1.07177.100) and 1,2 dibromopropane purchased from Sigma (140961-100 G) internal standard was added. A 15g of Na_2SO_4 purchased from Merck (SAAR5825260EM) was added to the vials and centrifuge on shaker at 2450 rpm for 8 min. From here onwards work was done in a fume-hood. The organic top layer was transferred using glass pasteur pipette into 20 mL amber vials. It was then stored in a freezer for at least 3 hours to remove the water. The organic phase of sample was transferred into auto sampler vial and capped before GC analysis.

2.4.5 Protocol for sampling transportation

The driver transporting the samples must ensure that a cooler box with ice bricks is was available in the vehicle and that the fridge in the back of the vehicle is functioning properly before commencing any deliveries and or collections. The driver must ensure that the temperature of samples is recorded on the cooler box temperature verification sheet before accepting samples.

2.4.6 Protocol of Sampling Handling

This procedure describes the process to be followed for the reception samples.
The laboratory does not take part in obtaining samples for test purposes; rather, the laboratory performs testing on samples on an “as received” basis. It is therefore the responsibility of the supplier that a representative sample portion must be supplied to the laboratory. Sampling protocol dictates that sampling must come from at least three different samples originating from the same production run, i.e. take one sample at the beginning of the production run, one at the middle and one before the end of the run. These products are then combined to form one representative sample. The laboratory also requires a sample portion of at least 500 mL for a full SANS 241 analysis.

Samples are submitted to the laboratory along with an analysis request form which identifies the required tests to be performed. Once a sample has arrived in

the laboratory it is logged into the Laboratory Information Management System (LIMS) by the administrative personnel member and is assigned a unique laboratory sample ID (e.g. AA43257). This sample ID number is printed on a set of sample labels, of which one is affixed to the sample packaging. The labeled sample (known as the laboratory sample), along with the second label and a copy of the request form is sent to the sampling area in the laboratory.

Particle size reduction is important to ensure that any chemicals or enzymes used during an analysis are in contact with the maximum surface area of the sample. Increasing the surface area of a substance generally increases the rate of a chemical reaction. It was also important to ensure that samples are properly homogenized to ensure a uniform distribution of sample components and nutrients throughout the sample (this is particularly important when working with fortified products or recipe dishes).

The first step performed by the analyst in the laboratory is to verify that the sample has been correctly logged for all of the required tests. This is done by opening the sample data in LIMS and crosschecking with the analysis request form. The request form will be signed by the personnel member checking the request form. The tests required for the sample must be carefully recorded as different tests require different handling of the sample. Sometimes as much as 20 sample portions are provided-spanning a certain production period and are used to form a composite sample.

The sample jars are labelled by attaching the sample label to the jar. A sample ID number (e.g. AA43256) must be clearly written on the lid of the sample with a permanent marker. After the sampling process, the analysis request form is filled in the appropriate record file. The sample jars get stored in the laboratory fridge. The appropriate sample must be recorded in the sample login record book to indicate that the sample has been sampled and is ready for analysis.

CHAPTER 3

Method Validation Results

3.1 Introduction

Validation of an analytical method is the process by which the laboratory establishes that the performance characteristics of the method meet the requirement for the intended analytical applications. The limitations of the method, as well as any factors which may influence its characteristics are also established.

Validation was done with optimized GC-ECD method (paragraph 2.2 pg 19-27). All information that was obtained from the GC-ECD instrument was stored on Laboratory Information Management Software (LIMS). The following water matrices were validated: river water, underground water, tap water (Woodstock), municipal water, chlorinated water, sea water, Johnson-Johnson medical water, unfiltered water and borehole water. All samples contain a unique barcode that makes it possible to retrieve any relevant information for any sample. The method will be validated in reference with Microchem Laboratory Services Standard Operating Procedure (SOP-C-002/07), which was established in conjunction with reference from the following documents:

1. ISO 17025:2005-Section 5.4.5
2. SOP-QM-016-method Validation
3. SANAS TG 41-Guidelines for the Verification of Methods in Forensic Chemistry
4. SANCO/10684/2009-Method Validation and Quality Control Procedures for Pesticides Residues Analysis in Food and Feed

Microchem Laboratory Service is an independent, SANAS accredited testing laboratory that provides a comprehensive range of chemical and microbiological analyses and related services to the food and non-food industries.

3.2 Method validation results

Method validation was established in the following order, limit of detection (LOD), limit of quantification (LOQ), instrument linearity, instrument precision, matrix effect based on recovery, matrix effect based on interferences, precision based

on reproducibility, accuracy based on recovery, selectivity based on recovery, selectivity based on interferences, inter-laboratory comparison, precision based on repeatability, robustness, measure of uncertainty (MOU) and proficiency testing.

3.2.1 Limit of detection and limit of quantification determined with use of blank method

The limit of detection is the lowest concentration of an analyte in a sample that can be detected. The limit of quantification is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under stated test conditions. For the determination of the limit of detection and limit of quantification the blank method was used. The blank method is a technique whereby a reagent blank is used as a test sample that undergoes the same treatment as the test sample without the addition of the test sample. To ensure that the chromatography water obtained from Merck is not contaminated, it was analysed (n=10) as a blank. A reagent blank, 0 µg/L trihalomethanes standard, was analysed after it went through the entire sample preparation steps. The results for chloroform are tabulated below in table 3.1. All the other LOD and LOQ values for THMs can be found in appendix: A pg: 105-106.

Table 3.1 Limit of Detection and Limit of Quantification for chloroform

	Determination No	Chromatography water as blank (µg/L)	Reagent blank (µg/L)
	1	0	0.427
	2	0	0.355
	3	0	0.496
	4	0	0.347
	5	0	0.157
	6	0	0.143
	7	0	0.134
	8	0	0.257
	9	0	0.228
	10	0	0.030
Mean			0.257
STD. DEV.			0.146
LOD			0.440
LOQ			1.469

Sample calculation for chloroform as reagent blank

LOD= Stdev × 3
LOQ= Stdev × 10

LOD chloroform= 0.1469 × 3 = 0.4407
LOQ chloroform= 0.1469 ×10 =14690

Table 3.2 Summary of Limit of Detection and Limit of Quantification for THMs

Trihalomethanes	Limit of Detection µg/L	Limit of Quantification µg/L
Chloroform	0.4407	1.4690
Bromodichloromethane	0.2927	0.9759
Chlorodibromomethane	0.1999	0.6664
Bromoform	0.2056	0.6854

From the values reported in table 3.2, it can be concluded that the analytes of interest can be quantified and detected at this stipulated values, which falls in the instrument quantification range.

Instrument Linearity

Instrument linearity is how well a plot of the analytical response versus the quantity of the interest follows a straight line. The calibration curves was obtained by preparing 7 different concentrations, that make up the working range, and these were analysed (n=7). The 7 concentrations ranged from 50 - 350 µg/L. This range was selected to cover the entire SANS 241:2015 range of limits that each trihalomethanes should not exceed, e.g. chloroform should not exceed 300 µg/L, bromodichloromethane 60 µg/L, chlorodibromomethane and bromoform 100 µg/L. The peak area of each analyte from 50 - 350 µg/L as well as the peak area of the internal standard was obtained from the instrument. The area ratios of each analyte were then calculated by dividing the peak area of the analytes by the peak area of the internal standard at different theoretical concentrations. This approach was taken to minimize errors and data manipulation. Errors can be minimized by comparing the internal standard peak area of each analyte to one another.

Data manipulation is much more difficult to implement when the peak area of analyte is divided by peak area of internal standard at different concentrations. Data manipulations can easily be implemented when, area of each analyte is manually integrated to make it a desired value; however, this is much more difficult to achieve when making use of the internal standard peak area. The internal standard peak area is directly proportional to that of the analyte. If the internal standard peak area is reduced, the peak area of the analyte increases and vice versa. A linearity graph was plotted by using the area ratio of each analyte against the concentration. All the other linearity values for THMs can be found in appendix: A pg. 107.

Sample calculation of area ratio for chloroform

$$\frac{\text{Peak Area Analyte}}{\text{Peak Area Internal Standard}} = \text{Area Ratio}$$

$$\text{Chloroform} = \frac{1052544357.86}{17002152714.29} = 0.06$$

Area ratio vs theoretical concentration = R^2

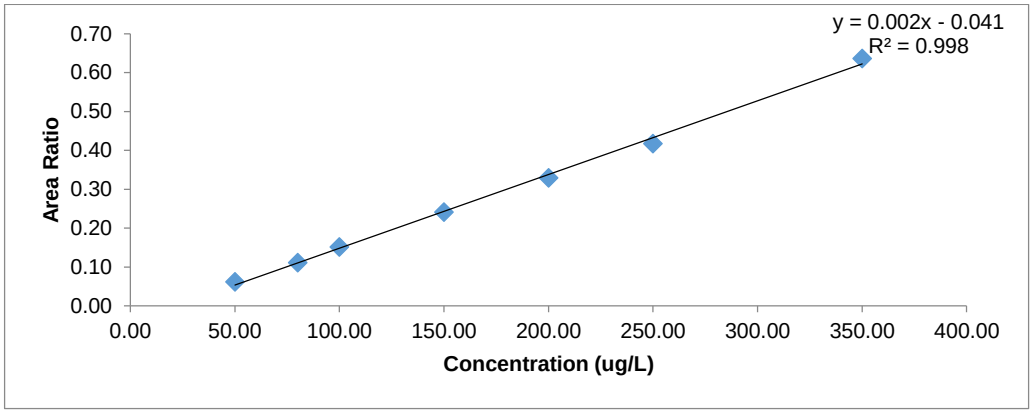


Figure 3.1 Calibration curve of chloroform (50-350 µg/L) with GC-ECD auto sampler injector (n-7)

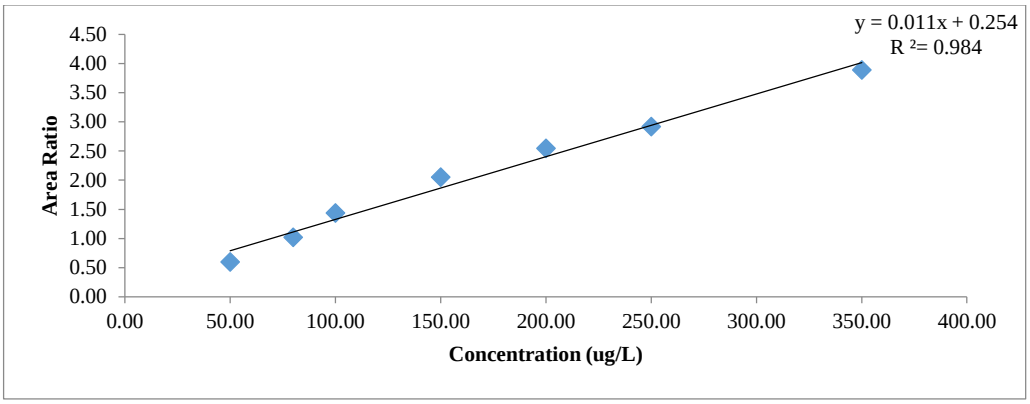


Figure 3.2 Calibration curve of BDCM (50-350 µg/L) with GC-ECD auto sampler injector (n-7)

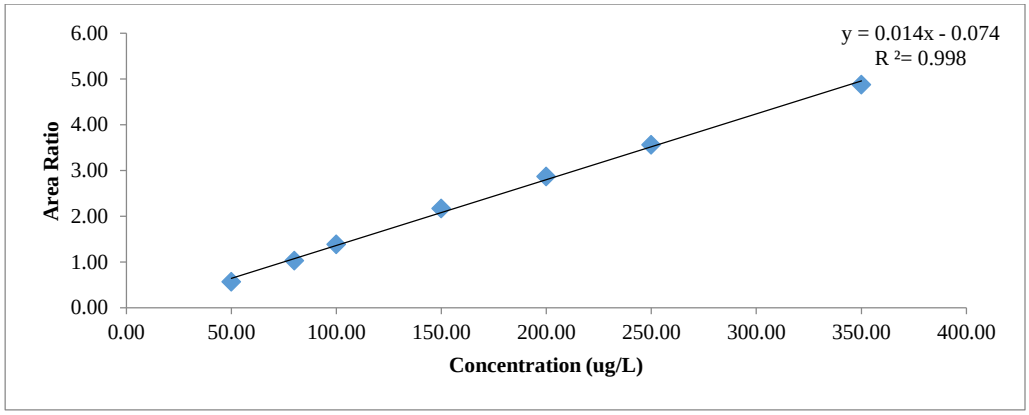


Figure 3.3 Calibration curve of CDBM (50-350 µg/L) with GC-ECD auto sampler injector (n-7)

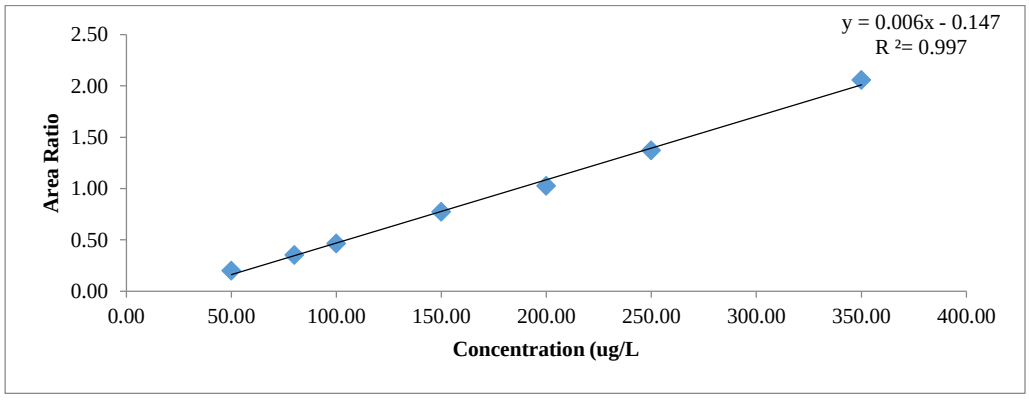


Figure 3.4 Calibration curve of bromoform (50-350 µg/L) with GC-ECD auto sampler injector (n-7)

Table 3.3 Linear regression and sensitivity for THMs

Trihalomethanes	Linearity	Instrument Sensitivity	Background Noise
Chloroform	0.998	0.002	0.041
Bromodichloromethane	0.984	0.011	0.254
Chlorodibromomethane	0.998	0.014	0.074
Bromoform	0.997	0.006	0.147

Calibration linear graphs (figure 3.1-3.4) were plotted and the correlation values (R^2) for each of the trihalomethanes was determination. Instrument sensitivity which is a representation of instrument noise was obtained from the linear regression. The instrument background noise was obtained, which is an indication of electrical or thermal noise generated from the instrument. In order for the R^2 value to be accepted, the correlation must be ≥ 0.98. The correlation values ranged from 0.984-0.998 for all the THMs. Therefore the linearity obtained indicates that the instrument response due to the change in analyte concentration is acceptable.

3.2.3 Instrument Precision

Instrument precision is the accuracy of the measured tolerance or transmission of the instrument and defines the limit of the errors made when the instrument is used in normal operating conditions. For instrument precision chromatographic water obtained from Merck was used as a blank and, to ensure that the chromatography water is not contaminated, it was analysed (n=10). A 50 µg/L trihalomethanes calibration standard, was analysed (n=10) after it went through the entire sample preparation steps. In order for the instrument precision to be accepted, the RSD value must be ≤ 10%. The results are tabulated below in table 3.4. All the other instrument precision values for THMs can be found in appendix: A pg. 108-109

Sample calculation for 50 µg/L chlorodibromomethane

$$\frac{\text{Standard Deviation}}{\text{Mean value}} \times 100\% = \text{Relative Standard deviation}$$

$$\text{Chlorodibromomethane} = \frac{1.51}{50.82} \times 100\% = 2.98\ \%$$

Table 3.4 Precision data for chlorodibromomethane at 50 µg/L

Standard	Conc (µg/L)	Inj. No	Sample Blank Amount (µg/L)	Spiked Sample Amount (µg/L)	Actual Sample Amount (µg/L)	Mean	Stdev	% RSD
CDBM	50.00	1	0.000	48.650	48.650	50.82	1.51	2.98
		2	0.000	50.993	50.993			
		3	0.000	52.301	52.301			
		4	0.000	52.105	52.105			
		5	0.000	51.709	51.709			
		6	0.000	49.792	49.792			
		7	0.000	51.025	51.025			
		8	0.000	53.037	53.037			
		9	0.000	49.797	49.797			
		10	0.000	48.769	48.769			

Table 3.5 Summary for precision data of THMs at 50 µg/L

Trihalomethanes	Concentration µg/L	Mean	Stdev	% RSD
Chloroform	50.00	41.34	0.78	1.89
Bromodichloromethane	50.00	57.75	1.57	2.72
Chlorodibromomethane	50.00	50.82	1.51	2.98
Bromoform	50.00	43.99	1.23	2.80

The RSD for instrument precision ranged from 1.89 % - 2.98 % for chloroform to chlorodibromomethane. In order for the instrument precision to be accepted, the RSD value must be ≤ 10%. Therefore from these results obtained, it can be concluded from Table 3.5 that the instrument precision for this method is acceptable.

3.2.4 Matrix effect on recovery

Matrix effect is the effect on an analytical method caused by all other components of the sample except the specific compound to be quantified. For recovery determination, eight different samples were analysed for matrix effect and recovery using the standard additional method. Each of the eight samples were analysed (n=3) times as unspiked samples, as well as a spiked sample at 3

different concentrations. These concentrations were 50 µg/L, 150 µg/L and 350 µg/L. These concentrations were selected to cover a lower range, middle range and upper range of the calibration standards. For recovery to be accepted, it needs to fall in a percentage range from 70-120 %. The results are tabulated below in table 3.6-3.8 for sea water. All the other listed matrixes based on recovery values for THMs can be found in appendix: A pg. 110-121.

Sample calculation for 50 µg/L sea water

$$\frac{\text{Sample Spiked with 50 µg/L}}{\text{Unspiked sample + theoretical concentration}} \times 100\% = \text{Recovery}$$

$$\text{Chloroform for sea water} = \frac{44.640}{2.60+52.640} \times 100\% = 84.80\ \%$$

Table 3.6 Recovery due to matrices effect for sea water at 50 µg/L

Sample	Trihalomethanes Standard	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Theoretical Conc (µg/L)	% Recovery
Sea water	Chloroform	2.640	44.640	52.640	84.80
Sea water	BDCM	2.941	62.972	52.941	118.95
Sea water	CDBM	15.601	76.390	65.601	116.45
Sea water	Bromoform	259.016	309.016	312.057	100.98

Table 3.7 Recovery due to matrices effect for sea water at 150 µg/L

Sample	Trihalomethanes Standard	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Theoretical Conc (µg/L)	% Recovery
Sea water	Chloroform	2.640	148.513	152.640	97.30
Sea water	BDCM	2.941	178.823	152.941	116.92
Sea water	CDBM	17.006	199.181	167.006	119.27
Sea water	Bromoform	259.016	424.016	409.016	103.67

Table 3.8 Recovery due to matrices effect for sea water at 350 µg/L

Sample	Trihalomethanes Standard	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Theoretical Conc (µg/L)	% Recovery
Sea water	Chloroform	2.640	349.000	352.640	98.97
Sea water	BDCM	2.941	288.000	352.941	81.60
Sea water	CDBM	15.601	316.005	365.601	86.43
Sea water	Bromoform	259.016	663.656	609.016	108.97

The recovery for 50 µg/L in sea water ranges from 84.80 % - 118.95 % for chloroform to bromodichloromethane, while recovery for 150 µg/L ranges from 97.30 % - 119.27 % for chloroform to chlorodibromomethane. The recovery for 350 µg/L ranges from 81.60 % - 108.97 % for bromodichloromethane to bromoform. The recovery for 50 µg/L in underground water ranges from 81.98 % - 98.52 % for chloroform to bromodichloromethane, while recovery for 150 µg/L ranges from 91.41 % - 115.81 % for chloroform to bromodichloromethane. The recovery for 350 µg/L ranges from 83.49 % - 118.24 % for bromodichloromethane to bromoform. The recovery for 50 µg/L in tap water ranges from 8.07 % - 111.57 % for bromoform to bromodichloromethane, while recovery for 150 µg/L ranges from 80.29 % - 110.26 % for bromoform to chlorodibromomethane. The recovery for 350 µg/L ranges from 80.06 – 108.01 % for chlorodibromomethane to chloroform. All other sample matrices for borehole water, municipal water, river water, Johnson-Johnson medical water, and unfiltered water all gave acceptable recoveries that were within 70 – 120 % recovery range for 50 µg/L, 150 µg/L and 350 µg/L as in appendix A pg. 110-121. This therefore concludes that there was an acceptable matrix effect within the sample matrices of interest.

3.2.5 Recovery and Matrix Effects based on Interferences

Matrix effect based on interferences is the effect on an analytical method caused by all other components of the sample except the specific compound to be quantified. In this case, the specific compound to be quantified is spiked with a direct interference substance. Three different interference reagents were used for

determining matrix effect and recovery based on interferences, using the standard addition method. Each of the three interference samples were analysed (n=5) as unspiked and spiked samples at different concentrations. This means that the unspiked sample only contains the interference analyte, while the spiked sample contains the interference analyte plus the known concentration standard it was spiked with.

The known concentrations were 50 µg/L, 150 µg/L and 350 µg/L trihalomethanes standards. The samples were spiked with 100 µL of 500 µg/L chloride, fluoride and SEPP-133 custom made heavy-metals which contains (As, Cd, Cu, Hg, Pb) interference standards. These concentrations were selected to cover the lower range, middle range and upper range of the calibration standards. For recovery to be accepted it needs to fall in a percentage range from 70-120 %. The results are tabulated below in table 3.9-3.11 for chloride standard. All the other matrixes based on interferences values for THMs can be found in appendix: A pg.122-130.

Sample calculation for 50 µg/L bromoform

$$\frac{\text{Sample Spiked with 50 µg/L}}{\text{Unspiked sample + theoretical concentration}} \times 100\% = \text{Recovery}$$

$$\text{Bromoform for Chloride standard} = \frac{40.249}{0.00+50.00} \times 100\% = 80.50\ \%$$

Table 3.9 Recovery matrices for chloride standard interferences at 50 µg/L

Sample	Trihalomethanes Standard	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Theoretical Conc (µg/L)	% Recovery
Chloride Standard	Chloroform	0.000	41.423	50.00	82.85
Chloride Standard	BDCM	0.000	54.739	50.00	109.48
Chloride Standard	CDBM	0.000	44.411	50.00	88.82
Chloride Standard	Bromoform	0.000	40.249	50.00	80.50

Table 3.10 Recovery matrices for chloride standard interferences at 150 µg/L

Sample	Trihalomethanes Standard	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Theoretical Conc (µg/L)	% Recovery
Chloride Standard	Chloroform	0.000	149.541	150.00	99.69
Chloride Standard	BDCM	0.000	178.541	150.00	118.45
Chloride Standard	CDBM	0.000	178.420	150.00	118.95
Chloride Standard	Bromoform	0.000	150.241	150.00	103.34

Table 3.11 Recovery matrices for chloride standard interferences at 350 µg/L

Sample	Trihalomethanes Standard	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Theoretical Conc (µg/L)	% Recovery
Chloride Standard	Chloroform	0.000	332.217	350.00	94.92
Chloride Standard	BDCM	0.000	284.428	350.00	80.64
Chloride Standard	CDBM	0.000	281.892	350.00	80.54
Chloride Standard	Bromoform	0.000	345.929	350.00	98.84

The recovery for 50 µg/L differs from 80.50 % - 109.48 % for bromoform to bromodichloromethane, while recovery for 150 µg/L differs from 99.69 % - 118.95 % for chloroform to chlorodibromomethane. The recovery for 350 µg/L differs from 80.54 % - 98.84 % for chlorodibromomethane to bromoform. All other sample matrices for fluoride standard and SEPP-133 custom (heavy metal) standard all gave acceptable recoveries that were within 70 – 120 % recovery range, for 50 µg/L, 150 µg/L and 350 µg/L as in appendix A pg.122-130. This therefore concludes that there was an acceptable matrix effect within the sample matrices of interest.

3.2.6 Precision based on Reproducibility

Reproducibility refers to the agreement between the results of experiment conducted by different analysts. It measures the ability to replicate the findings of

others. Precision based on reproducibility was determined by comparing the relative standard deviation of two analysts against one another. Two laboratory samples were used for this comparison, municipal water and chlorinated water. Each analyst analysed the samples with (n=5) times. The samples were run as unspiked samples as well as a spiked sample with a known concentration of 50 µg/L. For the repeatability to be accepted, the relative standard deviation must be less ≤ 10%. The results are tabulated below in table 3.12-3.15 for municipal water and chlorinated water. All the other precision based on reproducibility values for THMs can be found in appendix: A pg. 131-134.

Sample calculations for bromoform

Spiked sample of 50 µg/L - Unspiked sample = Actual concentration

$$\frac{\text{Standard Deviation}}{\text{Mean value}} \times 100\% = \text{Relative Standard deviation}$$

Bromoform for Midrand municipal water = 58.643-0.106 = 58.53

$$\frac{1.95}{58.19} \times 100\% = 3.35$$

Table 3.12 Reproducible data for analyst 1 at 50 µg/L

Sample	THMs	Conc (µg/L)	Inj No	Unspiked sample	Spiked Sample Amount	Actual Sample Amount	Mean	Stdev	% RSD
Municipal Water (Midrand)	Bromoform	50.00	1	0.106	58.643	58.64	58.19	1.95	3.35
			2	0.100	58.228	58.12			
			3	0.123	55.456	55.35			
			4	0.125	60.942	60.84			
			5	0.101	58.116	58.01			

Table 3.13 Reproducible data for analyst 2 at 50 µg/L

Sample	THMs	Conc (µg/L)	Inj. No	Unspiked sample	Spiked Sample Amount	Actual Sample Amount	Mean	Stdev	% RSD
Municipal Water (Midrand)	Bromoform	50.00	1	0.137	54.203	54.07	55.66	1.79	3.22
			2	0.160	55.617	55.46			
			3	0.146	55.870	55.72			
			4	0.171	58.766	58.60			
			5	0.134	54.600	54.47			

Table 3.14 Reproducible data for analyst 1 at 50 µg/L

Sample	THMs	Conc (µg/L)	Inj. No	Unspiked sample	Spiked Sample Amount	Actual Sample Amount	Mean	Stdev	% RSD
Chlorinated Water (Midrand)	Bromoform	50.00	1	0.120	55.638	55.52	55.73	3.70	6.64
			2	0.123	57.843	57.72			
			3	0.130	60.934	60.80			
			4	0.133	53.296	53.16			
			5	0.130	51.562	51.43			

Table 3.15 Reproducible data for analyst 2 at 50 µg/L

Sample	THMs	Conc (µg/L)	Inj. No	Unspiked sample	Spiked Sample Amount	Actual Sample Amount	Mean	Stdev	% RSD
Chlorinated Water (Midrand)	Bromoform	50.00	1	0.103	53.205	53.21	57.41	3.93	6.85
			2	0.106	54.219	54.12			
			3	0.125	62.017	61.91			
			4	0.120	60.963	60.86			
			5	0.135	57.065	56.96			

The RSD for municipal water ranges from 3.22 % - 3.35 %, while the RSD for chlorinated water ranges from 6.64 % - 6.85 %. The difference in results is not significant from one another. The RSD obtained for all other trihalomethanes analytes for municipal water ranges from 3.22 % - 8.91 % for bromoform to chloroform, while the RSD for chlorinated water the highest with values from 6.27 % - 7.42 %. In order for RSD to be accepted, the RSD value must be ≤ 10%. Therefore from the results in tables 3.12 to 3.15 above and in appendix: A pg.

131-134, it can be concluded that the precision based on reproducibility for this method is acceptable.

3.2.7 Accuracy based on recovery

Accuracy is how close the obtained results are to the initial theoretical concentration, while recovery is calculated by dividing the actual concentration by the theoretical concentration multiplied by hundred. Accuracy based on recovery was done on four different samples that were analysed (n=5) times. The four samples that were used for the accuracy was borehole water, municipal water, river water and underground water. The samples were spiked with a known concentration of 50 µg/L, 150 µg/L and 350 µg/L. The samples were run consistently one after another to determine accuracy of the method. For the accuracy to be accepted, the recovery needs to fall in a percentage range from 70-120 %, as well as having a relative standard deviation of ≤ 10%. The results are tabulated below in table 3.16-3.18 for borehole water. All the other accuracy based on recovery values for THMs can be found in appendix: A pg. 135-146.

Sample calculation for bromodichloromethane

$$\frac{\text{Actual sample amount}}{\text{Theoretical sample amount}} \times 100\% = \text{Recovery}$$

$$\frac{\text{Standard Deviation}}{\text{Mean value}} \times 100\% = \text{Relative Standard deviation}$$

$$\text{Bromodichloromethane for borehole water} = \frac{46.005}{50.00} \times 100\% = 92.01\ \%$$

$$= \frac{1.59}{92.43} \times 100\% = 1.72\ \%$$

Table 3.16 Accuracy recovered for borehole water at 50 µg/L

Sample	THMs	Inj. No	Sample Amount Obtained (µg/L)	Theoretical Conc (µg/L)	% Recovery	Mean	Stdev	% RSD
Borehole Water	Bromoform	1	46.005	50.00	92.01	92.43	1.59	1.72
		2	45.432	50.00	90.86			
		3	45.826	50.00	91.65			
		4	47.524	50.00	95.05			
		5	46.283	50.00	92.57			

Table 3.17 Accuracy recovered for borehole water at 150 µg/L

Sample	THMs	Inj. No	Sample Amount Obtained (µg/L)	Theoretical Conc (µg/L)	% Recovery	Mean	Stdev	% RSD
Borehole Water	Bromoform	1	130.710	150.00	87.14	95.15	5.74	6.03
		2	140.140	150.00	93.43			
		3	140.710	150.00	93.81			
		4	149.820	150.00	99.88			
		5	152.250	150.00	101.50			

Table 3.18 Accuracy recovered for borehole water at 350 µg/L

Sample	THMs	Inj. No	Sample Amount Obtained (µg/L)	Theoretical Conc (µg/L)	% Recovery	Mean	Stdev	% RSD
Borehole Water	Bromoform	1	419.5	350.00	119.86	116.17	3.98	3.42
		2	383.9	350.00	109.69			
		3	403.7	350.00	115.34			
		4	414.3	350.00	118.37			
		5	411.6	350.00	117.60			

The recovery for 50 µg/L bromoform was 90.86 % - 92.57 %, for 150 µg/L it was 87.14 % - 101.50 %, for 350 µg/L it was 109.69 % - 119.86 %. All other sample

matrices for municipal water, underground water and river water all gave acceptable recoveries that were within 70 – 120 % recovery range, for 50 µg/L, 150 µg/L and 350 µg/L as in appendix A page: 31-42. This therefore concludes that there was an acceptable accuracy within the sample matrices of interest for recovery. The relative standard deviation (RSD) for borehole water was calculated for each trihalomethanes based on the recovery, and was found to be 1.72 % for 50 µg/L, 6.03 % for 150 µg/L and 3.42 % for 350 µg/L. All other sample matrices for municipal water, underground water and river water was found to have RSD less than the ≤ 10% limit for RSD as in appendix A pg. 135-146.

It must be noted that the borehole water and underground water for 150 µg/L, showed consistently high RSD values for all four trihalomethanes. These values were reported as followed, for borehole and underground water in the range from 4.21 % - 6.23 %. These values are still less than the acceptable ≤ 10% RSD limit. This therefore concludes that the method is accurate in quantifying the trihalomethanes of interest within the sample matrices of the method.

3.2.8 Selectivity based on recovery

A method can be selective if it responds to a change in a specific analyte, while obtaining a selective recovery for that specific analyte. For selectivity, three different samples were analysed for matrix effect and recovery making use of the standard additional method. Each of the three samples was analysed (n=3) times as unspiked samples, as well as a spiked sample at 2 different concentrations. These concentrations were 50 µg/L for river and underground water, while 80 µg/L for municipal water. The selectivity test was conducted in order to analyze the ability of the method to respond to a particular analyte of interest. For the selectivity to be accepted, the recovery needs to fall in a percentage range from 70-120 %, as well as having proof on the chromatograms that the samples respond to a particular analyte. The results are tabulated below in table 3.19 for chloride standard. All the other selectivity based on recovery values for THMs can be found in appendix: A pg. 147-148. All the other selectivity based on recovery chromatograms for THMs can be found in appendix: B pg. 177-182.

Sample calculation for selectivity based on recovery for river water

$$\frac{\text{Sample Spiked with 50 µg/L}}{\text{Unspiked sample + theoretical concentration}} \times 100\% = \text{Recovery}$$

$$\text{Chloroform for river water} = \frac{57.029}{1.220+50.00} \times 100\% = 111.34\ \%$$

Table 3.19 Selective recovery for river water at 50 µg/L

Sample	Trihalomethanes Standard	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Theoretical Conc (µg/L)	% Recovery
River water	Chloroform	1.220	57.029	51.220	111.34
River water	BDCM	0.059	59.685	50.059	119.23
River water	CDBM	0.056	57.800	50.056	115.47
River water	Bromoform	0.144	59.033	50.144	117.73

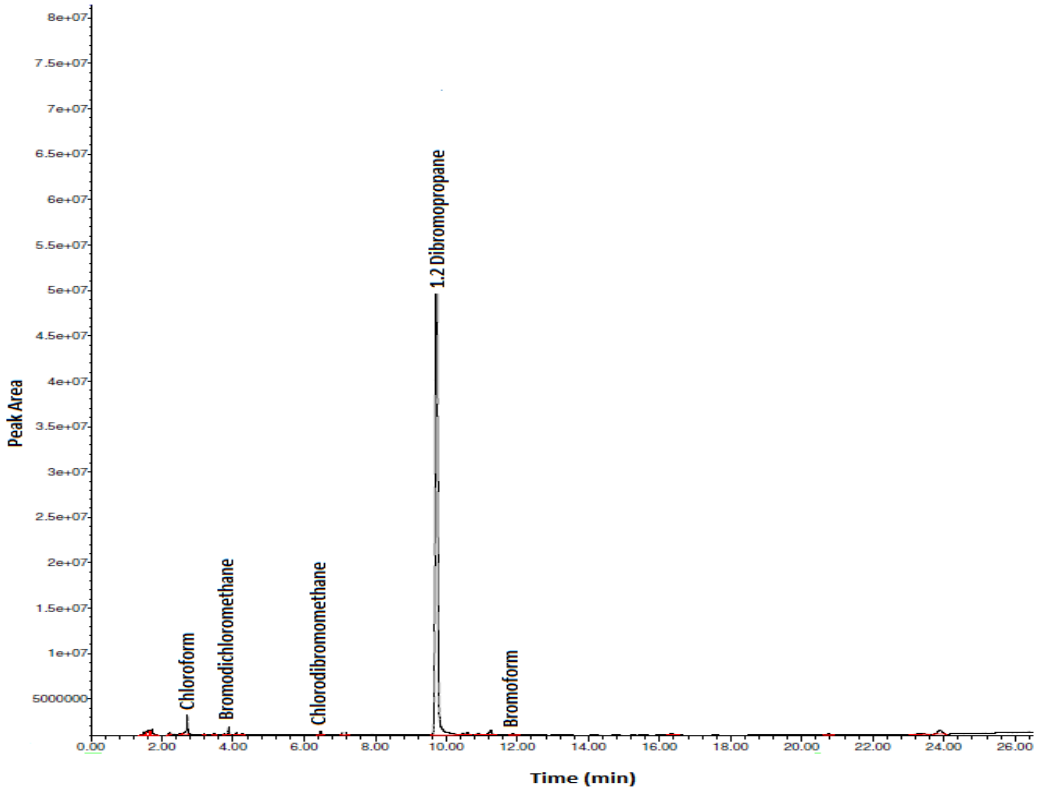


Figure 3.5 0 µg/L unspiked river water sample recoveries for the individual trihalomethanes GC-ECD HP 7683 auto-sampler series injector.

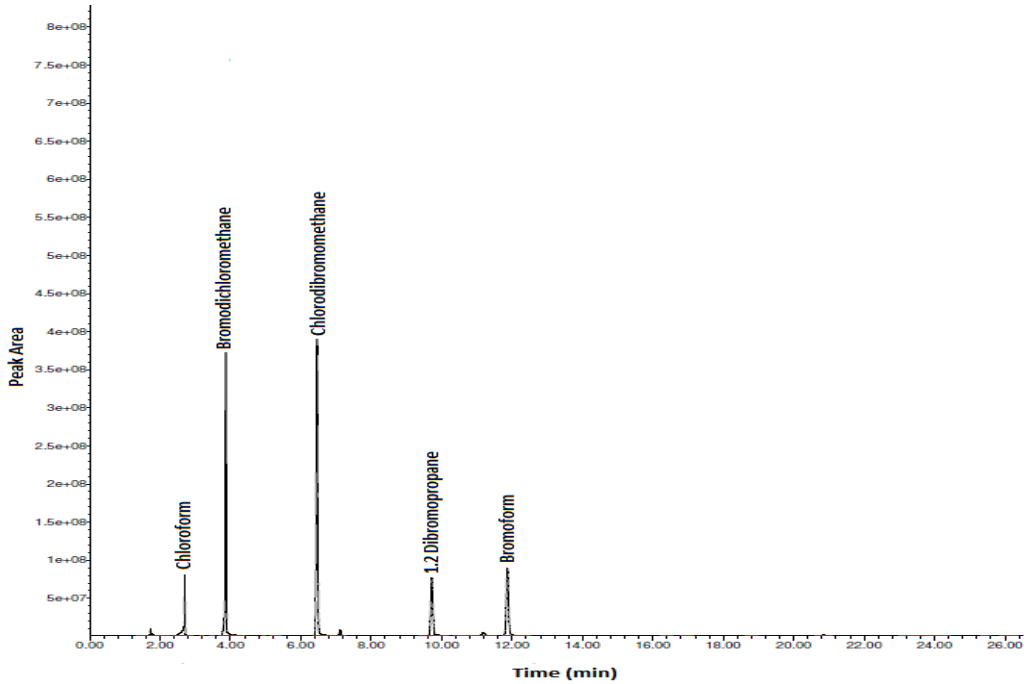


Figure 3.6 50µg/L spiked river water sample recoveries for the individual trihalomethanes GC-ECD HP 7683 auto-sampler series injector

The recovery for 50 µg/L river water differs from 111.34 % - 119.23 % for chloroform to bromodichloromethane, while the recovery for 50 µg/L underground water differs from 109.84 % - 119.97 %. The recovery for 80 µg/L municipal water differs from 82.82 % - 119.59 %. The % recoveries for all 4 trihalomethanes are acceptable within 70 % – 120 % range as in appendix A pg. 147-148. This then concludes that the method is selective to the analytes of interest as it can be seen on the chromatograms for respective sample matrices.

3.2.9 Selectivity based on Interferences

A method can be selective if it responds to a change in a specific analyte in the precessions of interference analyte. For selectivity based on interferences, three different samples were analysed making use of the standard addition method. Each of the three samples was analysed (n=5) times as unspiked samples, as well as a spiked sample at 3 different concentrations. These concentrations were 50 µg/L, 150 µg/L and 350 µg/L. These concentrations were selected to cover a lower range, middle range and upper range of the calibration standards. For the selectivity to be accepted with no interferences, the recovery needs to fall in a percentage range from 70-120 %, as well as having proof on the chromatograms that the samples are free from interference matrices. The results are tabulated

below in table 3.20 for chloride standard. All the other selectivity based on interferences values for THMs can be found in appendix: A pg. 149-157. All the other selectivity based on interferences chromatograms for THMs can be found in appendix: B pg. 183-194.

Sample calculation for chloride standard

$$\frac{\text{Sample Spiked with 250 µg/L}}{\text{Unspiked sample + theoretical concentration}} \times 100\% = \text{Recovery}$$

$$\text{Chlorodibromomethane} = \frac{281.892}{0.00+350.00} \times 100\% = 80.54$$

Table 3.20 Selective recovery matrices for chloride standard at 350 µg/L

Sample	Trihalomethanes Standard	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Theoretical Conc (µg/L)	% Recovery
Chloride Standard	Chloroform	0.000	332.217	350.00	94.92
Chloride Standard	BDCM	0.000	284.428	350.00	81.27
Chloride Standard	CDBM	0.000	281.892	350.00	80.54
Chloride Standard	Bromoform	0.000	345.929	350.00	98.84

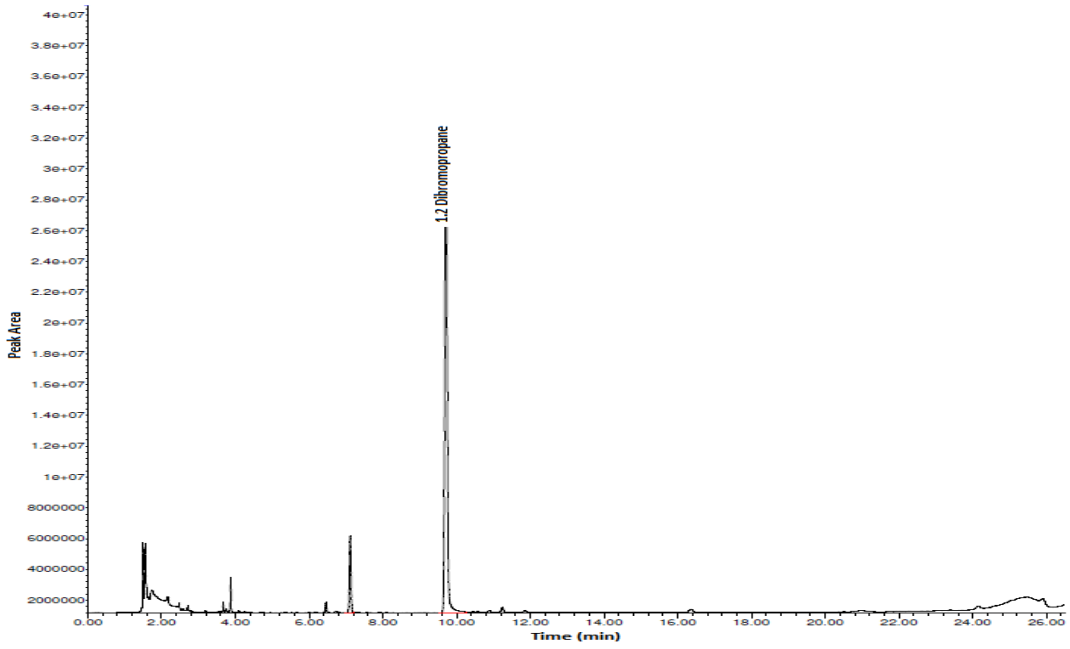


Figure 3.7 Unspiked chloride standard sample recoveries for the individual trihalomethanes GC-ECD HP 7683 auto-sampler series injector

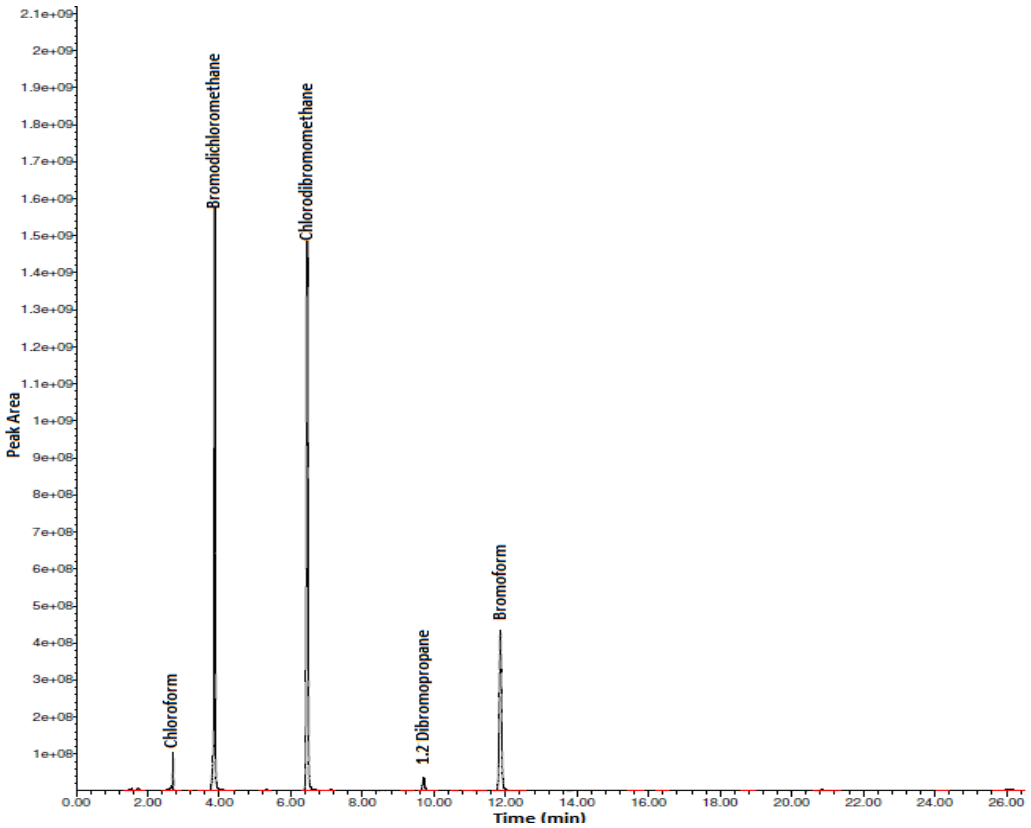


Figure 3.8 350µg/L spiked Chloride Standard sample recoveries for the individual trihalomethanes from GC-ECD HP 7683 auto-sampler series injector.

The recovery for 350 µg/L trihalomethanes differs from 80.54 % - 98.84 % for chlorodibromomethane to bromoform. All other sample matrixes for fluoride standard and SEPP-133 (custom) heavy metal standard gave acceptable recoveries that were within 70 – 120 % recovery range, for 50 µg/L, 150 µg/L and 350 µg/L as in appendix: A pg. 149-157. All the other selectivity based on recovery chromatograms for THMs can be found in appendix: B pg. 177-182. This then concludes that the method is selective to the analytes of interest in the presence of any possible interference, as it can be seen on the chromatograms for respective sample matrices.

3.2.10 Inter Laboratory Comparison

Inter laboratory comparison consists in testing and comparing the results of the same sample by different laboratories. Microchem Laboratory Services outsource its trihalomethanes samples to SANAS Accredited A.L Abbott and Association Laboratory Service. A.L Abbott and Association is a testing laboratory that

specializes in the testing of water and wastewater treatment plants throughout South Africa. Municipal water, tap water, Johnson-Johnson medical water and Wong on Fibre water samples were outsourced to A.L Abbott and Associations. These samples were outsourced under the following laboratory sample identification number for traceability purposes. Municipal water (AG 92981), tap water (AG85690), Johnson-Johnson medical water (AG 92982) and wong on fibre water (AG60080). These outsourced samples were then also tested in-house at Microchem Laboratory Services for inter-laboratory comparisons. The results that were obtained from A.L Abbott and Association were reported under the identification numbers. Table 3.21 and 3.22 shows the inter-laboratory comparison results for tap water and wong on fibre. All the other inter-laboratory comparison values for THMs can be found in appendix: A pg. 158-159. The certificate of analysis from A.L Abbott and Association can be found in appendix: C pg. 204-208.

Table 3.21 Laboratory Comparisons for Tap Water

Company	Sample	Analyte	Conc (µg/L)	Combined Trihalomethanes
Microchem	Tap Water (AG85690)	Chloroform	0.176	<1.00
		Bromodichloromethane	0.080	
		Chlorodibromomethane	0.044	
		Bromoform	0.045	
Company	**Sample**	**Analyte**	**Conc (µg/L)**	**Combined Trihalomethanes**
A.L.Abbott and Association	Tap Water (AG85690)	Chloroform	<1.00	<1.00
		Bromodichloromethane	<1.00	
		Chlorodibromomethane	<1.00	
		Bromoform	<1.00	

Table 3.22 Laboratory Comparisons for Wong on Fibre

Company	Sample	Analyte	Conc (µg/L)	Combined Trihalomethanes
Microchem	Wong on Fibre (AG60080)	Chloroform	12.300	<1.00
		Bromodichloromethane	1.050	
		Chlorodibromomethane	0.400	
		Bromoform	N/D	
Company	**Sample**	**Analyte**	**Conc (µg/L)**	**Combined Trihalomethanes**
A.L.Abbott and Association	Wong on Fibre (AG60080)	Chloroform	16.000	<1.00
		Bromodichloromethane	<1.00	
		Chlorodibromomethane	<1.00	
		Bromoform	<1.00	

The inter-laboratory comparison shows that there is not a significant difference between the two results that were obtained. A.L Abbott and Association report a significant amount of results ≤ 1.00 ie due to the result being lower than their limit of quantification even though it is in range of their limit of detection. Microchem has much lower limit of quantification than A.L Abbott and that is why it can report values less than <1.00. A.L Abbott and Association reported 16.00 µg/L chloroform for wong on fibre compared to Microchem 12.30 µg/L chloroform. However, this results does not show a significant difference in results between the two laboratories.

It must be considered that inter-laboratory test is of a significant importance, it has been shown above that there is no significant difference in results between the two laboratories for tap water and Wong on Fibre. The results obtained for Johnson-Johnson medical water and municipal water all report results as <1.00, for both A.L Abbott and Association and Microchem. However, this does not indicate that the method development for trihalomethanes is validated acceptable. Therefore participation in FAPAS proficiency (paragraph 3.2.14 pg 80) test provides a much more accurate indication of whether the method development validation data will be accepted.

3.2.11 Precision based on Repeatability

Precision based on repeatability or re-test reliability is the closeness of the agreement between the results of successive measurements of the same measurement carried out under the same conditions of measurement. Precision based on repeatability was done on four different laboratory samples that were analysed (n=5) times each. The four samples that were used for precision testing under the same conditions are borehole water, municipal water, river water and underground water. Each of the four samples were analysed (n=5) times as unspiked samples, as well as a spiked sample at 150 µg/L concentrations.

The samples were run consistently one after another to calculate how precise the results were under the same conditions. For precision based on repeatability to be accepted, it needs to have a relative standard deviation ≤ 10%. It must also have z-score value that falls between -3 ≤ lzl ≤ + 3. The results are tabulated below in table 3.23-3.26 for river water. All the other precision based on reproducibility values for THMs can be found in appendix: A pg. 160-163.

Sample calculation for river water

Spiked sample amount - unspiked sample amount= actual concentration

Sample calculation for river water= 156.00-0.072=155.93

$$\frac{\text{Standard Deviation}}{\text{Mean value}} \times 100\% = \text{Relative Standard deviation}$$

$$\text{Bromoform} = \frac{6.89 \times 100\%}{158.34} = 4.35\ \%$$

Table 3.23 Precision based data on repeatability with 150 µg/L standard

Sample	THMs	Conc (µg/L)	Inj. No	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Actual Sample Amount (µg/L)	Mean	Stdev	% RSD	Z-Score
River Water	Chloroform	150	1	1.657	135.800	134.143	142.77	6.47	4.53	0.5
			2	1.650	141.700	140.050				
			3	1.558	142.800	141.242				
			4	1.897	150.800	148.903				
			5	1.657	151.200	149.543				

Table 3.24 Precision based data on repeatability with 150 µg/L standard

Sample	THMs	Conc (µg/L)	Inj. No	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Actual Sample Amount (µg/L)	Mean	Stdev	% RSD	Z-Score
River Water	BDCM	150	1	0.110	164.000	163.890	170.21	3.85	2.26	0.5
			2	0.120	173.500	173.380				
			3	0.146	173.100	172.954				
			4	0.196	171.600	171.404				
			5	0.147	169.600	169.453				

Table 3.25 Precision based data on repeatability with 150 µg/L standard

Sample	THMs	Conc (µg/L)	Inj. No	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Actual Sample Amount (µg/L)	Mean	Stdev	% RSD	Z-Score
River Water	CDBM	150	1	0.052	135.900	135.848	148.58	9.00	6.06	0.5
			2	0.060	145.600	145.540				
			3	0.091	147.100	147.009				
			4	0.023	157.200	157.177				
			5	0.064	157.400	157.336				

Table 3.26 Precision based data on repeatability with 150 µg/L standard

Sample	THMs	Conc (µg/L)	Inj. No	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Actual Sample Amount (µg/L)	Mean	Stdev	% RSD	Z-Score
River Water	Bromoform	150	1	0.072	156.0	155.93	158.34	6.89	4.35	0.5
			2	0.060	147.5	147.44				
			3	0.054	162.7	162.65				
			4	0.049	164.7	164.65				
			5	0.081	161.1	161.02				

The RSD for river water differs from 2.26 % - 6.06 % for bromodichloromethane to chlorodibromomethane. All other sample matrices for municipal water, underground water and river water all gave acceptable RSD values that were below the ≤ 10% as in appendix. The z-score were then calculated making use of

the standard deviation and the mean value. For the z-score to be accepted at 95 % confidence level, it must obtain a z-score between -3 ≤ lzl ≤ + 3. From the tabulated z-score, it can be concluded that precision based on repeatability for river water, municipal water, underground water and borehole water is accepted for this method as in appendix A pg. 160-163.

3.2.12 Robustness

Robustness or ruggedness of analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provided an indication of its reliability during normal usage. Ruggedness was done on municipal and borehole water samples and were analysed (n=3) times each. Ruggedness was done in such a manager that some of the instrument parameters were altered as indicated in the table 13.27 below. The ultimate aim of ruggedness is to obtain a recovery in 70 % - 120% range.

Ruggedness testing were done making use of the present method (method develop table 2.1) as well as using a modified optimized method of the present method. Each of the two samples were analysed (n=3) times as unspiked samples, as well as a spiked samples at known concentration of 100 µg/L and the recovery was calculated All the other ruggedness values for THMs can be found in appendix: A pg. 164-165. All the other robustness chromatograms for THMs can be found in appendix: B pg. 195-202

Optimization of parameters

The following parameter was optimized for the robustness test. The injection volume, carrier flow, make up flow, injection temperature and oven program. The injection volume was optimized to get better analyte separation. The carrier flow was optimized to allow the carrier gas to flow at higher rate through the column, which led to shorter retention time theoretically for peak to elute. The make-up flow was optimized to reduce band broadening. Injection temperature parameters were selected to be as close as possible to the optimized method temperature. Too hot injection temperature may lead to partial destruction of the sample or to release it from the septum, it can also lead to back-flush of the sample if it exceeds the liner temperature. Oven program modifications are always seen as

temperature program effect that is used to reduce retention time. A reduction in the retention time leads to lesser consumable gas usage.

Table 3.27 Present method compared with modified method

Parameters	**Present Method**			**Modified Optimized Method**		
Analytical Column	HP-5MS UI			HP-5MS UI		
Length (m)	30			30		
Internal Diameter (mm)	0.25			0.25		
Film Thickness (µm)	1.0			1.0		
Injection volume (µL)	2			1		
Injection Type	Splitless			Splitless		
Split Flow	none			none		
Carrier Gas	Helium			Helium		
Split Flow	none			none		
Carrier Gas	Helium			Helium		
Carrier Flow (mL/min)	1.5			2.5		
Make-up Flow (mL/min)	30			40		
Injector Temp (°C)	175			190		
Detector Temp (°C)	275			275		
Oven Program	Rate ºC/min	Value ºC	Hold	Rate ºC/min	Value ºC	Hold
		40	10		50	10
	3	45	6	3	60	3
	5	81	0	5	85	3
	5.5	90	0	5.5	120	4
	Run Time :26.503			**Run Time :23.152**		

Sample calculations for municipal water

$$\frac{\text{Sample Spiked with 100 µg/L}}{\text{Unspiked sample + theoretical concentration}} \times 100\% = \text{Recovery}$$

$$\text{Chloroform for municipal water} = \frac{80.239 \times}{0.00+100}\ 100\% = 80.24\ \%$$

Table 3.28 Recovery for municipal water using modified optimized method at 100 µg/L

Sample	Trihalomethanes Standard	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Theoretical Conc (µg/L)	% Recovery
Municipal water	Chloroform	0.000	80.239	100.000	80.24
Municipal water	BDCM	0.000	107.349	100.000	107.35
Municipal water	CDBM	0.000	97.349	100.000	97.35
Municipal water	Bromoform	0.000	83.822	100.000	83.82

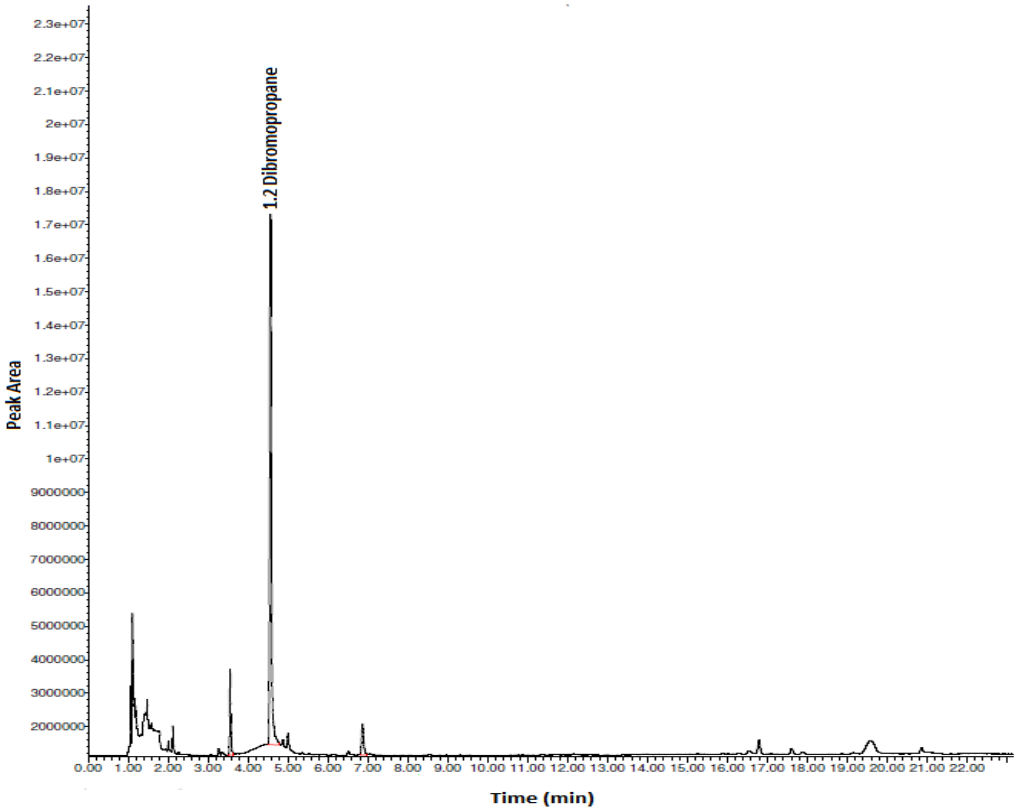


Figure 3.9 Municipal water unspiked sample chromatogram for modified method. GC-ECD HP 7683 auto-sampler series injector

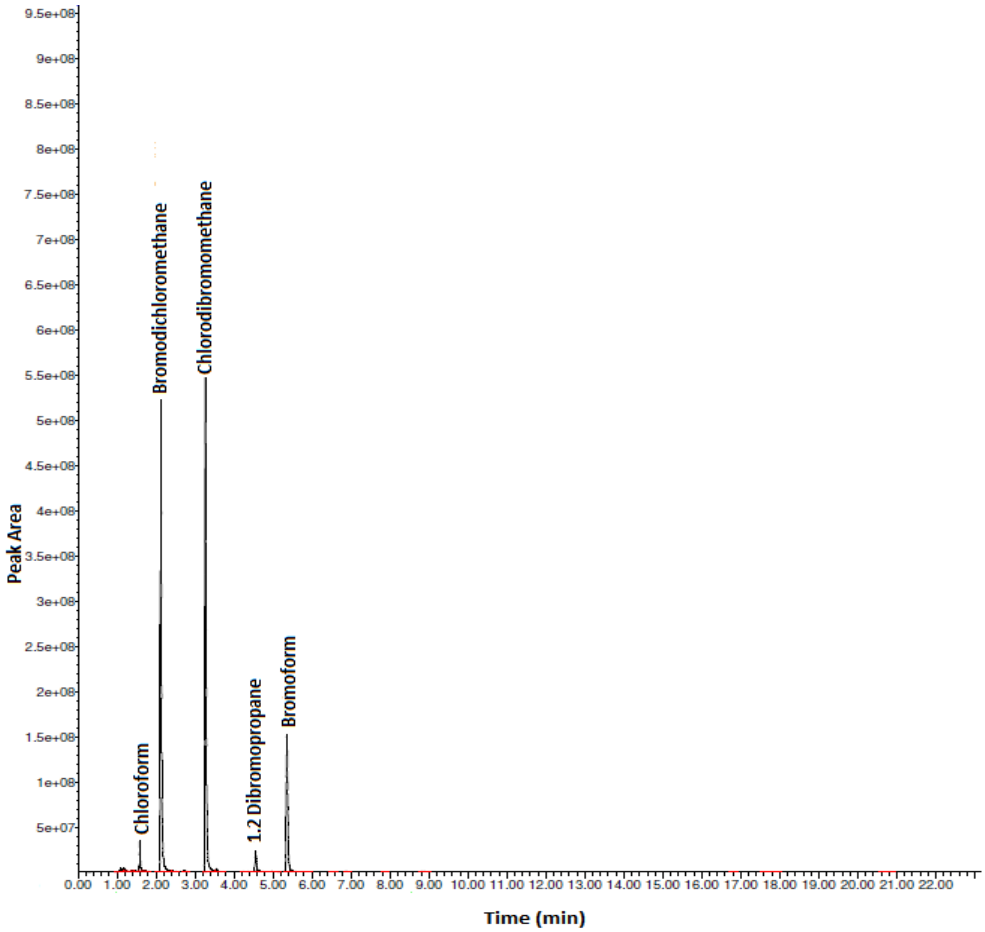


Figure 3.10 Municipal water 100 µg/L spiked sample chromatogram for modified method. GC-ECD HP 7683 auto-sampler series injector

Table 3.29 Recovery for municipal water using present method at 100 µg/L

Sample	Trihalomethanes Standard	Unspiked Sample Amount (µg/L)	Spiked Sample Amount (µg/L)	Theoretical Conc (µg/L)	% Recovery
Municipal water	Chloroform	0.000	84.500	100.000	84.50
Municipal water	BDCM	0.000	109.300	100.000	109.30
Municipal water	CDBM	0.000	83.690	100.000	83.69
Municipal water	Bromoform	0.000	108.300	100.000	108.30

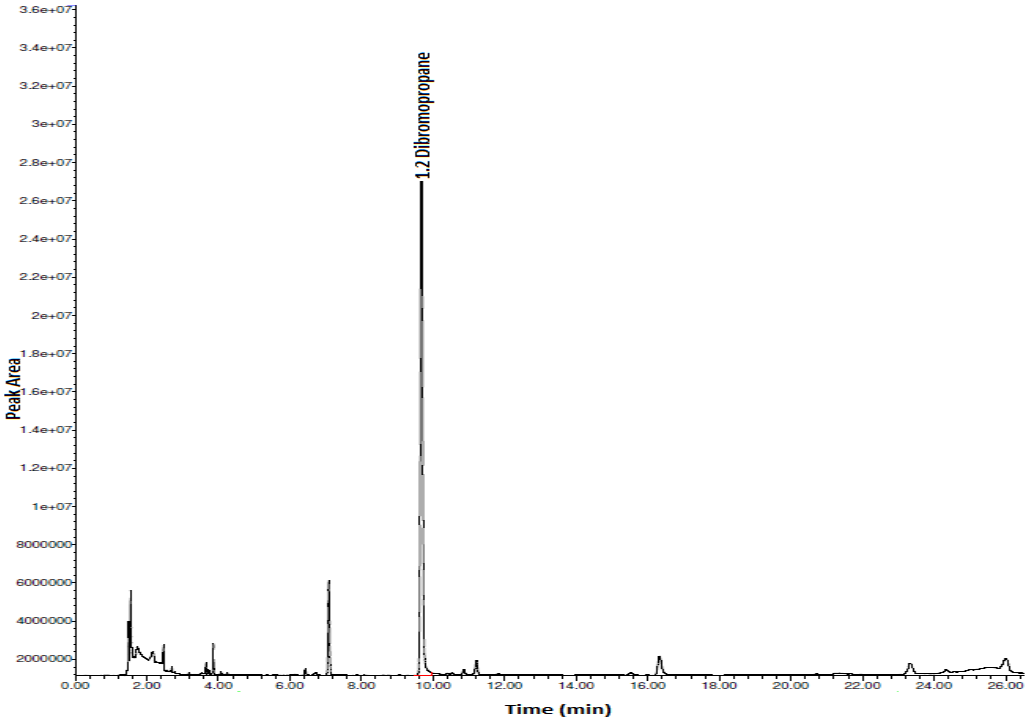


Figure 3.11 Municipal water Unspiked sample chromatogram for present method. GC-ECD HP 7683 auto-sampler series injector

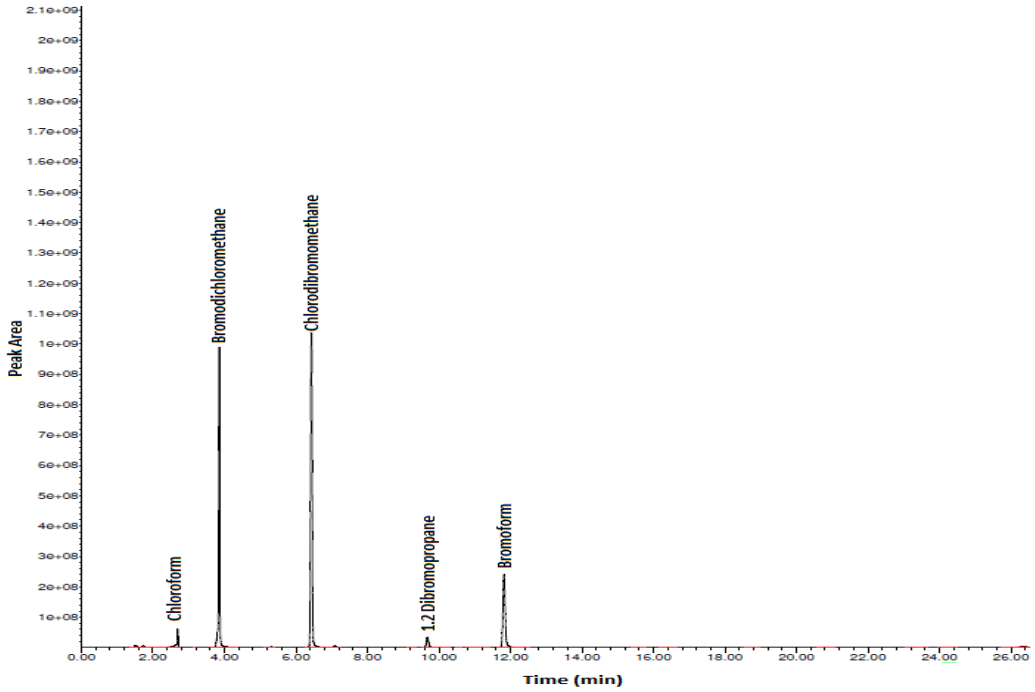


Figure 3.12 Municipal Water 100 µg/L spiked sample chromatogram for present method GC-ECD HP 7683 auto-sampler series injector

The recovery for 100 µg/L municipal water differs from 80.24 % - 107.35 % for chloroform to bromodichloromethane for the modified method, while the recoveries for the optimized method differ from 83.69 % - 109.30 % for chlorodibromomethane to bromodichloromethane. All other sample matrices for river water gave acceptable recoveries that were within 70 – 120 % recovery range as in appendix A pg. 164-165. This therefore concludes that the method is robust to give precise results even though significant changes were made to the method in quantifying the trihalomethanes of interest.

3.2.13 Measure of Uncertainty

The procedure for the estimation of measurement of uncertainty was based on the verification of traceability using reference standard. In order to do this, the following reference material was used 2000 mg/mL THMs calibration CRM standard from Sigma-Aldrich: chloroform (Purity 98.1 %), bromodichloromethane (Purity 97.6 %), chlorodibromomethane (Purity 97.1 %), and bromoform (Purity 99.7 %)

The aim of this procedure is to verify traceability (i.e. the absence of significant bias) using reference materials and to calculate the uncertainty of the analytical procedure with this data. The approach that was used is the uncertainty estimated from precision and bias. Measurement of accuracy is compared to the precision and trueness of the results that are obtained from the measure of uncertainty. Measurement of uncertainty therefore compares to intra-laboratory reproducibility as well as uncertainty on bias. Precision and bias estimates obtained using the within-laboratory validation approach are designed to cover all uncertainty factors impacting the measurement that would occur under normal operating conditions for the measurement procedure.

The following factors contributing to uncertainty of measurement: Analytical balance, mass pieces, 50 mL Erlenmeyer flask, 200 µL pipette, 1000 µL pipette, 5000 µL pipette, 10 mL volumetric flask, 100 ml volumetric flask, GC injection volume, Trihalomethanes CRM.

Measurement uncertainty (standard measurement uncertainty, ***u***) is estimated as a root sum of squares of a standard deviation (***s***) that characterizes the precision or imprecision of the measurement and an estimate (***b***) accounting for the measurement bias.

$$u = \sqrt{s^2 + b^2}$$

Within-laboratory reproducibility standard deviation (S_{RW}) was determined by carrying out replicate analysis under intermediate precision conditions. Six replicate analyses for trihalomethanes were performed to include all major and minor uncertainty contributions.

$$\mathrm{b} = \sqrt{\Delta^2 + u_{ref}^2 + \frac{s^2}{n}}$$

Measurement bias (b) is eliminated to the greatest extent possible. The residual bias that still remains consists of three different components. The first of these components, delta (Δ) represents the mean deviation of replicate measurement results from the stated reference value. The second component (u_{ref}) comprises the uncertainty estimate for the reference value while the third term comprises the standard deviation and the number of measurements made.

The uncertainty of measurement for 120 µg/L trihalomethanes was determined as explained below.

An in-house laboratory control of 120 µg/L trihalomethanes was prepared in six duplicates. The mean value between the two duplicates was calculated and reported as the average values at a designated % reference value. From the six average values, the mean was calculated from them and reported as the X_{mean} value. From the X_{mean} value, the standard deviation was calculated. The uncertainty of reference at 95 % was obtained from the certificate of analysis for 2000 mg/mL THMs CRM. All the other measure of uncertainty values for THMs can be found in appendix: A page: 166-173.

From this data the mean result (X_{mean}) is calculated as 117.29 µg/L.

The standard deviation (SD) of the results is determined as 4.88.

The uncertainty at 95 % confidence interval for the reference value is 0.014 %, which gives a standard uncertainty (u_{ref}) of 0.007.

Table 3.30 Results obtained for the 120 µg/L THMs in-house laboratory control for chloroform

No	Enter duplicate results below µg/L		Average values µg/L	% of the reference value
1	122.290	123.900	123.1	104.90%
2	116.331	117.090	116.71	99.50%
3	104.000	122.450	113.23	96.50%
4	113.241	107.347	110.29	94.00%
5	120.280	122.580	121.43	103.50%
6	119.070	118.900	118.99	101.40%
Xmean			117.29	
Stdev			4.88	
Uncertainty of reference at 95 %			0.014	

To determine the standard deviation of reproducibility ($SD_{(r)}$) the following equation is used:

$$SD_{(iR)} = \sqrt{(SD^2 + (\frac{SD_{(r)}}{2})^2} \qquad \text{Eq……1}$$

Thus reproducibility ($SD_{(r)}$) is calculated as:

$$\therefore SD_{(iR)} = \sqrt{((4.88^2 + (\frac{(0.017)}{2})^2}$$

$$= 6.31$$

The standard deviation of reproducibility ($SD_{(iR)}$) was calculated as 6.31

The uncertainty at 95 % confidence interval for the reference value is 0.014 %,

this is obtained from the THMs certificate, which gives a standard uncertainty (u_{ref}) of 0.007.

The mean deviation of replicate measurement results (Δ) from the stated reference value was determined to be 0.000

The bias of the method is calculated using the following equation:

Where

$$b = \sqrt{\Delta^2 + u_{ref}^2 + \frac{s^2}{n}} \qquad \text{Eq.....2}$$

Thus bias is calculated as:

$$\sqrt{((0.000)^2 + (0.007)^2 + (\frac{(6.31)^2}{6}))}$$

$$\boldsymbol{b} = 2.57$$

The overall standard uncertainty is calculated using the following equation:

$$u = \sqrt{s^2 + b^2} \qquad \text{Eq.....3}$$

Thus standard uncertainty is calculated as:

$$= \sqrt{((6.31)^2 + (2.57)^2)}$$

$$= 11.05$$

The expanded overall uncertainty (k=2) at 95% confidence interval is calculated as 11.05

Finally the expanded relative overall uncertainty (u %) for chloroform is calculated by dividing the expanded overall uncertainty by the reference value (in percentage) of the chloroform control sample. The expanded relative overall uncertainty (u%) for chloroform is calculated as 9.42 %.

Table 3.31 Measurement of uncertainty results obtained for chloroform in- house laboratory control sample.

Statistical calculations		
Number of valid values		
Detection of suspect values		
Tolerance interval		**Classical**
	Min	109.24
	Max	125.34
Conclusion		
There is no suspect value		
Analysis of trueness *vs.* Reference Sample		
		Classical
Median / mean of results		117.290
SD(results)		4.88906
SD(iR)		6.3157
Recovery		100.0%
SD(Recovery)		0.017
t test		0.000
p value		100.0%
Uncertainty of recovery		0.017
Relative uncertainty of recovery		1.70%
Confidence interval	Min	95.6%
	Max	104.4%
The recovery is NOT different from 100% (at 95% confidence)		
MU - Eurolab - Technical Report No.1/2007 March 2007 "Measurement uncertainty revisited: Alternative approaches to uncertainty evaluation"		
Delta - Δ		0.000
Bias - b		2.5784
uncertainty - u		5.5273
Expanded overall u (K=2):	**u =**	**11.0546**
Relative uncertainty		4.71%
Expanded overall relative u :	**u% =**	**9.42%**

$$b = \sqrt{\Delta^2 + u_{ref}^2 + \frac{s^2}{n}}$$

$$u = \sqrt{s^2 + b^2}$$

Table 3.32 Summary of Measurement of uncertainty for THMs

Trihalomethanes	Measure of Uncertainty
Chloroform	9.42%
Bromodichloromethane	3.81%
Chlorodibromomethane	3.10%
Bromoform	9.43%

The measurement of uncertainty associated with the determination of chloroform in trihalomethanes tested on a routine basis was determined as 9.42 %. These uncertainty estimations are based upon physical analysis of reference standard. This process includes all areas of uncertainty in the final results, and the calculated uncertainties meets the requirements of the test method as well as the requirements of the customer.

3.2.14 Proficiency Testing (Fapas drinking water proficiency test)

Proficiency testing aims to provide an independent assessment of the competence of participating laboratories. Together with the use of validation methods, proficiency testing is an essential element of laboratory quality assurance.

The laboratory participated in Fapas drinking water proficiency test in the month of June 2017. Chemistry proficiency test for drinking water DWC017 was dispatched in May 2017. Each participant received drinking water test material of spiking concentration to be analysed for a variety of analytes in a single run. The trihalomethanes results of the Fapas proficiency sample were obtained from the instrument. Results were submitted by 39 participants (95%) before the closing date for this test. Each participant was given a laboratory number, assigned in order of receipt of results. All the other Fapas proficiency values for THMs can be found in appendix: A pg: 174.

Fapas drinking water proficiency test calculations

An assigned value ($\chi_{\alpha)}$ was determined for each analyte and in conjunction with the standard deviation for proficiency ($\sigma_{p)}$ was used to calculate a z-score for each result.

- The results were statistically analysed in order to provide an assigned value for each analyte
- The assigned values were then used in combination with the standard deviation for proficiency, (σ_p) to calculate a z-score for each result
- Participants recovery values (rec) and percentage recovery value (% rec) were calculated as:

  Spiked results = recovery value = (rec)
  Recovery value / assigned value ×100 = % recovery

Table 3.33 Fapas drinking water proficiency test results

Bromodichloromethane			**Bromoform**		
Assigned value 46.2µg/L			Assigned value 28.7 µg/L		
Recovery	% Recovery	z-score	Recovery	% Recovery	z-score
41.505	90	-0.8	18.857	66	-2.7
Pass			**Pass**		

Table 3.34 Fapas drinking water proficiency test results

Chloroform			**Chlorodibromomethane**		
Assigned value 10.5µg/L			Assigned value 35.6µg/L		
Recovery	% Recovery	z-score	Recovery	% Recovery	z-score
4.334	**41**	**-3.9**	26.819	75	-2
Fail			**Pass**		

In normal circumstances, over time, about 95% of z-score will lie in the range -2 ≤ lzl ≤ 2. Occasional scores in the range of -3 ≤ lzl ≤ 3 are to be expected at a rate of 1 in 20. Whether or not such scores are of importance can only be decided by considering them in the content of the other scores obtained by that laboratory. Scores where -3 ≥ lzl ≥ 3 are to be expected at a rate of 1 in 300. Given this rarity, such z-scores very strongly indicate that the result is not fit-for-purpose and almost certainly requires investigation.

Proficiency testing was conducted by participating Fapas proficiency test. Three of the four analytes bromodichloromethane, chlorodibromomethane and bromoform scored a z-score in the range between -3 ≤ lzl ≤ + 3. lt can be concluded that these three analytes passed the proficiency test. However, chloroform failed the proficiency test with a z score of -3.9. The failure of chloroform can be due to sample transfer lost due to volatile sample technique, loss of liquid-liquid extraction due to transfer process, poor sample preparation technique, lack of experience by analyst in proficiency testing. It must be concluded that overall result of three out of four is quite satisfactory which gave a 75 % pass rate.

CHAPTER 4

Discussion, Conclusion and Recommendation

4.1 Introduction

South Africa is relatively poorly endowed with water resources and is considered a “water stressed country”. According to statistics South Africa’s 2016 general household survey, South Africa is the 39th “driest” country in the world. South Africa water supply is mainly from rivers and dams, but the quality of water supplied from the dams and rivers remains threatened from contaminated sources. According to the literature reviewed 132 towns were tested for water and sewerage quality. It was reported that seven municipalities did not meet the quality standards for drinking water in 2016 (BDS, 2016)**.** South Africa’s water quality has been on a decline, after a published report by the South African Water Caucus, (SAWC, 2017). It appears that the drinking water quality in South Africa has severely deteriorated from 2009 (DEA, 2013) and is now a cause for concern, after the Department of Water Affairs (DWA) failed to publish the countries Blue Drop/ Green Drop report for 2017, (SAWC, 2017).

The number of systems that were awarded Blue Drop status in 2012 has dropped from 98 to 44 in 2014 (SAWC, 2017). In 2009, the first Blue Drop report showed that the national microbiological compliance for tap water in South Africa was measured at 93.3 % against the national standard. Since then the Blue Drop score has significantly deteriorated dropping from 87.6 % in 2012 to 79.6 % in 2014. The minister of water and sanitation has responsibility to fulfil certain obligations related to the use, allocation and protection of and access to water. Many rural as well as urban communities have no choice but to collect water from rivers and dams for domestic use, irrigation, cattle, rituals, recreation and at times for drinking purposes because of erratic water supply There is huge risk involved when the public consumes untreated water. Untreated water from municipalities may results in a broad spectrum of toxic metals in high concentrations. Drinking water that contains metals in high concentration levels may result in impairment of cognitive functions, skin lesions, cancer and metal retardation in foetus since it affect the neural development. The Blue Drop and

Green Drop statuses give the public assurance that the water they drink is of a high quality standard.

Water treatment on its own brings significant problems. Water in South Africa is treated with chlorine or other disinfectants. They are used to control microbial contaminants in natural drinking water; however, this leads to formation of significant amount of disinfections known as THMs. Trihalomethanes have been known to cause cancer (Jamaleddin et al., 2016) in human population using chlorine or other disinfectant for water treatments, before being consuming by people, where chloroform is the most present THM. Consistent slight increases in the incidence of rectal, colon and bladder cancer was found with the strongest evidence for bladder cancer. This alone should serve as enough reason for the DWA affairs to publish the countries Blue Drop/Green report for 2017.

In South Africa, there are 9 chemical laboratories that are SANAS accredited (SANAS, 2017) and undisclosed amount of laboratories that are not SANAS accredited for THMs. There is significant need in South Africa for independent laboratories to obtain accreditation of THMs analysis in drinking water. Government laboratories also better known as State Owned Entities (SOE) laboratories are not required to obtain any accreditation for testing purposes. State Owned Entities laboratories need to compare their inter-laboratory testing results to SANAS accredited laboratory, whether it is for chemical testing or microbial testing. As there are only 9 accredited laboratories which limits SOE laboratories to the number of laboratories it can send their samples. Therefore with the development of this method it will be possible to become one of the accredited laboratories in the country that is able to test for THMs, and also be able to contribute to the improvement of better water quality in the country.

4.2 Optimization of gas chromatography method

The standard method of analysis for THMs was the US EPA method 551.1. This method was published in 1990, when gravimetric extraction techniques were still considered the most reliable technique for THMs. This technique is still considered one of the most reliable techniques, as it requires a significant amount of weighing small quantities and is a high chemical consumable

technique. Reckhow et al., (2012) derived a new analytical method for the determination of THMs. This method was known as “Analysis of Trihalomethanes and Related Pentane-Extractable Organic Halides”. This new method added significant advantages compared to US EPA method, such as, better analytical column, better carrier gasses, better carrier flow and shorter retention time. This is currently the most advanced liquid-liquid extraction technique available for determination of THMs.

The aim of this project was to optimize the method developed by Reckhow et al., (2012). Optimization normally requires changes being made to analytical methods that are currently in use. The first change was the analytical column. The DB-5 column was in use but a significant baseline noise was experienced after initial trial tests as shown in figure 2.1. The column was replaced with a more advanced analytical column HP-5 MS UI. This column offers major advantages such as more selective analytes, water robustness, low bleed and excellent inertness. This was all proven when the column was inserted and no baseline noise was experienced as shown in figure 2.2. The carrier gas was changed from nitrogen to helium, as there was a significant advantage of helium compared to nitrogen. Helium provides high column efficiency at moderate linear velocity flow rate, shorter retention time and less carrier gas being consumed during analysis. The oven program was the one where the most changes were made. When Reckhow et al., (2012) developed the method, it was developed for THMs and organic halides. This project is only focusing on THMs. After a considerate amount of testing trials, it was decided to optimize the method retention time from 37.50 min to 26.50 min. This decision was made because all THMs of interest has already eluted after 12.00 min. An additional 14.50 min was added to the time to discard all purities that remained trapped behind in the column after each analytical run. The initial oven temperature was increased from 27 ºC to 40 ºC. This decision was made because oven program temperature needs to be consistent from season to season. It was found that during summer seasons it becomes difficult for the oven to reach 27 ºC, because of the surrounding ambient temperatures.

4.3.1 Validation of Trihalomethanes

Validation is the core foundation for all analytical methods that are developed or modified. A method needs to be validated when significant changes are made to the current method which has already been validated, an in-house developed method is used or a method is published in scientific literature but no validation characteristics is included. Validation needs to be done based on scientific guidelines in order to be acceptable. The guideline characteristics that were used for validation purposes are found in the SANAS TR 28-01 document. The document states that for any method that is validated the following requirements need to be met: limit of detection, limit of quantification, instrument linearity, instrument precision, matrix effect based on recovery, matrix effect based on interferences, precision based on reproducibility, accuracy based on recovery, selectivity based on recovery, selectivity based on interferences, inter-laboratory comparison, precision based on repeatability, robustness, measure of uncertainty and proficiency testing. All of the above were done and complied with accepted levels according to SANAS TR 28-01.

4.3.2 Limit of detection (LOD) and limit of quantification (LOQ)

The limits of detection and quantification showed that the THMs content ranges from 0.199-0.440 for LOD and 0.666-1.469 for LOQ. As it can be seen from table 3.2 both the LOD and LOQ calculated from the reagent blank concentration concluded that this method will quantify the THMs of interest based on their respective limits.

4.3.3 Linearity

The linearity of the 7 concentrations working range showed linear graphs with acceptable correlation values (R^2) for each THM. For the R^2 value to be accepted, the R^2 must be ≥ 0.980. Therefore the R^2 obtained ranges from 0.984-0.998 as indicated in table 3.3 and showed that the instrument response due to the change in analyte concentration is acceptable.

4.3.4 Instrument Precision

The instrument precision test was conducted by injecting a 50 µg/L calibration standard sample 10 times. The RSD values ranges from 1.89 % - 2.98 % as indicated in table 3.5. All gave acceptable results falling below the 10% limit for RSD. It was concluded that the instrument precision for this method is acceptable for the sample matrices of interest.

4.3.5 Recovery and Matrix effect

In order to establish recovery and matrix effect for this method, eight samples of different matrices were analysed for each of the four THMs, with both the blank samples and the spiked samples prepared separately in three different concentrations. The recovery range from 81.60 % - 119.27 %, all the sample matrices gave an acceptable % recovery that is within the 70 – 120 %. This concludes that there were acceptable matrix effects within the sample matrices of interest.

4.3.6 Recovery and Matrix effect based on Interferences

The recovery and matrix based on interference were established by using three samples of different matrices were analysed for each of the four THMs, with both the blank samples and the spiked samples prepared separately in three different concentrations. The recoveries ranges from 80.50 % - 118.95 %, all the samples gave an acceptable % recovery that is within the 70 – 120 % recovery range. Therefore concludes that there were acceptable matrix effects based on interferences within the sample matrices of interest.

4.3.7 Precision based on Reproducibility

Precision based on reproducibility were established on municipal and chlorinated water samples were it was spiked with 50 µg/L THMs calibration standard respectively, and analyzed five times each by two different analysts. The RSD results obtained for municipal and chlorinated water ranges from 3.22 % - 6.85 %. The instrument gave acceptable RSD results falling below the 10% limit. It was concluded that the precision based on reproducibility for this method is acceptable for the sample matrices of interest.

4.3.8 Accuracy based on recovery

In order to establish accuracy based on recovery for this method, four samples of different matrices were analysed for THMs, with both the blank samples and the spiked samples prepared separately in three different concentrations. The recovery ranges from 87.14 % - 118.37 %. The % recoveries for THMs in each sample were acceptable (within 70 % – 120 % range). The RSD was calculated and ranges from 1.72 % - 6.03 %, they were accepted based on the RSD ≤ 10% limit. This then concludes that the method is accurate in quantifying the THMs of interest within the sample matrices of the method.

4.3.9 Selectivity based on recovery

Selectivity based on recovery is a very important validation test regarding selective analytes of interest. The sample matrices were spiked with the 50, 80 and 250 µg/L THMs standards, and ran on the instrument. The % recovery ranges from 82.82 % - 119.97 %. The % recoveries for THMs in each sample were acceptable (within 70 % – 120 % range). It was then concluded that the method is selective to the analytes of interest as the respective sample matrix chromatograms showed in figure 3.5-3.6 a proper separation of the analytes of interest.

4.3.10 Selectivity based on Interferences

Individual test were conducted to establish selectivity, the sample matrices were spiked with the 50, 150 and 350 µg/L THMs standards, as well as 100 µL of 500 µg/L chloride, fluoride and SEPP-133 custom made (Metals) standards and ran on the instrument. The % recovery ranges from 80.54 % - 98.84 %. The % recoveries for THMs were acceptable (within 70 % – 120 % range),. It was then concluded that the method is selective to the analytes of interest as the respective sample matrix chromatograms showed in figure 3.7-3.8 a proper separation of the analytes of interest and no interferences.

4.3.11 Laboratory comparison

To establish laboratory comparison, Microchem participate in an inter-laboratory comparison with A.L. Abbot and Associations, the samples that were outsourced

to A.L Abbott and Associations were in-house tested at Microchem laboratory service. The combined THMs showed that there were no significant differences between the results of the two laboratories. This then concludes that the method is capable of quantifying the THMs of interest.

4.3.12 Precision based on Repeatability

In order to establish precision based on repeatability for this method, four samples of different matrices were analysed for THMs, with both the blank samples and the spiked sample prepared separately at 150 µg/L concentration. The RSD results ranges from 2.26 % - 6.06 %. The RSD results obtained, for the respective THMs were acceptable below the 10% value. The z-scores were found to be 0.5 for all respected THMs. This is in range with the acceptable z-score range which falls between $-3 \leq |z| \leq +3$. Therefore, it was concluded that the precision based on repeatability for this method is acceptable for the sample matrices of interest.

4.3.13 Robustness

Robustness for this method consists of two samples of different matrices were analysed for THMs, with both the blank samples and the spiked samples prepared separately. The recovery ranges from 80.24 % - 109.30 %, all the samples gave an acceptable % recovery that is within the 70 – 120 % recovery range. It was concluded that this method is robust enough to withstand significant changes made to the method and would still give reliable results.

4.3.14 Estimation of uncertainty of measurement

The calculation of measurement of uncertainty was based on the analysis of the spiked control sample, for any factors that contributes to the change in results of the same sample. This process includes all areas of uncertainty in the final results. Due to this serial analysis it was concluded that the calculated uncertainties ranges from 3.10 % - 9.43 %, it meets the requirements of the test method as well as the requirements of the customer.

4.3.15 Proficiency Testing

Proficiency testing was conducted by participating FAPAS proficiency test. Three of the four analytes bromodichloromethane, chlorodibromomethane and bromoform scored a z-score in the range between $-3 \leq |z| \leq +3$, which concluded that these three analytes pass the proficiency test. However, chloroform failed the proficiency test with a z score of -3.9. The failure of chloroform can be due to sample transfer lost due to volatile sample technique, loss of liquid-liquid extraction due to transfer process, poor sample preparation technique, lack of experience by analyst in proficiency testing. It must be mentioned that this was the first proficiency testing participating for THMs. Thus, it must be concluded that overall result of three out of four is quite satisfactory which gave 75 % pass rate.

4.4 Real life water

A selective number of natural water samples were selected for application of the validation method: such as river water, underground water, sea water, unfiltered water and borehole water. These are waters that are natural in nature and are accessible to humans. This is untreated water that is being made used of on a daily basis in the rural areas. Most of these communities collect these waters from rivers for domestic use, irrigation, cattle, rituals, recreation and at times for drinking purposes. A question that needs to be asked is how safe are these natural waters for consumption. It must be considered that municipalities have a difficult time reaching out to these communities, as they are very far away from cities and small townships where municipalities are situated, to implement water treatment protocols. It must be taken into account of how well these waters measure up to drinking water limits set up by SANS 241:2015 standards. SANS 241:2015 states that no water should exceed the following limits for THMs, 300 µg/L for chloroform, 60 µg/L bromodichloromethane, 100 µg/L chlorodibromomethane and bromoform. If water exceeds these limits, it is considered a chronic risk and is not safe for human consumption, whereby water treatment needs to be taken care of immediately.

None of the water these was tested exceeded this limits except sea-water. The water that did not exceed the limits can be classified as partially safe for human consumption. The reason it is classified as partially safe for human consumption is because it only passed the SANS 241:2015 THMs drinking water regulations and levels. However, SANS 241:2015 does not just consist of trihalomethanes, it also consists of physical and aesthetic determination (TDS, color, conductivity, turbidity etc.), macro determinations (sulphate, nitrate, monochloramine, chlorides and etc.), micro determinations (lead, mercury, and selenium etc.), and organic determinations (TOC, phenols and microcystin). For the water to be considered safe for human consumption, it needs to pass all these SANS 241:2015 tests. Sea water has shown very high levels of THMs content in them. It has shown very high levels of bromoform which was reported as 259.016, 260.397 and 260.631 µg/L as untreated bromoform. This exceeds the limits of 100 µg/L set out by SANS 241:2015. This is a very strong indication that sea water is not safe for human consumption, due to the significant high levels of bromoform in it. This study has confirmed that all other natural water is safe for human consumption in the presence of THMs. It is hoping that this study could fill the significant hole left by municipalities that are more centralized to small townships and larger cities than rural areas.

4.5 Municipal drinking water samples

Municipalities have a major responsibility of ensuring that the water quality that they supply to rural areas, small towns or major cities is of safe standard for human consumption. Pre-treated water samples were selected from a variety of municipalities to ensure that municipalities are delivering water qualities of the highest standards. The following water samples were selected from the following municipalities: municipal and chlorinated water (Midrand), municipal water (Sasko-Ladysmith KZN), Johnson-Johnson medical water, (Cape Town) and tap water (Woodstock). Midrand municipality water showed low levels of THMs for chloroform, bromodichloromethane, chlorodibromomethane and bromoform. They all showed values that vary from 11.740 µg/L - 11.981 µg/L for chloroform 3.210 µg/L – 3.564 µg/L for bromodichloromethane, 0.820 µg/L - 0.890 µg/L for chlorodibromomethane and 0.100 µg/L - 0.171 µg/L for bromoform. Sasko – Ladysmith municipality showed very low values as well for THMs content. The

values verified from 2.208 µg/L- 2.300 µg/L for chloroform, 0.138 µg/L - 0.145 µg/L for bromodichloromethane, 0.060 µg/L - 0.068 µg/L for chlorodibromomethane and 0.073 µg/L - 0.074 µg/L for bromoform. These are all municipal waters that are used for human consumption and were supplied directly from municipalities. These waters all show very low content of THMs that are way below the permitted limits.

Chlorinated water is water that has been presumably been treated for bacteria and fungi growth. These are normally the type of water that produces high content of THMs in them, due to chlorination treatment. The results that were obtained for chlorinated water were reported as follows: 10.120 µg/L – 11.654 µg/L for chloroform, 2.214 µg/L - 2.666 µg/L for bromodichloromethane, 0.819 µg/L – 0.895 µg/L chlorodibromomethane and 0.103 µg/L - 0.135 µg/L for bromoform. This does conform to the permissible levels set out by WHO and SANS 2015:241.

It has been observed that natural water shows a very high affinity for chloroform. This is what is expected under normal conditions as chloroform is the most abundant THM of all THMs present in natural water. Johnson-Johnson medical water is very well-known natural water that is consistently used in medical facilities like hospitals for treatment of sick patients. It is water that must be free of any organic matter, bacterial growth or microbial contamination. The results that were obtained from this water source are reported as follows, 0.511 µg/L – 0.580 µg/L for chloroform, 0.758 µg/L – 0.775 µg/L for bromodichloromethane, 0.550 µg/L – 0561 µg/L for chlorodibromomethane and 0.220 µg/L – 0231 µg/L for bromoform. This low concentration levels of THMs are what are expected because this water undergoes significant purification steps to eliminate all traces of THMs, microbial contaminations, fungi and bacterial growth from them.

Tap water in Woodstock is water that was directly from a tap. Woodstock is known for its fragile infrastructure in general. The results that were reported for tap water are as follows: 31.410 µg/L – 32.665 µg/L for chloroform, 15.358 µg/L – 15.987 µg/L for bromodichloromethane, 5.260 µg/L – 5.963 µg/L for chlorodibromomethane and 0.597 µg/L – 0.654 µg/L for bromoform. High content of THMs was reported for chloroform and chlorodibromomethane. This high

content can be due to fragile quality of infrastructures of pipes that contains high content of organic or inorganic matter that carries water to taps, which contributes to high content of THMs. It could be due to poor quality of water supplied by the municipality. This high content of THMs should be a concern for the municipality. It must be noted that this high content of THMs for chloroform and chlorodibromomethane is still far below the critical limits, for it to be considered a chronic risk.

4.6 Fapas Proficiency Test and inter laboratory comparison

Inter laboratory comparisons are done to check the ability of a laboratory to deliver accurate testing results to their customers or to find out whether a certain analytical method performed well and is fit for its intended purposes. The former is normally termed “proficiency testing” and the latter “collaborative method validation study”. Laboratories involved in official control activities are required to provide evidence for their competence in carrying out testing. This process is called accreditation. Accredited laboratories should use standardized methods of analysis and are required to participate in proficiency test for demonstrating their technical competence to their customers and to ensure comparability and acceptability of the testing results produced by them.

A proficiency sample was dispatched under the identification name ‘drinking water DWC017’ in May 2017. The proficiency testing was for all four THMs: chloroform, bromodichloromethane, chlorodibromomethane and bromoform. Proficiency tests are scored based on z-scores. The following z-scores were reported for THMs: chloroform -3.9, bromodichloromethane -0.8, chlorodibromomethane -2.0 and bromoform -2.7. In normal circumstances, over time, about 95% of z-score will lie in the range $-2 \leq |z| \leq 2$. Occasional scores in the range of $-3 \leq |z| \leq 3$ are to be expected at a rate of 1 in 20. Whether or not such scores are of importance can only be decided by considering them in the context of the other scores obtained by that laboratory. Scores where $-3 \geq |z| \geq 3$ are to be expected at a rate of 1 in 300. Given this rarity, such z-scores very strongly indicate that the result is not fit-for-purpose and almost certainly requires investigation.

Under normal circumstances at 95 % confidence level bromodichloromethane and chlorodibromomethane is acceptable base on z-score results. Occasionally z-scores in range of -3 ≤ lzl ≤ 3 can be accepted. In this case the result of bromoform can be accepted, based on the following indications. For instances, the result can be accepted due to the overall low recovery percentage of all four THMs. In this way, chloroform obtained a result of 4.334 µg/L, while the actual result is 10.50 µg/L, this gives a recovery of 41 %. Bromodichloromethane obtained a result of 41.505 µg/L, while the actual result is 46.2 µg/L, this gives a recovery of 90 %. Chlorodibromomethane obtained a result of 26.819 µg/L, while the actual result is 35.6 µg/L, this gives a recovery of 75 %. Bromoform obtained result is 18.857 µg/L, while the actual result 28.7 µg/L, this gives a recovery of 66 %.

Low recovery can be due to loss of THMs during the extraction process. Scores where z-score values are -3 ≥ lzl ≥ 3 strongly indicated that the result is not fit for purpose use. In this case, the result for chloroform is unacceptable and is not fit for purpose use and failed the proficiency test. The proficiency test has an overall pass rate of 75 % under acceptable circumstance. However, it must be mentioned that this is the first proficiency test carried out for THMs. Hopefully in the near future more proficiency tests will be carried out to improve the overall results.

4.7 Conclusion

South Africa is a water scarce country, and its water sources needs to be protected at all cost. There are guideline limits for THMs in the SANS 241:2015 document, but they are not continuously being monitored and their levels in natural water are not known. The results obtained for natural water e.g. river, borehole and underground water have shown low levels of THMs concentrations. These low levels need to be monitored consistently with precision. The water supplied from municipal sources e.g. chlorinated, municipal and tap waters were within the specification limits stipulated in SANS 241:2015, and need to be monitored on a daily basis as these are the waters that are supplied to the communities for consumption. The liquid-liquid micro-extraction technique that was used for the determination of THMs is a rapid, simple and inexpensive

technique that provides low limits of detection and wide dynamic range for the determination of THMs. The modified validated method for determination of THMs in drinking water in South Africa is a suitable effective method which can be used to determine trihalomethanes and combined content in water samples.

4.8 Recommendations for future studies

The primary objective of this project was to develop a fast and reliable method for THMs, so that it can detect and quantify the THMs content present in natural water. In order to improve monitoring of THM levels in natural water content, the following recommendations need to be implemented.

- Looking at alternative ways other than chlorination for water treatment plants.
- Looking at other DBPs in drinking water content, not just THMs in South Africa.
- Complete detailed study needs to be done regarding the effect of THMs, it has been stipulated that it leads to chronic effect which can lead to cancer in laboratory animals.
- More extensive monitoring of THMs in municipal drinking water.
- The method is in processes of getting SANAS accredited

Chapter 5

References

Booi, X. (2013) Perfluorinated Compounds and Trihalomethanes in Drinking Water Sources of the Western Cape, South Africa, Magister Technologiae: Chemical Engineering. Cape Peninsula University of Technology.

Capri, M., Zufall, C. (2003) Gas Chromatography Analysis of Trihalomethanes in Drinking Water –A rapid and Direct Quantitative Method. LCGC ASIA Pacific, 6(1), 36-39

Chang, H.H., Tung, H.H., Chao, C.C., Wang, G.S. (2010) Occurrence of Haloacetic acids (HAAs) and Trihalomethanes (THMs) in drinking water of Taiwan. Environmental Monitoring and Assessment, 162(1-4), 237-250

Chowdhury, S. (2013) Trihalomethanes in drinking water: effect of natural organic matter distribution. Water SA, 39 (1)

Chowdhury, S., Champagne, P. (2008) An investigation on parameters for modeling THMs formation. Global NEST Journal, 10(1), 80-91

Cristina, M.V., Ester, G.L., Cristina, B., Elena, R. (2017) Colorectal cancer and long-term exposure to trihalomethanes in drinking water. Environ Health Prospect. 125 (1), 56-65

Department of Water Affairs Directorate: Water Services Planning & Information. (2013) Strategic Overview of the Water Sector in South Africa, Department of Water Affairs. Version 1.5.

Department of Water Affair Republic of South Africa. (2013) Strategic overview of the Water Sector in South Africa, 1(5), 10-22

FDA, 2013 Food and Drug Administration United States of America.

Guyo, U., Moyo, M., Nyamundal, B., Shumbai, M., Chigondo, F. (2013) Determination of trihalomethanes in raw and treaded water supply to a local city

in Zimbabwe. International Journal of Engineering Research & Technology, 2(2), 1-7

Hasan, A., Thacker, N.P., Bassin, J. (2010) Trihalomethane formation potential in treated water supplies in urban metro city. Environmental Monitoring & Assessment, 168, 489-497

Health Services Executive & Environmental Protective Agency. (2011) Joint Position Statement: Trihalomethanes in Drinking Water, 1-9

Kozani, R. R., Assadi, Y., Shemirani, F., Hosseini, M. R., Jamali, M. R. (2007) Determination of Trihalomethanes in Drinking Water by Dispersive Liquid-Liquid Micro-Extraction then Gas Chromatography with Electron-Capture Detection. Chromatographia, 66 (1-2), 81-86

Loyola-Sepulveda, R., Mudge, S.M., Bravo-Linares, C. (2013) Seasonal Variation of Disinfection By-products in Drinking Water in Central Chile. Water Quality, Exposure and Health, 5(1), 1-9

Madabhushi, B. S. (1999) What are Trihalomethanes: 18-19

Mamba, B.B., Rietveld, L.C., Verberk, JQ. JC. (2008) SA Drinking Water Standards Under The Microscope. The Water Wheel: 1-3

Munch, D. J., Hautman, D. P. (1995) Determination of Chlorination Disinfection By-products, Chlorinated Solvents, And Halogenated Pesticides/ Herbicides in Drinking Water by Liquid- liquid Extraction and Gas Chromatography with Electron-Capture Detection, National Exposure research Laboratory Office Of research And Development U.S Environmental Protection Agency Cincinnati Ohio. Method 551.1

Oelofse, S., Strydom, W. (2010) A CSIR Perspective on water in South Africa. CSIR Report No: CSIR/NRE/PW/IR/2011/0012/A.

Pavon, J.LP., Martin, S. H., Pinto, C. G., Cordero, B. M. (2008) Determination of Trihalomethanes in water samples: A review. Analytica Chemica Acta 629, 6-23

Rand Water & Water Wise. (2011) The Water Situation:1-1

Rahnama, K.R., Assadi, Y., Shemirani, F,. Milani Hosseini, M.R., Jamali, M.R. (2007) Determination of Trihalomethanes in Drinking Water by Dispersive Liquid-Liquid Micro extraction then Gas Chromatography with Electron-Capture Detection. Chromatographia. 66 (1-2)

Reckhow, D. A. (2012) Analysis of Trihalomethanes and Related Pentane-Extraction Organic Halides, University of Massachusetts, Environmental Engineering Research Laboratory. THM SOP

Rodriquez-Cabo, T., Ramil, M,. Rodriquez, I., Cela, R. (2012) Dispersive Liquid–Liquid Micro-Extraction with Non-Halogenated Extractants for Trihalomethanes Determination in tap and swimming pool water. 99, 846-852

Ristoiu, D., Von Gunten, U., Haydee Kovacs, M. and Chira, R. (2009) Factors affecting THM formation in the distribution system of Cluj, Romania. Romanian Research Programme on Environmental Science and Technology (ESTROM), 73-79

Rivette, U., Champanis, M ., Wilson-Jones, T. (2012) Monitoring Drinking Water Quality in South: Designing information systems for local needs. Water SA. 39(3), 409-410

Seager, S.L., and Slabaugh, M.R. 2010 Chemistry for Today: General, Organic and Biochemistry, 7th Edition. Publisher: Brooks/Cole, Cengage Learning. Page 584

South Africa National Standards. (2005) Drinking Water Quality Management Guide for Water Services Authorities. SANS 241

South Africa National Standards. (2011) Drinking Water Quality Management Guide for Water Services Authorities. SANS 241

South Africa National Standards. (2015) Drinking Water Quality Management Guide for Water Services Authorities. SANS 241

South Africa National Standard. (2015) Drinking Water Part 1: Microbiological, physical, aesthetic and chemical determinands: SANS 241-1, 8-9
The group 17 elements: fluorine, chlorine, bromine and iodine, Chemistry 2810 Laboratory Manual. 26 June 2012

Van Der Molen, L. (2015) Microchem Specialized Lab Services: Method Validation-Chemistry, Standard Operating Procedure, No: SOP-C-002/08

Van Steenderen, R.A., Pieterse, M.J., Bourne, D. (1991) Trihalomethanes Formation in Portable Water with Reference to Related Variable and Health Data Bases, Water South Africa, 17(4)

World Health Organization. (2004) Background document for development of WHO Guideline for Drinking water Quality, Trihalomethanes in Drinking Water, 2-39

WHO Guidelines for Drinking-water Quality 4th Edition, 2011

Appendix A Chapter 3

3.1.1 Limit of Detection and Limit of Quantification

Table 3.1 Limit of detection and limit of quantification for chloroform

	Determination No	Chromatography water as blank (µg/L)	Reagent blank (µg/L)
	1	0	0.427
	2	0	0.355
	3	0	0.496
	4	0	0.347
	5	0	0.157
	6	0	0.143
	7	0	0.134
	8	0	0.257
	9	0	0.228
	10	0	0.030
Mean			0.257
STD. DEV.			0.146
LOD			0.440
LOQ			1.469

Table 3.2 Limit of detection and Limit of quantification for BDCM

	Determination No	Chromatography water as blank (µg/L)	Reagent blank (µg/L)
	1	0	0.229
	2	0	0.074
	3	0	0.226
	4	0	0.229
	5	0	0.014
	6	0	0.017
	7	0	0.017
	8	0	0.051
	9	0	0.020
	10	0	0.016
Mean		0	0.089
STD. DEV.			0.097
LOD			0.292
LOQ			0.975

Table 3.3 Limit of detection and limit of quantification for CDBM

	Determination No	Chromatography water as blank (µg/L)	Reagent blank (µg/L)
	1	0	0.157
	2	0	0.064
	3	0	0.163
	4	0	0.157
	5	0	0.014
	6	0	0.022
	7	0	0.022
	8	0	0.031
	9	0	0.015
	10	0	0.006
Mean			0.065
STD. DEV.			0.066
LOD			0.199
LOQ			0.666

Table 3.4 Limit of detection and limit of quantification for bromoform

	Determination No	Chromatography water as blank (µg/L)	Reagent blank (µg/L)
	1	0	0.189
	2	0	0.117
	3	0	0.189
	4	0	0.186
	5	0	0.078
	6	0	0.092
	7	0	0.090
	8	0	0.038
	9	0	0.025
	10	0	0.007
Mean			0.101
STD. DEV.			0.068
LOD			0.205
LOQ			0.685

3.2.2 Instrument Linearity

Table 3.5: Trihalomethanes Linearity Data

Standard Concentration (µg/L)	Area of Internal Standard (1.2-dibromopropane) peak	Area of Chloroform peak	Area of Bromodichloromethane peak	Area of Chlorodibromomethane peak	Area of Bromoform peak	Area ratio of Chloroform	Area Ratio of Bromodichloromethane	Area ratio of Chlorodibromomethane peak	Area Ratio of Bromoform
50.00	17002152714.29	1052544357.86	10137706198.43	23176829178.71	7771815744.71	0.15	1.44	1.39	0.47
80.00	16986564379.43	1889285089.43	17355066729.71	17429092036.14	5956088268.86	0.11	1.02	1.03	0.35
100.00	16701283169.57	2524916486.57	23973521509.29	23176829178.71	7771815744.71	0.15	1.44	1.39	0.47
150.00	17189881066.57	4151104995.14	35256167296.00	37311870444.86	13290102073.43	0.24	2.05	2.17	0.77
200.00	17578769849.57	5788526587.43	44741044564.29	50461036703.00	18015563936.00	0.33	2.55	2.87	1.02
250.00	16800165003.00	7007085668.29	49062180262.57	59798604425.86	23018697805.00	0.42	2.92	3.56	1.37
350.00	16460013577.00	10478687361.14	63957490209.14	80185012427.14	33852866827.29	0.64	3.89	4.87	2.06

3.2.3 Instrument Precision

Table 3.6 Instrument precision for chloroform at 50 µg/L

THMs	Conc µg/L	Injection No	Sample Blank Amount µg/L	Spiked Sample Amount µg/L	Actual Sample Amount µg/L	Mean	Stdev	% RSD
Chloroform	50.00	1	0.000	40.466	40.466	41.34	0.78	1.89
		2	0.000	42.118	42.118			
		3	0.000	41.949	41.949			
		4	0.000	41.379	41.379			
		5	0.000	41.837	41.837			
		6	0.000	41.131	41.131			
		7	0.000	40.185	40.185			
		8	0.000	42.449	42.449			
		9	0.000	41.498	41.498			
		10	0.000	40.391	40.391			

Table 3.7 Instrument precision for bromodichloromethane at 50 µg/L

THMs	Conc µg/L	Injection No	Sample Blank Amount µg/L	Spiked Sample Amount µg/L	Actual Sample Amount µg/L	Mean	Stdev	% RSD
BDCM	50.00	1	0.000	55.466	55.466	57.75	1.57	2.72
		2	0.000	58.613	58.613			
		3	0.000	59.062	59.062			
		4	0.000	58.578	58.578			
		5	0.000	59.022	59.022			
		6	0.000	56.806	56.806			
		7	0.000	57.726	57.726			
		8	0.000	60.063	60.063			
		9	0.000	56.622	56.622			
		10	0.000	55.589	55.589			

Table 3.8 Instrument precision for chlorodibromomethane at 50 µg/L

THMs	Conc µg/L	Injection No	Sample Blank Amount µg/L	Spiked Sample Amount µg/L	Actual Sample Amount µg/L	Mean	Stdev	% RSD
CDBM	50.00	1	0.000	48.650	48.650	50.82	1.51	2.98
		2	0.000	50.993	50.993			
		3	0.000	52.301	52.301			
		4	0.000	52.105	52.105			
		5	0.000	51.709	51.709			
		6	0.000	49.792	49.792			
		7	0.000	51.025	51.025			
		8	0.000	53.037	53.037			
		9	0.000	49.797	49.797			
		10	0.000	48.769	48.769			

Table 3.9 Instrument precision for bromoform at 50 µg/L

THMs	Conc µg/L	Injection No	Sample Blank Amount µg/L	Spiked Sample Amount µg/L	Actual Sample Amount µg/L	Mean	Stdev	% RSD
Bromoform	50.00	1	0.000	42.391	42.391	43.99	1.23	2.80
		2	0.000	43.999	43.999			
		3	0.000	45.218	45.218			
		4	0.000	44.901	44.901			
		5	0.000	44.289	44.289			
		6	0.000	43.098	43.098			
		7	0.000	44.115	44.115			
		8	0.000	46.211	46.211			
		9	0.000	43.177	43.177			
		10	0.000	42.490	42.490			

3.2.4 Matrix effect on Recovery

Table 3.10 Recovery based on matrices at 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Borehole Water	Chloroform	50	1	1.657	44.059	51.657	85.29
			2	1.500	45.000	51.500	87.38
			3	1.658	44.693	51.658	86.52
Borehole Water	BDCM	50	1	0.110	46.005	50.110	91.81
			2	0.120	45.321	50.120	90.42
			3	0.132	46.665	50.132	93.08
Borehole Water	CDBM	50	1	0.052	41.052	50.052	82.02
			2	0.060	42.000	50.060	83.90
			3	0.065	41.031	50.065	81.96
Borehole Water	Bromoform	50	1	0.000	40.696	50.000	81.39
			2	0.000	40.700	50.000	81.40
			3	0.000	40.650	50.000	81.30

Table 3.11 Recovery based on matrices at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Borehole Water	Chloroform	150	1	1.657	151.200	151.657	99.70
			2	1.500	151.300	151.500	99.87
			3	1.658	151.640	151.658	99.99
Borehole Water	BDCM	150	1	0.110	169.600	150.110	112.98
			2	0.120	167.000	150.120	111.24
			3	0.132	166.100	150.132	110.64
Borehole Water	CDBM	150	1	0.052	157.400	150.052	104.90
			2	0.060	157.600	150.060	105.02
			3	0.065	156.321	150.065	104.17
Borehole Water	Bromoform	150	1	0.000	153.400	150.000	102.27
			2	0.000	153.640	150.000	102.43
			3	0.000	153.900	150.000	102.60

Table 3.12 Recovery based on matrices at 350 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Borehole Water	Chloroform	350	1	1.657	379.700	351.657	107.97
			2	1.500	380.600	351.500	108.28
			3	1.658	379.602	351.658	107.95
Borehole Water	BDCM	350	1	0.110	285.410	350.110	81.52
			2	0.120	286.400	350.120	81.80
			3	0.132	286.320	350.132	81.77
Borehole Water	CDBM	350	1	0.052	354.850	350.052	101.37
			2	0.060	352.100	350.060	100.58
			3	0.065	351.230	350.065	100.33
Borehole Water	Bromoform	350	1	0.000	397.420	350.000	113.55
			2	0.000	397.300	350.000	113.51
			3	0.000	375.300	350.000	107.23

Table 3.13 Recovery based on matrices at 50 µg/L

Sample	THMs	Conc µg/L	Inj No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Municipality Water (Sasko Ladysmith)	Chloroform	50	1	2.208	44.000	52.208	84.28
			2	2.300	44.521	52.300	85.13
			3	2.220	44.560	52.220	85.33
Municipality Water (Sasko Ladysmith)	BDCM	50	1	0.138	53.800	50.138	107.30
			2	0.140	52.990	50.140	105.68
			3	0.145	52.996	50.145	105.69
Municipality Water (Sasko Ladysmith)	CDBM	50	1	0.068	48.700	50.068	97.27
			2	0.060	49.000	50.060	97.88
			3	0.065	48.561	50.065	97.00
Municipality Water (Sasko Ladysmith)	Bromoform	50	1	0.073	52.100	50.073	104.05
			2	0.073	52.100	50.073	104.05
			3	0.074	52.101	50.073	102.01

Table 3.14 Recovery based on matrices at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Municipality Water (Sasko Ladysmith)	Chloroform	150	1	2.208	162.600	152.208	106.83
			2	2.300	163.600	152.300	107.42
			3	2.220	164.600	152.220	108.13
Municipality Water (Sasko Ladysmith)	BDCM	150	1	0.138	176.100	150.138	117.29
			2	0.140	174.300	150.140	116.09
			3	0.145	176.100	150.145	117.29
Municipality Water (Sasko Ladysmith)	CDBM	150	1	0.068	169.400	150.068	112.88
			2	0.060	168.000	150.060	111.96
			3	0.065	169.000	150.065	112.62
Municipality Water (Sasko Ladysmith)	Bromoform	150	1	0.073	161.100	150.073	107.35
			2	0.073	160.320	150.073	106.82
			3	0.074	161.990	150.074	107.94

Table 3.15 Recovery based on matrices at 350 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Municipality Water (Sasko Ladysmith)	Chloroform	350	1	2.208	396.040	352.208	112.44
			2	2.300	395.300	352.300	112.21
			3	2.220	396.100	352.220	112.46
Municipality Water (Sasko Ladysmith)	BDCM	350	1	0.138	285.410	350.138	81.51
			2	0.140	284.900	350.140	81.37
			3	0.145	289.310	350.145	82.63
Municipality Water (Sasko Ladysmith)	CDBM	350	1	0.068	354.850	350.068	101.37
			2	0.060	353.210	350.060	100.90
			3	0.065	354.610	350.065	101.30
Municipality Water (Sasko Ladysmith)	Bromoform	350	1	0.073	397.420	350.073	113.52
			2	0.073	396.300	350.073	113.20
			3	0.074	397.800	350.074	113.63

Table 3.16 Recovery based on matrices at 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Underground Water	Chloroform	50	1	2.437	44.119	52.437	84.14
			2	3.463	44.200	53.463	82.67
			3	2.455	43.000	52.455	81.98
Underground Water	BDCM	50	1	0.079	48.356	50.079	96.56
			2	0.090	49.000	50.090	97.82
			3	0.060	49.321	50.060	98.52
Underground Water	CDBM	50	1	0.052	44.864	50.052	89.63
			2	0.050	45.000	50.050	89.91
			3	0.033	45.369	50.033	90.68
Underground Water	Bromoform	50	1	0.069	45.296	50.069	90.47
			2	0.056	45.330	50.056	90.56
			3	0.060	45.630	50.060	91.15

Table 3.17 Recovery based on matrices at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Underground Water	Chloroform	150	1	2.437	139.400	152.437	91.45
			2	3.463	140.321	153.463	91.44
			3	2.455	139.357	152.455	91.41
Underground Water	BDCM	150	1	0.079	173.800	150.079	115.81
			2	0.090	171.320	150.090	114.14
			3	0.060	170.321	150.060	113.50
Underground Water	CDBM	150	1	0.052	146.800	150.052	97.83
			2	0.050	144.600	150.050	96.37
			3	0.033	146.900	150.033	97.91
Underground Water	Bromoform	150	1	0.069	143.100	150.069	95.36
			2	0.056	144.321	150.056	96.18
			3	0.060	140.358	150.060	93.53

Table 3.18 Recovery based on matrices at 350 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Underground Water	Chloroform	350	1	2.437	412.100	352.437	116.93
			2	3.463	410.300	353.463	116.08
			3	2.455	411.980	352.455	116.89
Underground Water	BDCM	350	1	0.079	294.900	350.079	84.24
			2	0.090	292.300	350.090	83.49
			3	0.060	293.600	350.060	83.87
Underground Water	CDBM	350	1	0.052	362.800	350.052	103.64
			2	0.050	361.320	350.050	103.22
			3	0.033	360.300	350.033	102.93
Underground Water	Bromoform	350	1	0.069	412.300	350.069	117.78
			2	0.056	413.600	350.056	118.15
			3	0.060	413.900	350.060	118.24

Table 3.19 Recovery based on matrices at 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Sea water	Chloroform	50	1	2.640	44.640	52.640	84.80
			2	2.932	45.632	52.932	86.21
			3	2.745	44.98	52.745	85.28
Sea water	BDCM	50	1	2.941	62.972	52.941	118.95
			2	2.966	63.91	52.966	120.66
			3	2.974	61.789	52.974	116.64
Sea water	CDBM	50	1	15.601	76.390	65.601	116.45
			2	15.631	76.65	65.631	116.79
			3	15.996	76.987	65.996	116.65
Sea water	Bromoform	50	1	259.016	312.057	309.016	100.98
			2	260.397	311.1	310.397	100.23
			3	260.631	312.74	310.631	100.68

Table 3.20 Recovery based on matrices at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Sea water	Chloroform	150	1	2.640	148.513	152.640	97.30
			2	2.932	148.321	152.932	96.98
			3	2.745	147.998	152.745	96.89
Sea water	BDCM	150	1	2.941	178.823	152.941	116.92
			2	2.966	176.321	152.966	115.27
			3	2.974	173.651	152.974	113.52
Sea water	CDBM	150	1	15.601	199.181	165.601	120.28
			2	15.631	198.61	165.631	119.91
			3	15.996	199.756	165.996	120.34
Sea water	Bromoform	150	1	259.016	424.016	409.016	103.67
			2	260.397	423.65	410.397	103.23
			3	260.631	425.698	410.631	103.67

Table 3.21 Recovery based on matrices at 350 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Sea water	Chloroform	350	1	2.640	349.000	352.640	98.97
			2	2.932	350.361	352.932	99.27
			3	2.745	349.651	352.745	99.12
Sea water	BDCM	350	1	2.941	288.000	352.941	81.60
			2	2.966	289.321	352.966	81.97
			3	2.974	288.634	352.974	81.77
Sea water	CDBM	350	1	15.601	316.005	365.601	86.43
			2	15.631	314.97	365.631	86.14
			3	15.996	315.97	365.996	86.33
Sea water	Bromoform	350	1	259.016	663.656	609.016	108.97
			2	260.397	660.874	610.397	108.27
			3	260.631	663.111	610.631	108.59

Table 3.22 Recovery based on matrices at 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Woodstock Tap-Water	Chloroform	50	1	32.356	75.811	82.356	92.05
			2	32.665	76.111	82.665	92.07
			3	31.410	76.367	81.410	93.81
Woodstock Tap-Water	BDCM	50	1	15.729	72.986	65.729	111.04
			2	15.358	72.369	65.358	110.73
			3	15.987	73.631	65.987	111.58
Woodstock Tap-Water	CDBM	50	1	5.260	63.856	55.260	115.56
			2	5.393	63.987	55.393	115.51
			3	5.963	63.987	55.963	114.34
Woodstock Tap-Water	Bromoform	50	1	0.597	44.368	50.597	87.69
			2	0.601	44.056	50.601	87.07
			3	0.654	45.999	50.654	90.81

Table 3.23 Recovery based on matrices at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Woodstock (Tap-Water)	Chloroform	150	1	32.356	186.486	182.356	102.26
			2	32.665	185.601	182.665	101.61
			3	31.410	186.11	181.410	102.59
Woodstock (Tap-Water)	BDCM	150	1	15.729	171.263	165.729	103.34
			2	15.358	170.996	165.358	103.41
			3	15.987	170.658	165.987	102.81
Woodstock (Tap-Water)	CDBM	150	1	5.260	171.263	155.260	110.31
			2	5.393	171.987	155.393	110.68
			3	5.963	171.963	155.963	110.26
Woodstock (Tap-Water)	Bromoform	150	1	0.597	121.012	150.597	80.35
			2	0.601	121.013	150.601	80.35
			3	0.654	120.963	150.654	80.29

Table 3.24 Recovery based on matrices at 350 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Woodstock Tap-Water	Chloroform	350	1	32.356	413.000	382.356	108.01
			2	32.665	410.321	382.665	107.23
			3	31.410	409.555	381.410	107.38
Woodstock Tap-Water	BDCM	350	1	15.729	358.616	365.729	98.06
			2	15.358	359.61	365.358	98.43
			3	15.987	356.122	365.987	97.30
Woodstock Tap-Water	CDBM	350	1	5.260	285.886	355.260	80.47
			2	5.393	286.321	355.393	80.56
			3	5.963	284.999	355.963	80.06
Woodstock Tap-Water	Bromoform	350	1	0.597	358.195	350.597	102.17
			2	0.601	359.61	350.601	102.57
			3	0.654	359.74	350.654	102.59

Table 3.25 Recovery based on matrices at 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Johnson-Johnson Medical	Chloroform	50	1	0.580	46.101	50.580	91.14
			2	0.560	45.21	50.560	89.42
			3	0.511	45.631	50.511	90.34
Johnson-Johnson Medical	BDCM	50	1	0.775	59.235	50.775	116.66
			2	0.758	58.77	50.758	115.78
			3	0.761	59.111	50.761	116.45
Johnson-Johnson Medical	CDBM	50	1	0.550	59.463	50.550	117.63
			2	0.561	59.321	50.561	117.33
			3	0.557	56.789	50.557	112.33
Johnson-Johnson Medical	Bromoform	50	1	0.229	52.148	50.229	103.82
			2	0.220	51.369	50.220	102.29
			3	0.231	51.654	50.231	102.83

Table 3.26 Recovery based on matrices at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Johnson-Johnson Medical	Chloroform	150	1	0.580	170.896	150.580	113.49
			2	0.560	171.669	150.560	114.02
			3	0.511	170.961	150.511	113.59
Johnson-Johnson Medical	BDCM	150	1	0.775	175.550	150.775	116.43
			2	0.758	174.631	150.758	115.84
			3	0.761	172.651	150.761	114.52
Johnson-Johnson Medical	CDBM	150	1	0.550	166.356	150.550	110.50
			2	0.561	166.678	150.561	110.70
			3	0.557	166.980	150.557	110.91
Johnson-Johnson Medical	Bromoform	150	1	0.229	180.227	150.229	119.97
			2	0.220	179.698	150.220	119.62
			3	0.231	180.657	150.231	120.25

Table 3.27 Recovery based on matrices at 350 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Johnson-Johnson Medical	Chloroform	350	1	0.580	401.916	350.580	114.64
			2	0.560	400.321	350.560	114.19
			3	0.511	398.771	350.511	113.77
Johnson-Johnson Medical	BDCM	350	1	0.775	284.025	350.775	80.97
			2	0.758	285.961	350.758	81.53
			3	0.761	287.963	350.761	82.10
Johnson-Johnson Medical	CDBM	350	1	0.550	290.836	350.550	82.97
			2	0.561	393.540	350.561	112.26
			3	0.557	291.690	350.557	83.21
Johnson-Johnson Medical	Bromoform	350	1	0.229	406.611	350.229	116.10
			2	0.220	407.651	350.220	116.40
			3	0.231	407.114	350.231	116.24

Table 3.28 Recovery based on matrices at 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Unfilted Water	Chloroform	50	1	27.822	70.298	77.822	90.33
			2	26.900	69.994	76.900	91.02
			3	27.669	70.321	77.669	90.54
Unfilted Water	BDCM	50	1	10.140	60.726	60.140	100.97
			2	10.143	60.321	60.143	100.30
			3	11.031	60.963	61.031	99.89
Unfilted Water	CDBM	50	1	1.230	60.723	51.230	118.53
			2	1.254	59.365	51.254	115.83
			3	1.120	57.691	51.120	112.85
Unfilted Water	Bromoform	50	1	0.350	51.860	50.350	103.00
			2	0.401	51.011	50.401	101.21
			3	0.451	52.367	50.451	102.20

Table 3.29 Recovery based on matrices at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Unfilted Water	Chloroform	150	1	27.822	178.767	177.822	100.53
			2	26.900	175.336	176.900	99.12
			3	27.669	174.999	177.669	98.50
Unfilted Water	BDCM	150	1	10.140	183.348	160.140	114.49
			2	10.143	183.1	160.143	114.34
			3	11.031	181.971	161.031	113.00
Unfilted Water	CDBM	150	1	1.230	174.380	151.230	115.31
			2	1.254	172.963	151.254	114.35
			3	1.120	172.478	151.120	114.13
Unfilted Water	Bromoform	150	1	0.350	167.331	150.350	111.29
			2	0.401	165.1	150.401	109.77
			3	0.451	160.369	150.451	106.59

Table 3.30 Recovery based on matrices at 350 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Unfilted Water	Chloroform	350	1	27.822	408.779	377.822	108.19
			2	26.900	400.691	376.900	106.31
			3	27.669	399.987	377.669	105.91
Unfilted Water	BDCM	350	1	10.140	291.444	360.140	80.93
			2	10.143	298.654	360.143	82.93
			3	11.031	297.364	361.031	82.37
Unfilted Water	CDBM	350	1	1.230	301.755	351.230	85.91
			2	1.254	305.639	351.254	87.01
			3	1.120	309.367	351.120	88.11
Unfilted Water	Bromoform	350	1	0.350	415.999	350.350	118.74
			2	0.401	410.321	350.401	117.10
			3	0.451	410.987	350.451	117.27

Table 3.31 Recovery based on matrices at 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
River Water	Chloroform	50	1	2.367	51.550	52.367	98.44
			2	2.314	51.697	52.314	98.82
			3	2.147	52.397	52.147	100.48
River Water	BDCM	50	1	0.072	53.151	50.072	106.15
			2	0.078	53.987	50.078	107.81
			3	0.061	53.915	50.061	107.70
River Water	CDBM	50	1	0.049	47.019	50.049	93.95
			2	0.059	47.201	50.059	94.29
			3	0.06	49.987	50.060	99.85
River Water	Bromoform	50	1	0.072	46.049	50.072	91.97
			2	0.079	47.894	50.079	95.64
			3	0.061	49.111	50.061	98.10

Table 3.32 Recovery based on matrices at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
River Water	Chloroform	150	1	2.367	148.500	152.367	97.46
			2	2.314	146.654	152.314	96.28
			3	2.147	149.647	152.147	98.36
River Water	BDCM	150	1	0.072	179.200	150.072	119.41
			2	0.078	176.333	150.078	117.49
			3	0.061	176.781	150.061	117.81
River Water	CDBM	150	1	0.049	167.600	150.049	111.70
			2	0.059	165.146	150.059	110.05
			3	0.060	168.964	150.060	112.60
River Water	Bromoform	150	1	0.072	164.700	150.072	109.75
			2	0.079	167.931	150.079	111.90
			3	0.061	169.456	150.061	112.92

Table 3.33 Recovery based on matrices at 350 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
River Water	Chloroform	350	1	2.367	391.900	352.367	111.22
			2	2.314	395.123	352.314	112.15
			3	2.147	388.647	352.147	110.36
River Water	BDCM	350	1	0.072	284.800	350.072	81.35
			2	0.078	289.741	350.078	82.76
			3	0.061	285.654	350.061	81.60
River Water	CDBM	350	1	0.049	346.800	350.049	99.07
			2	0.059	346.987	350.059	99.12
			3	0.060	347.111	350.060	99.16
River Water	Bromoform	350	1	0.072	373.500	350.072	106.69
			2	0.079	371.951	350.079	106.25
			3	0.061	373.159	350.061	106.60

3.2.5 Recovery and Matrix Effects based on Interferences

Table 3.34 Recovery based on interferences for chloride standard at 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Chloride Standard	Chloroform	50	1	0.000	41.423	50.000	82.85
			2	0.000	40.877	50.000	81.75
			3	0.000	40.208	50.000	80.42
			4	0.000	40.064	50.000	80.13
			5	0.000	40.595	50.000	81.19
Chloride Standard	BDCM	50	1	0.000	54.739	50.000	109.48
			2	0.000	55.445	50.000	110.89
			3	0.000	56.228	50.000	112.46
			4	0.000	56.195	50.000	112.39
			5	0.000	55.348	50.000	110.70
Chloride Standard	CDBM	50	1	0.000	44.411	50.000	88.82
			2	0.000	46.619	50.000	93.24
			3	0.000	46.975	50.000	93.95
			4	0.000	47.056	50.000	94.11
			5	0.000	46.598	50.000	93.20
Chloride Standard	Bromoform	50	1	0.000	40.249	50.000	80.50
			2	0.000	40.102	50.000	80.20
			3	0.000	40.975	50.000	81.95
			4	0.000	40.032	50.000	80.06
			5	0.000	46.049	50.000	92.10

Table 3.35 Recovery based on interferences for fluoride standard at 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Fluoride Standard	Chloroform	50	1	0.000	42.565	50.000	85.13
			2	0.000	40.177	50.000	80.35
			3	0.000	40.422	50.000	80.84
			4	0.000	40.792	50.000	81.58
			5	0.000	40.948	50.000	81.90
Fluoride Standard	BDCM	50	1	0.000	59.602	50.000	119.20
			2	0.000	52.882	50.000	105.76
			3	0.000	58.732	50.000	117.46
			4	0.000	56.499	50.000	113.00
			5	0.000	59.191	50.000	118.38
Fluoride Standard	CDBM	50	1	0.000	59.998	50.000	120.00
			2	0.000	59.243	50.000	118.49
			3	0.000	55.416	50.000	110.83
			4	0.000	56.499	50.000	113.00
			5	0.000	59.767	50.000	119.53
Fluoride Standard	Bromoform	50	1	0.000	44.970	50.000	89.94
			2	0.000	44.228	50.000	88.46
			3	0.000	40.237	50.000	80.47
			4	0.000	40.792	50.000	81.58
			5	0.000	45.494	50.000	90.99

Table 3.36 Recovery based on interferences for SEPP-133 at 50 µg/L

Sample	THMs	Conc. µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
SEPP-133	Chloroform	50	1	0.000	40.488	50.000	80.98
			2	0.000	40.524	50.000	81.05
			3	0.000	40.978	50.000	81.96
			4	0.000	41.302	50.000	82.60
			5	0.000	40.326	50.000	80.65
SEPP-133	BDCM	50	1	0.000	58.385	50.000	116.77
			2	0.000	58.485	50.000	116.97
			3	0.000	55.884	50.000	111.77
			4	0.000	59.049	50.000	118.10
			5	0.000	51.398	50.000	102.80
SEPP-133	CDBM	50	1	0.000	59.896	50.000	119.79
			2	0.000	49.741	50.000	99.48
			3	0.000	55.904	50.000	111.81
			4	0.000	58.766	50.000	117.53
			5	0.000	51.836	50.000	103.67
SEPP-133	Bromoform	50	1	0.000	43.420	50.000	86.84
			2	0.000	40.859	50.000	81.72
			3	0.000	45.243	50.000	90.49
			4	0.000	43.424	50.000	86.85
			5	0.000	44.358	50.000	88.72

Table 3.37 Recovery based on interferences for chloride standard at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Chloride Standard	Chloroform	150	1	0.000	149.541	150.000	99.69
			2	0.000	153.115	150.000	102.08
			3	0.000	149.833	150.000	99.89
			4	0.000	151.474	150.000	100.98
			5	0.000	147.803	150.000	98.54
Chloride Standard	BDCM	150	1	0.000	178.541	150.000	119.03
			2	0.000	177.675	150.000	118.45
			3	0.000	175.808	150.000	117.21
			4	0.000	178.474	150.000	118.98
			5	0.000	177.087	150.000	118.06
Chloride Standard	CDBM	150	1	0.000	178.420	150.000	118.95
			2	0.000	176.336	150.000	117.56
			3	0.000	179.413	150.000	119.61
			4	0.000	176.105	150.000	117.40
			5	0.000	179.583	150.000	119.72
Chloride Standard	Bromoform	150	1	0.000	150.241	150.000	100.16
			2	0.000	152.055	150.000	101.37
			3	0.000	155.138	150.000	103.43
			4	0.000	155.181	150.000	103.45
			5	0.000	155.006	150.000	103.34

Table 3.38 Recovery based on interferences for fluoride standard at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Fluoride Standard	Chloroform	150	1	0.000	130.950	150.000	87.30
			2	0.000	130.648	150.000	87.10
			3	0.000	139.896	150.000	93.26
			4	0.000	125.784	150.000	83.86
			5	0.000	148.748	150.000	99.17
Fluoride Standard	BDCM	150	1	0.000	177.855	150.000	118.57
			2	0.000	175.350	150.000	116.90
			3	0.000	178.502	150.000	119.00
			4	0.000	174.051	150.000	116.03
			5	0.000	174.624	150.000	116.42
Fluoride Standard	CDBM	150	1	0.000	171.523	150.000	114.35
			2	0.000	173.156	150.000	115.44
			3	0.000	174.800	150.000	116.53
			4	0.000	158.865	150.000	105.91
			5	0.000	175.146	150.000	116.76
Fluoride Standard	Bromoform	150	1	0.000	142.916	150.000	95.28
			2	0.000	131.967	150.000	87.98
			3	0.000	145.337	150.000	96.89
			4	0.000	122.079	150.000	81.39
			5	0.000	145.865	150.000	97.24

Table 3.39 Recovery based on interferences for SEPP-133 at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
SEPP-133	Chloroform	150	1	0.000	125.061	150.000	83.37
			2	0.000	144.637	150.000	96.42
			3	0.000	139.542	150.000	93.03
			4	0.000	133.441	150.000	88.96
			5	0.000	139.294	150.000	92.86
SEPP-133	BDCM	150	1	0.000	179.258	150.000	119.51
			2	0.000	169.828	150.000	113.22
			3	0.000	172.964	150.000	115.31
			4	0.000	174.082	150.000	116.05
			5	0.000	170.528	150.000	113.69
SEPP-133	CDBM	150	1	0.000	164.992	150.000	109.99
			2	0.000	174.910	150.000	116.61
			3	0.000	173.423	150.000	115.62
			4	0.000	164.900	150.000	109.93
			5	0.000	170.127	150.000	113.42
SEPP-133	Bromoform	150	1	0.000	125.960	150.000	83.97
			2	0.000	146.905	150.000	97.94
			3	0.000	138.119	150.000	92.08
			4	0.000	132.717	150.000	88.48
			5	0.000	136.644	150.000	91.10

Table 3.40 Recovery based on interferences for chloride standard at 350 µg/L

Sample	THMs	Conc. µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Chloride Standard	Chloroform	350	1	0.000	332.217	350.000	94.92
			2	0.000	280.176	350.000	80.05
			3	0.000	304.408	350.000	86.97
			4	0.000	282.356	350.000	80.67
			5	0.000	373.850	350.000	106.81
Chloride Standard	BDCM	350	1	0.000	284.428	350.000	81.27
			2	0.000	282.244	350.000	80.64
			3	0.000	289.172	350.000	82.62
			4	0.000	281.872	350.000	80.53
			5	0.000	284.101	350.000	81.17
Chloride Standard	CDBM	350	1	0.000	281.892	350.000	80.54
			2	0.000	285.560	350.000	81.59
			3	0.000	285.748	350.000	81.64
			4	0.000	286.884	350.000	81.97
			5	0.000	286.078	350.000	81.74
Chloride Standard	Bromoform	350	1	0.000	345.929	350.000	98.84
			2	0.000	292.435	350.000	83.55
			3	0.000	319.806	350.000	91.37
			4	0.000	384.759	350.000	109.93
			5	0.000	356.472	350.000	101.85

Table 3.41 Recovery based on interferences for chloride standard at 350 µg/L

Sample	THMs	Conc .µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Fluoride Standard	Chloroform	350	1	0.000	363.025	350.000	103.72
			2	0.000	375.870	350.000	107.39
			3	0.000	376.212	350.000	107.49
			4	0.000	382.362	350.000	109.25
			5	0.000	333.229	350.000	95.21
Fluoride Standard	BDCM	350	1	0.000	282.055	350.000	80.59
			2	0.000	285.027	350.000	81.44
			3	0.000	280.155	350.000	80.04
			4	0.000	280.153	350.000	80.04
			5	0.000	283.150	350.000	80.90
Fluoride Standard	CDBM	350	1	0.000	285.756	350.000	81.64
			2	0.000	287.458	350.000	82.13
			3	0.000	280.244	350.000	80.07
			4	0.000	280.130	350.000	80.04
			5	0.000	284.302	350.000	81.23
Fluoride Standard	Bromoform	350	1	0.000	348.583	350.000	99.60
			2	0.000	357.452	350.000	102.13
			3	0.000	368.271	350.000	105.22
			4	0.000	368.254	350.000	105.22
			5	0.000	333.229	350.000	95.21

Table 3.42 Recovery based on interferences for SEPP-133 at 350 µg/L

Sample	THMs	Conc. µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
SEPP-133	Chloroform	350	1	0.000	285.811	350.000	81.66
			2	0.000	339.766	350.000	97.08
			3	0.000	361.990	350.000	103.43
			4	0.000	369.620	350.000	105.61
			5	0.000	353.857	350.000	101.10
SEPP-133	BDCM	350	1	0.000	283.637	350.000	81.04
			2	0.000	280.253	350.000	80.07
			3	0.000	287.371	350.000	82.11
			4	0.000	280.733	350.000	80.21
			5	0.000	284.742	350.000	81.35
SEPP-133	CDBM	350	1	0.000	281.427	350.000	80.41
			2	0.000	284.876	350.000	81.39
			3	0.000	280.641	350.000	80.18
			4	0.000	283.021	350.000	80.86
			5	0.000	288.396	350.000	82.40
SEPP-133	Bromoform	350	1	0.000	298.204	350.000	85.20
			2	0.000	308.060	350.000	88.02
			3	0.000	332.167	350.000	94.90
			4	0.000	341.514	350.000	97.58
			5	0.000	354.778	350.000	101.37

3.2.6 Precision based on Reproducibility

Table 3.43 Precision based on reproducibility for analyst 1 water 50 µg/L

Sample Description	THMs	Conc. µg/L	Inj. No	Unspiked sample µg/L	Spiked Sample Amount µg/L	Actual Sample Amount µg/L	Mean	Stdev	% RSD
Municipal Water (Midrand)	Chloroform	50	1	11.702	68.361	56.66	55.76	1.98	3.56
			2	11.711	66.980	55.28			
			3	11.690	64.477	52.78			
			4	11.569	69.866	58.16			
			5	11.740	67.626	55.92			
Municipal Water (Midrand)	BDCM	50	1	3.266	70.010	66.74	66.23	2.25	3.39
			2	3.564	69.372	66.11			
			3	3.265	66.318	63.05			
			4	3.210	72.612	69.35			
			5	3.330	69.172	65.91			
Municipal Water (Midrand)	CDBM	50	1	0.823	59.220	58.40	57.87	1.95	3.37
			2	0.832	58.646	57.82			
			3	0.835	55.827	55.00			
			4	0.845	61.274	60.45			
			5	0.845	58.504	57.68			
Municipal Water (Midrand)	Bromoform	50	1	0.106	58.643	58.64	58.19	1.95	3.35
			2	0.100	58.228	58.12			
			3	0.123	55.456	55.35			
			4	0.125	60.942	60.84			
			5	0.101	58.116	58.01			

Table 3.44 Precision based on reproducibility for analyst 2 water 50 µg/L

Sample Description	THMs	Conc µg/L	Inj. No	Unspiked sample µg/L	Spiked Sample Amount µg/L	Actual Sample Amount µg/L	Mean	Stdev	% RSD
Municipal Water (Midrand)	Chloroform	50	1	11.959	58.334	46.38	49.90	4.44	8.90
			2	11.954	63.456	51.50			
			3	11.880	64.295	52.42			
			4	11.950	66.964	55.01			
			5	11.981	56.203	44.22			
Municipal Water (Midrand)	BDCM	50	1	3.422	61.042	57.62	60.75	4.64	7.64
			2	3.420	65.900	62.48			
			3	3.401	66.333	62.93			
			4	3.366	65.648	62.28			
			5	3.433	57.950	54.52			
Municipal Water (Midrand)	CDBM	50	1	0.887	51.900	51.01	53.47	3.90	7.29
			2	0.890	55.684	54.79			
			3	0.863	56.082	55.22			
			4	0.852	58.970	58.12			
			5	0.820	49.005	48.19			
Municipal Water (Midrand)	Bromoform	50	1	0.137	54.203	54.07	55.66	1.79	3.22
			2	0.160	55.617	55.46			
			3	0.146	55.870	55.72			
			4	0.171	58.766	58.60			
			5	0.134	54.600	54.47			

Table 3.45 Precision based on reproducibility for analyst 1 water 50 µg/L

Sample Description	THMs	Conc. µg/L	Inj. No	Unspiked sample µg/L	Spiked Sample Amount µg/L	Actual Sample Amount µg/L	Mean	Stdev	% RSD
Chlorinated Water (MIDRAND)	Chloroform	50	1	10.770	65.163	54.39	53.83	3.99	7.42
			2	10.653	66.884	56.23			
			3	10.365	69.044	58.68			
			4	10.555	60.967	50.41			
			5	10.120	59.531	49.41			
Chlorinated Water (MIDRAND)	BDCM	50	1	2.594	66.730	64.14	63.38	4.19	6.61
			2	2.600	68.420	65.82			
			3	2.445	71.147	68.70			
			4	2.654	62.724	60.07			
			5	2.666	60.837	58.17			
Chlorinated Water (MIDRAND)	CDBM	50	1	0.888	56.411	55.52	55.48	3.85	6.94
			2	0.895	58.585	57.69			
			3	0.890	61.471	60.58			
			4	0.870	53.380	52.51			
			5	0.865	51.965	51.10			
Chlorinated Water (MIDRAND)	Bromoform	50	1	0.120	55.638	55.52	55.73	3.70	6.64
			2	0.123	57.843	57.72			
			3	0.130	60.934	60.80			
			4	0.133	53.296	53.16			
			5	0.130	51.562	51.43			

Table 3.46 Precision based on reproducibility for analyst 2 water 50 µg/L

Sample Description	THMs	Conc. µg/L	Inj. No	Unspiked sample µg/L	Spiked Sample Amount µg/L	Actual Sample Amount µg/L	Mean	Stdev	% RSD
Chlorinated Water (MIDRAND)	Chloroform	50	1	11.442	63.025	51.58	55.33	3.47	6.27
			2	11.402	64.335	52.89			
			3	11.330	71.064	59.62			
			4	11.354	69.716	58.27			
			5	11.654	65.716	54.27			
Chlorinated Water (MIDRAND)	BDCM	50	1	2.419	64.177	61.76	66.36	4.58	6.90
			2	2.314	64.988	62.57			
			3	2.360	74.149	71.73			
			4	2.540	73.017	70.60			
			5	2.451	67.542	65.12			
Chlorinated Water (MIDRAND)	CDBM	50	1	0.819	54.581	53.76	57.80	3.79	6.56
			2	0.830	55.665	54.85			
			3	0.835	63.078	62.26			
			4	0.836	62.046	61.23			
			5	0.822	57.734	56.92			
Chlorinated Water (MIDRAND)	Bromoform	50	1	0.103	53.205	53.21	57.41	3.93	6.85
			2	0.106	54.219	54.12			
			3	0.125	62.017	61.91			
			4	0.120	60.963	60.86			
			5	0.135	57.065	56.96			

3.2.7 Accuracy based on recovery

Table 3.47 Accuracy based on recovery for 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Sample Amount Obtained µg/L	Theoretical Concentration µg/L	% Recovery	Mean	Stdev	% RSD
Borehole Water	Chloroform	50	1	44.059	50.000	88.12	88.14	1.16	1.32
			2	43.468	50.000	86.94			
			3	43.719	50.000	87.44			
			4	45.002	50.000	90.00			
			5	44.100	50.000	88.20			
Borehole Water	BDCM	50	1	46.005	50.000	92.01	92.43	1.59	1.72
			2	45.432	50.000	90.86			
			3	45.826	50.000	91.65			
			4	47.524	50.000	95.05			
			5	46.283	50.000	92.57			
Borehole Water	CDBM	50	1	41.052	50.000	82.10	82.60	1.39	1.68
			2	40.654	50.000	81.31			
			3	40.945	50.000	81.89			
			4	42.446	50.000	84.89			
			5	41.406	50.000	82.81			
Borehole Water	Bromoform	50	1	40.696	50.000	81.39	81.90	1.43	1.75
			2	40.360	50.000	80.72			
			3	40.529	50.000	81.06			
			4	42.154	50.000	84.31			
			5	41.022	50.000	82.04			

Table 3.48 Accuracy based on recovery for 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Sample Amount Obtained µg/L	Theoretical Concentration µg/L	% Recovery	Mean	Stdev	% RSD
Borehole Water	Chloroform	150	1	141.570	150.000	94.38	101.52	5.23	5.15
			2	148.900	150.000	99.27			
			3	151.058	150.000	100.71			
			4	159.210	150.000	106.14			
			5	160.664	150.000	107.11			
Borehole Water	BDCM	150	1	157.150	150.000	104.77	112.54	5.35	4.75
			2	166.620	150.000	111.08			
			3	167.469	150.000	111.65			
			4	176.620	150.000	117.75			
			5	176.180	150.000	117.45			
Borehole Water	CDBM	150	1	134.544	150.000	89.70	98.39	6.13	6.23
			2	144.560	150.000	96.37			
			3	145.980	150.000	97.32			
			4	156.170	150.000	104.11			
			5	156.680	150.000	104.45			
Borehole Water	Bromoform	150	1	130.710	150.000	87.14	95.15	5.74	6.03
			2	140.140	150.000	93.43			
			3	140.710	150.000	93.81			
			4	149.820	150.000	99.88			
			5	152.250	150.000	101.50			

Table 3.49 Accuracy based on recovery for 350 µg/L

Sample	THMs	Conc µg/L	Inj. No	Sample Amount Obtained µg/L	Theoretical Concentration µg/L	% Recovery	Mean	Stdev	% RSD
Borehole Water	Chloroform	350	1	405.500	350.000	115.86	112.23	4.95	4.41
			2	379.700	350.000	108.49			
			3	417.000	350.000	119.14			
			4	380.900	350.000	108.83			
			5	380.900	350.000	108.83			
Borehole Water	BDCM	350	1	302.700	350.000	86.49	84.85	1.31	1.54
			2	290.600	350.000	83.03			
			3	294.500	350.000	84.14			
			4	298.200	350.000	85.20			
			5	298.800	350.000	85.37			
Borehole Water	CDBM	350	1	366.400	350.000	104.69	102.86	1.57	1.53
			2	351.600	350.000	100.46			
			3	358.500	350.000	102.43			
			4	361.500	350.000	103.29			
			5	362.100	350.000	103.46			
Borehole Water	Bromoform	350	1	419.500	350.000	119.86	116.17	3.98	3.43
			2	383.900	350.000	109.69			
			3	403.700	350.000	115.34			
			4	414.300	350.000	118.37			
			5	411.600	350.000	117.60			

Table 3.50 Accuracy based on recovery for 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Sample Amount Obtained µg/L	Theoretical Concentration µg/L	% Recovery	Mean	Stdev	% RSD
Municipal Water	Chloroform	50	1	43.500	50.000	87.00	86.88	0.78	0.90
			2	43.000	50.000	86.00			
			3	43.200	50.000	86.40			
			4	43.500	50.000	87.00			
			5	44.000	50.000	88.00			
Municipal Water	BDCM	50	1	53.400	50.000	106.80	106.36	1.04	0.98
			2	52.500	50.000	105.00			
			3	52.800	50.000	105.60			
			4	53.400	50.000	106.80			
			5	53.800	50.000	107.60			
Municipal Water	CDBM	50	1	48.700	50.000	97.40	97.36	0.46	0.47
			2	48.300	50.000	96.60			
			3	48.800	50.000	97.60			
			4	48.900	50.000	97.80			
			5	48.700	50.000	97.40			
Municipal Water	Bromoform	50	1	50.700	50.000	101.40	102.12	0.12	0.12
			2	50.600	50.000	101.20			
			3	51.000	50.000	102.00			
			4	50.900	50.000	101.80			
			5	52.100	50.000	104.20			

Table 3.51 Accuracy based on recovery for 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Sample Amount Obtained µg/L	Theoretical Concentration µg/L	% Recovery	Mean	Stdev	% RSD
Municipal Water	Chloroform	150	1	165.110	150.000	110.07	109.41	3.21	2.93
			2	160.150	150.000	106.77			
			3	169.910	150.000	113.27			
			4	167.100	150.000	111.40			
			5	158.310	150.000	105.54			
Municipal Water	BDCM	150	1	170.360	150.000	113.57	114.68	1.73	1.51
			2	169.500	150.000	113.00			
			3	171.000	150.000	114.00			
			4	175.910	150.000	117.27			
			5	173.360	150.000	115.57			
Municipal Water	CDBM	150	1	168.600	150.000	112.40	116.12	3.35	2.88
			2	169.030	150.000	112.69			
			3	177.170	150.000	118.11			
			4	179.510	150.000	119.67			
			5	176.595	150.000	117.73			
Municipal Water	Bromoform	150	1	160.347	150.000	106.90	110.69	3.35	3.03
			2	161.050	150.000	107.37			
			3	169.170	150.000	112.78			
			4	171.511	150.000	114.34			
			5	168.082	150.000	112.05			

Table 3.52 Accuracy based on recovery for 350 µg/L

Sample	THMs	Conc µg/L	Inj. No	Sample Amount Obtained µg/L	Theoretical Concentration µg/L	% Recovery	Mean	Stdev	% RSD
Municipal Water	Chloroform	350	1	404.980	350.000	115.71	114.72	0.98	0.85
			2	402.470	350.000	114.99			
			3	403.430	350.000	115.27			
			4	396.040	350.000	113.15			
			5	400.750	350.000	114.50			
Municipal Water	BDCM	350	1	288.920	350.000	82.55	81.59	0.91	1.12
			2	281.030	350.000	80.29			
			3	284.230	350.000	81.21			
			4	285.410	350.000	81.55			
			5	288.260	350.000	82.36			
Municipal Water	CDBM	350	1	358.810	350.000	102.52	100.69	1.91	1.90
			2	341.820	350.000	97.66			
			3	350.310	350.000	100.09			
			4	354.850	350.000	101.39			
			5	356.230	350.000	101.78			
Municipal Water	Bromoform	350	1	409.060	350.000	116.87	112.28	4.29	3.82
			2	369.410	350.000	105.55			
			3	388.810	350.000	111.09			
			4	397.420	350.000	113.55			
			5	400.196	350.000	114.34			

Table 3.53 Accuracy based on recovery for 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Sample Amount Obtained µg/L	Theoretical Concentration µg/L	% Recovery	Mean	Stdev	% RSD
Underground Water	Chloroform	50	1	44.119	50.000	88.24	94.99	4.00	4.21
			2	48.047	50.000	96.09			
			3	47.857	50.000	95.71			
			4	47.986	50.000	95.97			
			5	49.470	50.000	98.94			
Underground Water	BDCM	50	1	48.356	50.000	96.71	105.31	5.24	4.98
			2	53.422	50.000	106.84			
			3	52.706	50.000	105.41			
			4	53.321	50.000	106.64			
			5	55.482	50.000	110.96			
Underground Water	CDBM	50	1	44.864	50.000	89.73	98.14	5.09	5.19
			2	49.612	50.000	99.22			
			3	49.299	50.000	98.60			
			4	49.780	50.000	99.56			
			5	51.791	50.000	103.58			
Underground Water	Bromoform	50	1	45.296	50.000	90.59	99.06	5.08	5.13
			2	50.291	50.000	100.58			
			3	49.778	50.000	99.56			
			4	50.127	50.000	100.25			
			5	52.156	50.000	104.31			

Table 3.54 Accuracy based on recovery for 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Sample Amount Obtained µg/L	Theoretical Concentration µg/L	% Recovery	Mean	Stdev	% RSD
Underground Water	Chloroform	150	1	135.400	150.000	90.27	92.67	2.22	2.40
			2	138.700	150.000	92.47			
			3	137.200	150.000	91.47			
			4	139.400	150.000	92.93			
			5	144.300	150.000	96.20			
Underground Water	BDCM	150	1	169.400	150.000	112.93	115.33	2.45	2.12
			2	173.400	150.000	115.60			
			3	170.300	150.000	113.53			
			4	173.100	150.000	115.40			
			5	178.800	150.000	119.20			
Underground Water	CDBM	150	1	142.200	150.000	94.80	97.37	2.73	2.80
			2	145.900	150.000	97.27			
			3	142.900	150.000	95.27			
			4	146.800	150.000	97.87			
			5	152.500	150.000	101.67			
Underground Water	Bromoform	150	1	138.400	150.000	92.27	94.68	2.62	2.77
			2	142.000	150.000	94.67			
			3	138.600	150.000	92.40			
			4	143.100	150.000	95.40			
			5	148.000	150.000	98.67			

Table 3.55 Accuracy based on recovery for 350 µg/L

Sample	THMs	Conc µg/L	Inj. No	Sample Amount Obtained µg/L	Theoretical Concentration µg/L	% Recovery	Mean	Stdev	% RSD
Underground Water	Chloroform	350	1	392.600	350.000	112.17	113.50	3.05	2.69
			2	384.600	350.000	109.89			
			3	393.500	350.000	112.43			
			4	403.500	350.000	115.29			
			5	412.100	350.000	117.74			
Underground Water	BDCM	350	1	289.700	350.000	82.77	82.79	1.84	2.22
			2	287.700	350.000	82.20			
			3	280.200	350.000	80.06			
			4	296.400	350.000	84.69			
			5	294.900	350.000	84.26			
Underground Water	CDBM	350	1	355.100	350.000	101.46	101.84	1.46	1.43
			2	349.900	350.000	99.97			
			3	354.200	350.000	101.20			
			4	360.200	350.000	102.91			
			5	362.800	350.000	103.66			
Underground Water	Bromoform	350	1	397.600	350.000	113.60	113.99	2.88	2.53
			2	383.100	350.000	109.46			
			3	394.300	350.000	112.66			
			4	407.600	350.000	116.46			
			5	412.300	350.000	117.80			

Table 3.56 Accuracy based on recovery for 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Sample Amount Obtained µg/L	Theoretical Concentration µg/L	% Recovery	Mean	Stdev	% RSD
River Water	Chloroform	50	1	50.305	50.000	100.61	102.56	2.16	2.11
			2	51.550	50.000	103.10			
			3	50.125	50.000	100.25			
			4	51.671	50.000	103.34			
			5	52.752	50.000	105.50			
River Water	BDCM	50	1	51.863	50.000	103.73	105.73	2.14	2.02
			2	53.151	50.000	106.30			
			3	51.736	50.000	103.47			
			4	53.272	50.000	106.54			
			5	54.292	50.000	108.58			
River Water	CDBM	50	1	45.957	50.000	91.91	93.77	2.05	2.19
			2	47.019	50.000	94.04			
			3	45.852	50.000	91.70			
			4	47.251	50.000	94.50			
			5	48.338	50.000	96.68			
River Water	Bromoform	50	1	45.191	50.000	90.38	92.11	1.64	1.78
			2	46.049	50.000	92.10			
			3	45.332	50.000	90.66			
			4	46.562	50.000	93.12			
			5	47.139	50.000	94.28			

Table 3.57 Accuracy based on recovery for 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Sample Amount Obtained µg/L	Theoretical Concentration µg/L	% Recovery	Mean	Stdev	% RSD
River Water	Chloroform	150	1	144.200	150.000	96.13	96.97	2.16	2.23
			2	137.600	150.000	91.73			
			3	149.300	150.000	99.53			
			4	148.500	150.000	99.00			
			5	147.700	150.000	98.47			
River Water	BDCM	150	1	176.800	150.000	117.87	118.40	2.14	1.81
			2	175.200	150.000	116.80			
			3	178.200	150.000	118.80			
			4	179.200	150.000	119.47			
			5	178.600	150.000	119.07			
River Water	CDBM	150	1	159.300	150.000	106.20	107.93	2.05	1.90
			2	151.200	150.000	100.80			
			3	166.400	150.000	110.93			
			4	167.600	150.000	111.73			
			5	165.000	150.000	110.00			
River Water	Bromoform	150	1	156.000	150.000	104.00	105.60	1.64	1.55
			2	147.500	150.000	98.33			
			3	162.700	150.000	108.47			
			4	164.700	150.000	109.80			
			5	161.100	150.000	107.40			

Table 3.58 Accuracy based on recovery for 350 µg/L

Sample	THMs	Conc µg/L	Inj. No	Sample Amount Obtained µg/L	Theoretical Concentration µg/L	% Recovery	Mean	Stdev	% RSD
River Water	Chloroform	350	1	400.000	350.000	114.29	114.18	2.16	1.89
			2	398.200	350.000	113.77			
			3	399.700	350.000	114.20			
			4	408.300	350.000	116.66			
			5	391.900	350.000	111.97			
River Water	BDCM	350	1	289.900	350.000	82.83	82.66	2.14	2.59
			2	290.300	350.000	82.94			
			3	287.400	350.000	82.11			
			4	294.200	350.000	84.06			
			5	284.800	350.000	81.37			
River Water	CDBM	350	1	350.800	350.000	100.23	100.31	2.05	2.04
			2	354.100	350.000	101.17			
			3	351.200	350.000	100.34			
			4	352.600	350.000	100.74			
			5	346.800	350.000	99.09			
River Water	Bromoform	350	1	376.400	350.000	107.54	109.01	1.64	1.50
			2	385.700	350.000	110.20			
			3	383.000	350.000	109.43			
			4	389.100	350.000	111.17			
			5	373.500	350.000	106.71			

3.2.8 Selectivity based on recovery

Table 3.59 Selectivity based on recovery for 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
River Water	Chloroform	50	1	1.220	57.029	51.220	111.34
			2	1.121	56.987	51.121	111.47
			3	1.147	57.111	51.147	111.66
River Water	BDCM	50	1	0.059	59.685	50.059	119.23
			2	0.064	57.697	50.064	115.25
			3	0.067	55.937	50.067	111.72
River Water	CDBM	50	1	0.056	57.800	50.056	115.47
			2	0.059	56.987	50.059	113.84
			3	0.060	58.951	50.060	117.76
River Water	Bromoform	50	1	0.144	59.033	50.144	117.73
			2	0.179	59.657	50.079	119.13
			3	0.161	59.369	50.061	118.59

Table 3.60 Selectivity based on recovery for 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Underground Water	Chloroform	50	1	0.763	55.760	50.763	109.84
			2	0.698	54.123	50.698	106.76
			3	0.680	55.976	50.680	110.45
Underground Water	BDCM	50	1	0.081	59.815	50.081	119.44
			2	0.077	58.367	50.077	116.55
			3	0.074	58.369	50.074	116.57
Underground Water	CDBM	50	1	0.051	59.427	50.051	118.73
			2	0.059	58.324	50.059	116.51
			3	0.060	58.951	50.060	117.76
Underground Water	Bromoform	50	1	0.092	60.094	50.092	119.97
			2	0.087	59.674	50.087	119.14
			3	0.077	56.774	50.077	113.37

Table 3.61 Selectivity based on recovery for 80 µg

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Municipal Water	Chloroform	80	1	0.349	66.542	80.349	82.82
			2	0.698	65.974	80.698	81.75
			3	0.68	65.784	80.680	81.54
Municipal Water	BDCM	80	1	0.065	95.753	80.065	119.59
			2	0.077	93.478	80.077	116.74
			3	0.074	94.781	80.074	118.37
Municipal Water	CDBM	80	1	0.052	89.298	80.052	111.55
			2	0.059	88.324	80.059	110.32
			3	0.060	87.324	80.060	109.07
Municipal Water	Bromoform	80	1	0.000	68.770	80.000	85.96
			2	0.000	65.147	80.000	81.43
			3	0.000	67.684	80.000	84.61

3.2.9 Selectivity based on Interferences

Table 3.62 Recovery based on interferences for chloride standard at 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Chloride Standard	Chloroform	50	1	0.000	41.423	50.000	82.85
			2	0.000	40.877	50.000	81.75
			3	0.000	40.208	50.000	80.42
			4	0.000	40.064	50.000	80.13
			5	0.000	40.595	50.000	81.19
Chloride Standard	BDCM	50	1	0.000	54.739	50.000	109.48
			2	0.000	55.445	50.000	110.89
			3	0.000	56.228	50.000	112.46
			4	0.000	56.195	50.000	112.39
			5	0.000	55.348	50.000	110.70
Chloride Standard	CDBM	50	1	0.000	44.411	50.000	88.82
			2	0.000	46.619	50.000	93.24
			3	0.000	46.975	50.000	93.95
			4	0.000	47.056	50.000	94.11
			5	0.000	46.598	50.000	93.20
Chloride Standard	Bromoform	50	1	0.000	40.249	50.000	80.50
			2	0.000	40.102	50.000	80.20
			3	0.000	40.975	50.000	81.95
			4	0.000	40.032	50.000	80.06
			5	0.000	46.049	50.000	92.10

Table 3.63 Recovery based on interferences for fluoride standard at 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Fluoride Standard	Chloroform	50	1	0.000	42.565	50.000	85.13
			2	0.000	40.177	50.000	80.35
			3	0.000	40.422	50.000	80.84
			4	0.000	40.792	50.000	81.58
			5	0.000	40.948	50.000	81.90
Fluoride Standard	BDCM	50	1	0.000	59.602	50.000	119.20
			2	0.000	52.882	50.000	105.76
			3	0.000	58.732	50.000	117.46
			4	0.000	56.499	50.000	113.00
			5	0.000	59.191	50.000	118.38
Fluoride Standard	CDBM	50	1	0.000	59.998	50.000	120.00
			2	0.000	59.243	50.000	118.49
			3	0.000	55.416	50.000	110.83
			4	0.000	56.499	50.000	113.00
			5	0.000	59.767	50.000	119.53
Fluoride Standard	Bromoform	50	1	0.000	44.970	50.000	89.94
			2	0.000	44.228	50.000	88.46
			3	0.000	40.237	50.000	80.47
			4	0.000	40.792	50.000	81.58
			5	0.000	45.494	50.000	90.99

Table 3.64 Recovery based on interferences for SEPP-133 at 50 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
SEPP-133	Chloroform	50	1	0.000	40.488	50.000	80.98
			2	0.000	40.524	50.000	81.05
			3	0.000	40.978	50.000	81.96
			4	0.000	41.302	50.000	82.60
			5	0.000	40.326	50.000	80.65
SEPP-133	BDCM	50	1	0.000	58.385	50.000	116.77
			2	0.000	58.485	50.000	116.97
			3	0.000	55.884	50.000	111.77
			4	0.000	59.049	50.000	118.10
			5	0.000	51.398	50.000	102.80
SEPP-133	CDBM	50	1	0.000	59.896	50.000	119.79
			2	0.000	49.741	50.000	99.48
			3	0.000	55.904	50.000	111.81
			4	0.000	58.766	50.000	117.53
			5	0.000	51.836	50.000	103.67
SEPP-133	Bromoform	50	1	0.000	43.420	50.000	86.84
			2	0.000	40.859	50.000	81.72
			3	0.000	45.243	50.000	90.49
			4	0.000	43.424	50.000	86.85
			5	0.000	44.358	50.000	88.72

Table 3.65 Recovery based on interferences for chloride standard at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Chloride Standard	Chloroform	150	1	0.000	149.541	150.000	99.69
			2	0.000	153.115	150.000	102.08
			3	0.000	149.833	150.000	99.89
			4	0.000	151.474	150.000	100.98
			5	0.000	147.803	150.000	98.54
Chloride Standard	BDCM	150	1	0.000	178.541	150.000	119.03
			2	0.000	177.675	150.000	118.45
			3	0.000	175.808	150.000	117.21
			4	0.000	178.474	150.000	118.98
			5	0.000	177.087	150.000	118.06
Chloride Standard	CDBM	150	1	0.000	178.420	150.000	118.95
			2	0.000	176.336	150.000	117.56
			3	0.000	179.413	150.000	119.61
			4	0.000	176.105	150.000	117.40
			5	0.000	179.583	150.000	119.72
Chloride Standard	Bromoform	150	1	0.000	150.241	150.000	100.16
			2	0.000	152.055	150.000	101.37
			3	0.000	155.138	150.000	103.43
			4	0.000	155.181	150.000	103.45
			5	0.000	155.006	150.000	103.34

Table 3.66 Recovery based on interferences for fluoride standard at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Fluoride Standard	Chloroform	150	1	0.000	130.950	150.000	87.30
			2	0.000	130.648	150.000	87.10
			3	0.000	139.896	150.000	93.26
			4	0.000	125.784	150.000	83.86
			5	0.000	148.748	150.000	99.17
Fluoride Standard	BDCM	150	1	0.000	177.855	150.000	118.57
			2	0.000	175.350	150.000	116.90
			3	0.000	178.502	150.000	119.00
			4	0.000	174.051	150.000	116.03
			5	0.000	174.624	150.000	116.42
Fluoride Standard	CDBM	150	1	0.000	171.523	150.000	114.35
			2	0.000	173.156	150.000	115.44
			3	0.000	174.800	150.000	116.53
			4	0.000	158.865	150.000	105.91
			5	0.000	175.146	150.000	116.76
Fluoride Standard	Bromoform	150	1	0.000	142.916	150.000	95.28
			2	0.000	131.967	150.000	87.98
			3	0.000	145.337	150.000	96.89
			4	0.000	122.079	150.000	81.39
			5	0.000	145.865	150.000	97.24

Table 3.67 Recovery based on interferences for SEPP-133 at 150 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
SEPP-133	Chloroform	150	1	0.000	125.061	150.000	83.37
			2	0.000	144.637	150.000	96.42
			3	0.000	139.542	150.000	93.03
			4	0.000	133.441	150.000	88.96
			5	0.000	139.294	150.000	92.86
SEPP-133	BDCM	150	1	0.000	179.258	150.000	119.51
			2	0.000	169.828	150.000	113.22
			3	0.000	172.964	150.000	115.31
			4	0.000	174.082	150.000	116.05
			5	0.000	170.528	150.000	113.69
SEPP-133	CDBM	150	1	0.000	164.992	150.000	109.99
			2	0.000	174.910	150.000	116.61
			3	0.000	173.423	150.000	115.62
			4	0.000	164.900	150.000	109.93
			5	0.000	170.127	150.000	113.42
SEPP-133	Bromoform	150	1	0.000	125.960	150.000	83.97
			2	0.000	146.905	150.000	97.94
			3	0.000	138.119	150.000	92.08
			4	0.000	132.717	150.000	88.48
			5	0.000	136.644	150.000	91.10

Table 3.68 Recovery based on interferences for chloride standard at 350 µg/L

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Chloride Standard	Chloroform	350	1	0.000	332.217	350.000	94.92
			2	0.000	280.176	350.000	80.05
			3	0.000	304.408	350.000	86.97
			4	0.000	282.356	350.000	80.67
			5	0.000	373.850	350.000	106.81
Chloride Standard	BDCM	350	1	0.000	284.428	350.000	81.27
			2	0.000	282.244	350.000	80.64
			3	0.000	289.172	350.000	82.62
			4	0.000	281.872	350.000	80.53
			5	0.000	284.101	350.000	81.17
Chloride Standard	CDBM	350	1	0.000	281.892	350.000	80.54
			2	0.000	285.560	350.000	81.59
			3	0.000	285.748	350.000	81.64
			4	0.000	286.884	350.000	81.97
			5	0.000	286.078	350.000	81.74
Chloride Standard	Bromoform	350	1	0.000	345.929	350.000	98.84
			2	0.000	292.435	350.000	83.55
			3	0.000	319.806	350.000	91.37
			4	0.000	384.759	350.000	109.93
			5	0.000	356.472	350.000	101.85

Table 3.69 Recovery based on interferences for chloride standard at 350 µg/L

Sample	THMs	Conc. µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
Fluoride Standard	Chloroform	350	1	0.000	363.025	350.000	103.72
			2	0.000	375.870	350.000	107.39
			3	0.000	376.212	350.000	107.49
			4	0.000	382.362	350.000	109.25
			5	0.000	333.229	350.000	95.21
Fluoride Standard	BDCM	350	1	0.000	282.055	350.000	80.59
			2	0.000	285.027	350.000	81.44
			3	0.000	280.155	350.000	80.04
			4	0.000	280.153	350.000	80.04
			5	0.000	283.150	350.000	80.90
Fluoride Standard	CDBM	350	1	0.000	285.756	350.000	81.64
			2	0.000	287.458	350.000	82.13
			3	0.000	280.244	350.000	80.07
			4	0.000	280.130	350.000	80.04
			5	0.000	284.302	350.000	81.23
Fluoride Standard	Bromoform	350	1	0.000	348.583	350.000	99.60
			2	0.000	357.452	350.000	102.13
			3	0.000	368.271	350.000	105.22
			4	0.000	368.254	350.000	105.22
			5	0.000	333.229	350.000	95.21

Table 3.70 Recovery based on interferences for SEPP-133 at 350 µg/L

Sample	THMs	Conc. µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Theoretical Concentration µg/L	% Recovery
SEPP-133	Chloroform	350	1	0.000	285.811	350.000	81.66
			2	0.000	339.766	350.000	97.08
			3	0.000	361.990	350.000	103.43
			4	0.000	369.620	350.000	105.61
			5	0.000	353.857	350.000	101.10
SEPP-133	BDCM	350	1	0.000	283.637	350.000	81.04
			2	0.000	280.253	350.000	80.07
			3	0.000	287.371	350.000	82.11
			4	0.000	280.733	350.000	80.21
			5	0.000	284.742	350.000	81.35
SEPP-133	CDBM	350	1	0.000	281.427	350.000	80.41
			2	0.000	284.876	350.000	81.39
			3	0.000	280.641	350.000	80.18
			4	0.000	283.021	350.000	80.86
			5	0.000	288.396	350.000	82.40
SEPP-133	Bromoform	350	1	0.000	298.204	350.000	85.20
			2	0.000	308.060	350.000	88.02
			3	0.000	332.167	350.000	94.90
			4	0.000	341.514	350.000	97.58
			5	0.000	354.778	350.000	101.37

3.2.10 Inter Laboratory Comparison

Table 3.71 Inter-Laboratory Comparison for Tap water

Company	Sample Identification	Analyte	Concentration (µg/L)	Combined THMs
Microchem	Tap Water AG 85690	Chloroform	0.176	<1.00
		Bromodichloromethane	0.080	
		Chlorodibromomethane	0.044	
		Bromoform	0.045	
Company	**Sample Identification**	**Analyte**	**Concentration (µg/L)**	**Combined THMs**
A.L.Abbott and Association	Tap Water AG 85690	Chloroform	<1.00	<1.00
		Bromodichloromethane	<1.00	
		Chlorodibromomethane	<1.00	
		Bromoform	<1.00	

Table 3.72 Inter-Laboratory Comparison for Municipal Water

Company	Sample Identification	Analyte	Concentration (µg/L)	Combined THMs
Microchem	Municipal Water AG 92981	Chloroform	0.869	<1.00
		Bromodichloromethane	0.167	
		Chlorodibromomethane	0.900	
		Bromoform	0.885	
Company	**Sample Identification**	**Analyte**	**Concentration (µg/L)**	**Combined THMs**
A.L.Abbott and Association	Municipal Water AG 92981	Chloroform	<1.00	<1.00
		Bromodichloromethane	<1.00	
		Chlorodibromomethane	<1.00	
		Bromoform	<1.00	

Table 3.72 Inter-Laboratory Comparison for Johnson-Johnson Medical Water

Company	Sample Identification	Analyte	Concentration (µg/L)	Combined THMs
Microchem	Johnson-Johnson Medical Water AG 92982	Chloroform	0.580	<1.00
		Bromodichloromethane	0.775	
		Chlorodibromomethane	0.550	
		Bromoform	0.229	
Company	**Sample Identification**	**Analyte**	**Concentration (µg/L)**	**Combined THMs**
A.L.Abbott and Association	Johnson-Johnson Medical Water AG 92982	Chloroform	<1.00	<1.00
		Bromodichloromethane	<1.00	
		Chlorodibromomethane	<1.00	
		Bromoform	<1.00	

Table 3.73 Inter-Laboratory Comparison for Johnson-Johnson Medical Water

Company	Sample Identification	Analyte	Concentration (µg/L)	Combined THMs
Microchem	Wong on Fibre AG 60080	Chloroform	12.300	<1.00
		Bromodichloromethane	1.050	
		Chlorodibromomethane	0.400	
		Bromoform	N/D	
Company	**Sample Identification**	**Analyte**	**Concentration (µg/L)**	**Combined THMs**
A.L.Abbott and Association	Wong on Fibre AG 60080	Chloroform	16.000	<1.00
		Bromodichloromethane	<1.00	
		Chlorodibromomethane	<1.00	
		Bromoform	<1.00	

3.2.11 Precision based on Repeatability

Table 3.74 Precision based on reproducibility for river water

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Actual Sample Amount µg/L	Mean	Stdev	% RSD	Z-Score
River Water	Chloroform	150	1	2.367	144.200	141.83	143.09	4.81	3.36	0.50
			2	2.365	137.600	135.24				
			3	2.510	149.300	146.79				
			4	2.340	148.500	146.16				
			5	2.270	147.700	145.43				
River Water	BDCM	150	1	0.072	176.800	176.73	177.54	1.61	0.90	0.50
			2	0.063	175.200	175.14				
			3	0.051	178.200	178.15				
			4	0.079	179.200	179.12				
			5	0.053	178.600	178.55				
River Water	CDBM	150	1	0.049	159.300	159.25	161.86	6.82	4.21	0.50
			2	0.046	151.200	151.15				
			3	0.051	166.400	166.35				
			4	0.410	167.500	167.09				
			5	0.067	165.500	165.43				
River Water	Bromoform	150	1	0.072	156.000	155.93	158.34	6.89	4.35	0.50
			2	0.060	147.500	147.44				
			3	0.054	162.700	162.65				
			4	0.049	164.700	164.65				
			5	0.081	161.100	161.02				

Table 3.75 Precision based on reproducibility for municipal water

Sample	THMs	Conc µg/L	Inj No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Actual Sample Amount µg/L	Mean	Stdev	% RSD	Z-Score
Municipal Water	Chloroform	150	1	2.208	165.110	162.902	161.85	4.82	2.98	0.50
			2	2.230	160.150	157.920				
			3	2.230	169.910	167.680				
			4	2.258	167.100	164.842				
			5	2.391	158.310	155.919				
Municipal Water	BDCM	150	1	0.138	170.360	170.222	171.89	2.60	1.51	0.50
			2	0.139	169.500	169.361				
			3	0.149	171.000	170.851				
			4	0.147	175.910	175.763				
			5	0.123	173.360	173.237				
Municipal Water	CDBM	150	1	0.068	168.600	168.532	174.11	5.02	2.88	0.50
			2	0.070	169.030	168.960				
			3	0.091	177.170	177.079				
			4	0.075	179.510	179.435				
			5	0.067	176.595	176.528				
Municipal Water	Bromoform	150	1	0.073	160.347	160.274	165.96	5.03	3.03	0.50
			2	0.080	161.050	160.970				
			3	0.069	169.170	169.101				
			4	0.050	171.511	171.461				
			5	0.091	168.082	167.991				

Table 3.76 Precision based on reproducibility for underground water

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Actual Sample Amount µg/L	Mean	Stdev	% RSD	Z-Score
Underground Water	Chloroform	150	1	2.437	135.400	132.963	136.58	3.34	2.44	0.50
			2	2.464	138.700	136.236				
			3	2.480	137.200	134.720				
			4	2.360	139.400	137.040				
			5	2.361	144.300	141.939				
Underground Water	BDCM	150	1	0.079	169.400	169.321	172.93	3.68	2.13	0.50
			2	0.069	173.400	173.331				
			3	0.051	170.300	170.249				
			4	0.056	173.100	173.044				
			5	0.081	178.800	178.719				
Underground Water	CDBM	150	1	0.052	142.200	142.148	146.00	4.09	2.80	0.50
			2	0.053	145.900	145.847				
			3	0.061	142.900	142.839				
			4	0.060	146.800	146.740				
			5	0.071	152.500	152.429				
Underground Water	Bromoform	150	1	0.069	138.400	138.331	141.96	3.93	2.77	0.50
			2	0.070	142.000	141.930				
			3	0.051	138.600	138.549				
			4	0.045	143.100	143.055				
			5	0.049	148.000	147.951				

Table 3.77 Precision based on reproducibility for river water

Sample	THMs	Conc µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	Actual Sample Amount µg/L	Mean	Stdev	% RSD	Z-Score
River Water	Chloroform	150	1	2.367	144.200	141.833	143.09	4.81	3.36	0.50
			2	2.365	137.600	135.235				
			3	2.510	149.300	146.790				
			4	2.340	148.500	146.160				
			5	2.270	147.700	145.430				
River Water	BDCM	150	1	0.072	176.800	176.728	177.54	1.61	0.90	0.50
			2	0.063	175.200	175.137				
			3	0.051	178.200	178.149				
			4	0.079	179.200	179.121				
			5	0.053	178.600	178.547				
River Water	CDBM	150	1	0.049	159.300	159.251	161.85	6.77	4.19	0.50
			2	0.046	151.200	151.154				
			3	0.051	166.400	166.349				
			4	0.041	167.600	167.559				
			5	0.067	165.000	164.933				
River Water	Bromoform	150	1	0.072	156.000	155.928	158.34	6.89	4.35	0.50
			2	0.060	147.500	147.440				
			3	0.054	162.700	162.646				
			4	0.049	164.700	164.651				
			5	0.081	161.100	161.019				

3.2.11 Robustness

Table 3.78 Robustness using modified optimized method

Sample	THMs	Theoretical Concentration µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	% Recovery
Borehole Water	Chloroform	100	1	0	83.507	83.51
			2	0	84.112	84.11
			3	0	83.771	83.77
Borehole Water	BDCM	100	1	0	95.570	95.57
			2	0	92.132	92.13
			3	0	93.555	93.56
Borehole Water	CDBM	100	1	0	97.349	97.35
			2	0	93.702	93.7
			3	0	91.931	91.93
Borehole Water	Bromoform	100	1	0	83.822	83.82
			2	0	87.084	87.08
			3	0	85.397	85.39

Table 3.79 Robustness using present method

Sample	THMs	Theoretical Concentration µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	% Recovery
Borehole Water	Chloroform	100	1	0	80.330	80.33
			2	0	83.126	83.13
			3	0	82.654	82.65
Borehole Water	BDCM	100	1	0	95.390	95.39
			2	0	93.110	93.11
			3	0	93.555	93.56
Borehole Water	CDBM	100	1	0	98.000	98.00
			2	0	97.555	97.56
			3	0	96.324	96.32
Borehole Water	Bromoform	100	1	0	81.821	81.82
			2	0	83.147	83.15
			3	0	83.660	83.66

Table 3.80 Robustness using modified optimized method

Sample	THMs	Theoretical Concentration µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	% Recovery
Municipal Water	Chloroform	100	1	0	80.239	80.24
			2	0	82.110	82.11
			3	0	82.670	82.67
Municipal Water	BDCM	100	1	0	107.349	107.35
			2	0	106.980	106.98
			3	0	106.987	106.99
Municipal Water	CDBM	100	1	0	97.349	97.35
			2	0	97.550	97.55
			3	0	96.999	97.00
Municipal Water	Bromoform	100	1	0	83.822	83.82
			2	0	83.813	83.81
			3	0	83.647	83.65

Table 3.81 Robustness using present method

Sample	THMs	Theoretical Concentration µg/L	Inj. No	Unspiked Sample Amount µg/L	Spiked Sample Amount µg/L	% Recovery
Municipal Water	Chloroform	100	1	0	84.500	84.50
			2	0	83.964	83.96
			3	0	83.114	83.11
Municipal Water	BDCM	100	1	0	109.300	109.3
			2	0	108.770	108.77
			3	0	107.332	107.33
Municipal Water	CDBM	100	1	0	83.690	83.69
			2	0	83.770	83.77
			3	0	83.874	83.87
Municipal Water	Bromoform	100	1	0	108.300	108.30
			2	0	107.987	107.99
			3	0	107.102	107.10

3.2.12 Measure of Uncertainty

Table 3.82 120 µg/L THMs in-house laboratory control for chloroform

No	Enter duplicate results below µg/L		Average values µg/L	% of the reference value
1	122.290	123.900	123.10	104.90%
2	116.331	117.090	116.71	99.50%
3	104.000	122.450	113.23	96.50%
4	113.241	107.347	110.29	94.00%
5	120.280	122.580	121.43	103.50%
6	119.070	118.900	118.99	101.40%
Xmean			117.29	
Stdev			4.88	
Uncertainty of reference at 95 %			0.014	

Table 3.83 Measure of Uncertainty for Chloroform

Statistical calculations		
Number of valid values		
Detection of suspect values		
Tolerance interval		**Classical**
	Min	109.24
	Max	125.34
Conclusion		
There is no suspect value		
Analysis of trueness *vs.* Reference Sample		
		Classical
Median / mean of results		117.290
SD(results)		4.88906
SD(iR)		6.3157
Recovery		100.0%
SD(Recovery)		0.017
t test		0.000
p value		100.0%
Uncertainty of recovery		0.017
Relative uncertainty of recovery		1.70%
Confidence interval	Min	95.6%
	Max	104.4%
The recovery is NOT different from 100% (at 95% confidence)		
MU - Eurolab - Technical Report No.1/2007 March 2007 "Measurement uncertainty revisited: Alternative approaches to uncertainty evaluation"		
Delta -		0.000
Bias - b		2.5784
uncertainty - u		5.5273
Expanded overall u (K=2):	**u =**	**11.0546**
Relative uncertainty		4.71%
Expanded overall relative u :	**u% =**	**9.42%**

$$b = \sqrt{\Delta^2 + u_{ref}^2 + \frac{s^2}{n}}$$

$$u = \sqrt{s^2 + b^2}$$

Table 3.84 120 µg/L THMs in-house laboratory control for BDCM

No	Enter duplicate results below µg/L		Average values µg/L	% of the reference value
1	118.660	124.190	121.43	102.3%
2	118.000	125.490	121.75	102.6%
3	122.060	116.000	119.03	100.3%
4	126.900	120.000	123.45	104.0%
5	123.780	118.500	121.14	102.1%
6	119.100	117.900	118.50	99.9%
Xmean			120.88	
Stdev			1.83	
Uncertainty of reference at 95 %			0.019	

Table 3.85 Measure of Uncertainty for bromodichloromethane

Statistical calculations		
Number of valid values		
Detection of suspect values		
Tolerance interval		**Classical**
	Min	117.86
	Max	123.90
Conclusion		
There is no suspect value		
Analysis of trueness *vs.* Reference Sample		
		Classical
Median / mean of results		120.882
SD(results)		1.83328
SD(iR)		3.4217
Recovery		100.0%
SD(Recovery)		0.006
t test		0.000
p value		100.0%
Uncertainty of recovery		0.006
Relative uncertainty of recovery		0.62%
Confidence interval	Min	98.4%
	Max	101.6%
The recovery is NOT different from 100% (at 95% confidence)		
MU - Eurolab - Technical Report No.1/2007 March 2007 "Measurement uncertainty revisited: Alternative approaches to uncertainty evaluation"		
Delta -		0.000
Bias - b		1.3969
uncertainty - u		2.3049
Expanded overall u (K=2):	**u =**	**4.6097**
Relative uncertainty		1.91%
Expanded overall relative u :	**u% =**	**3.81%**

$$b = \sqrt{\Delta^2 + u_{ref}^2 + \frac{s^2}{n}}$$

$$u = \sqrt{s^2 + b^2}$$

Table 3.86 120 µg/L THMs in-house laboratory control for CDBM

No	Enter duplicate results below µg/L		Average values µg/L	% of the reference value
1	122.950	124.440	123.70	99.3%
2	126.470	122.040	124.26	99.7%
3	125.360	126.680	126.02	101.2%
4	121.570	128.820	125.20	100.5%
5	128.020	124.600	126.31	101.4%
6	122.000	121.870	121.94	97.9%
Xmean			124.57	
Stdev			1.63	
Uncertainty of reference at 95 %			0.025	

Table 3.87 Measure of Uncertainty for chlorodibromomethane

Statistical calculations		
Number of valid values		
Detection of suspect values		
Tolerance interval		**Classical**
	Min	121.88
	Max	127.26
Conclusion		
There is no suspect value		
Analysis of trueness *vs.* Reference Sample		
		Classical
Median / mean of results		124.568
SD(results)		1.63271
SD(iR)		2.5153
Recovery		100.0%
SD(Recovery)		0.005
t test		0.000
p value		100.0%
Uncertainty of recovery		0.005
Relative uncertainty of recovery		0.54%
Confidence interval	Min	98.6%
	Max	101.4%
The recovery is NOT different from 100% (at 95% confidence)		
MU - Eurolab - Technical Report No.1/2007 March 2007 "Measurement uncertainty revisited: Alternative approaches to uncertainty evaluation"		
Delta -		0.000
Bias - b		1.0269
uncertainty - u		1.9288
Expanded overall u (K=2):	**u =**	**3.8577**
Relative uncertainty		1.55%
Expanded overall relative u :	**u% =**	**3.10%**

$$b = \sqrt{\Delta^2 + u_{ref}^2 + \frac{s^2}{n}}$$

$$u = \sqrt{s^2 + b^2}$$

Table 3.88 120 µg/L THMs in-house laboratory control for Bromoform

No	Enter duplicate results below µg/L		Average values µg/L	% of the reference value
1	117.020	117.090	117.06	102.4%
2	112.540	110.016	111.28	97.3%
3	109.906	117.820	113.86	99.6%
4	105.900	106.708	106.30	93.0%
5	120.000	119.710	119.86	104.8%
6	118.200	116.900	117.55	102.8%
Xmean			114.32	
Stdev			4.94	
Uncertainty of reference at 95 %			0.031	

Table 3.89 Measure of Uncertainty for bromoform

Statistical calculations		
Number of valid values		
Detection of suspect values		
Tolerance interval		**Classical**
	Min	106.18
	Max	122.46
Conclusion		
There is no suspect value		
Analysis of trueness *vs.* Reference Sample		
		Classical
Median / mean of results		114.318
SD(results)		4.94506
SD(iR)		5.2374
Recovery		100.0%
SD(Recovery)		0.018
t test		0.000
p value		100.0%
Uncertainty of recovery		0.018
Relative uncertainty of recovery		1.77%
Confidence interval	Min	95.5%
	Max	104.5%
The recovery is NOT different from 100% (at 95% confidence)		
MU - Eurolab - Technical Report No.1/2007 March 2007 "Measurement uncertainty revisited: Alternative approaches to uncertainty evaluation"		
Delta -		0.000
Bias - b		2.1382
uncertainty - u		5.3875
Expanded overall u (K=2):	**u =**	**10.7751**
Relative uncertainty		4.71%
Expanded overall relative u :	**u% =**	**9.43%**

$$b = \sqrt{\Delta^2 + u_{ref}^2 + \frac{s^2}{n}}$$

$$u = \sqrt{s^2 + b^2}$$

3.2.1 Fapas Proficiency Test

Table 3.90 Fapas drinking water results

Bromodichloromethane			Bromoform		
Assigned value 46.2µg/L			Assigned value 28.7 µg/L		
Recovery	% Recovery	z-score	Recovery	% Recovery	z-score
41.505	90	-0.8	18.857	66	-2.7
Pass			**Pass**		

Table 3.91 Fapas drinking water results

Chloroform			Dibromochloromethane		
Assigned value 10.5µg/L			Assigned value 35.6µg/L		
Recovery	% Recovery	z-score	Recovery	% Recovery	z-score
4.334	**41**	**-3.9**	26.819	75	-2
Fail			**Pass**		

Appendix B Chapter 3

3.2.2 Instrument Linearity

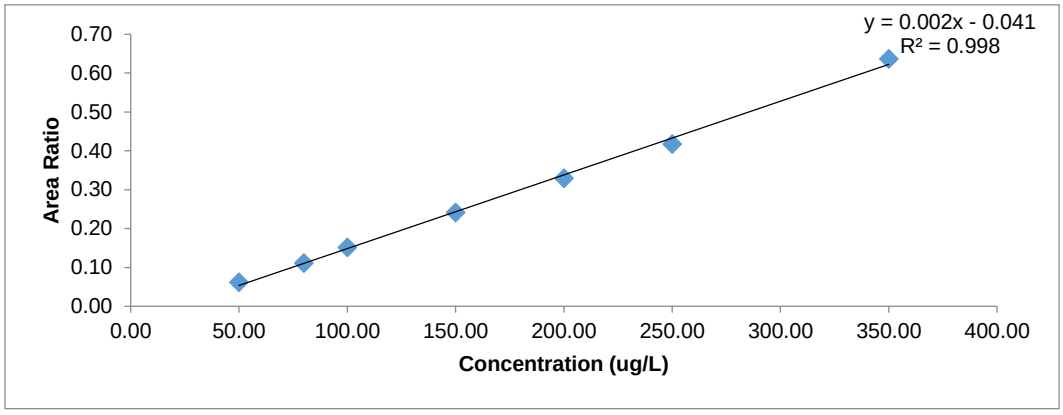


Figure 3.1 Calibration curve of chloroform (50-350 µg/L) with GC-ECD auto sampler injector (n-7)

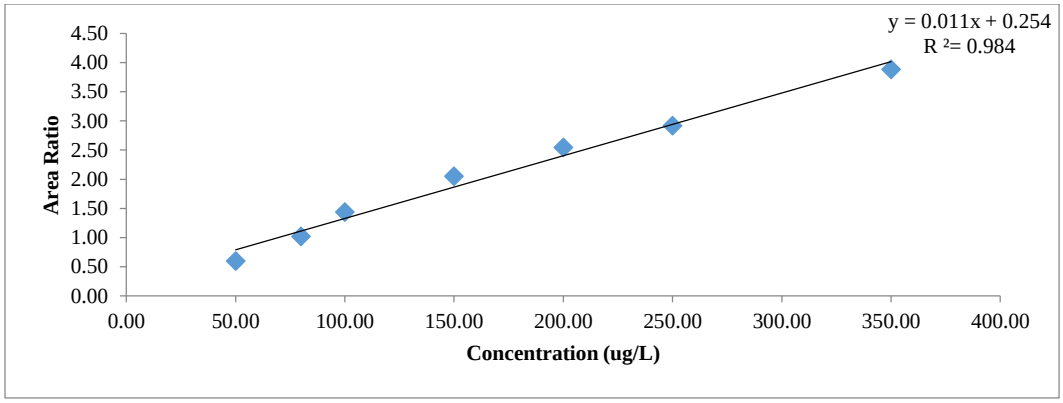


Figure 3.2 Calibration curve of BDCM (50-350 µg/L) with GC-ECD auto sampler injector (n-7)

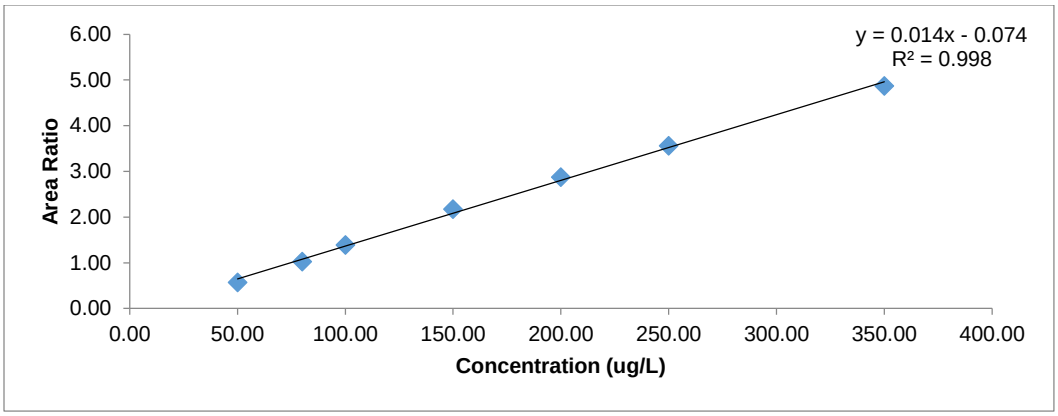


Figure 3.3 Calibration curve of CDBM (50-350 µg/L) with GC-ECD auto sampler injector (n-7)

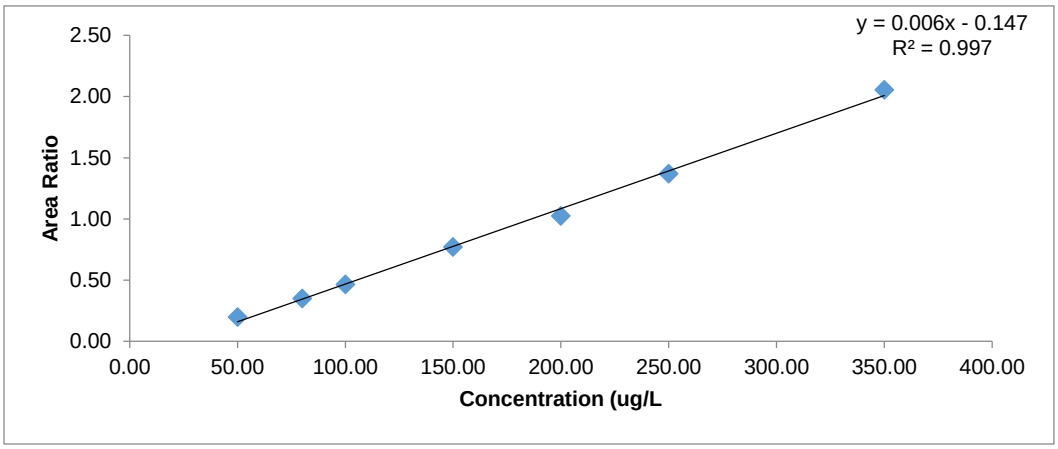


Figure 3.4 Calibration curve of bromoform (50-350 µg/L) with GC-ECD auto sampler injector (n-7)

3.2.8 Selectivity based on recovery

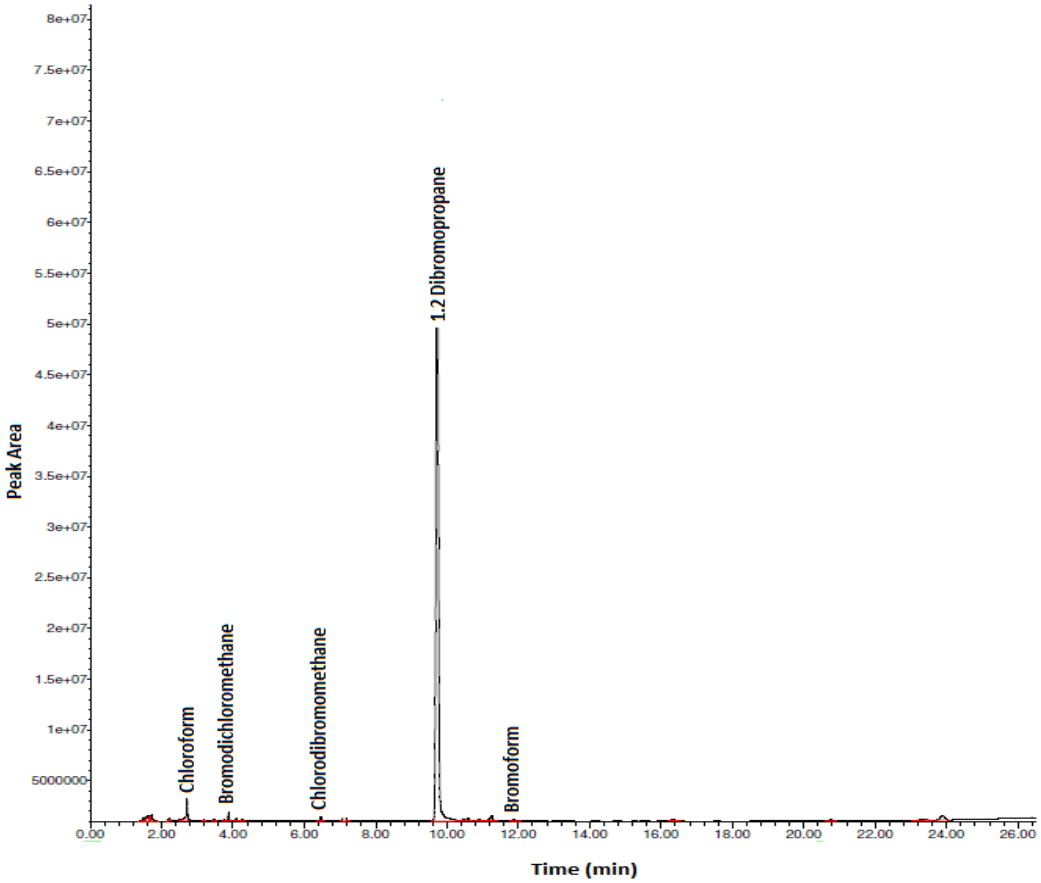


Figure 3.5 0 µg/L unspiked river water sample recoveries for the individual trihalomethanes GC-ECD HP 7683 auto-sampler series injector.

`

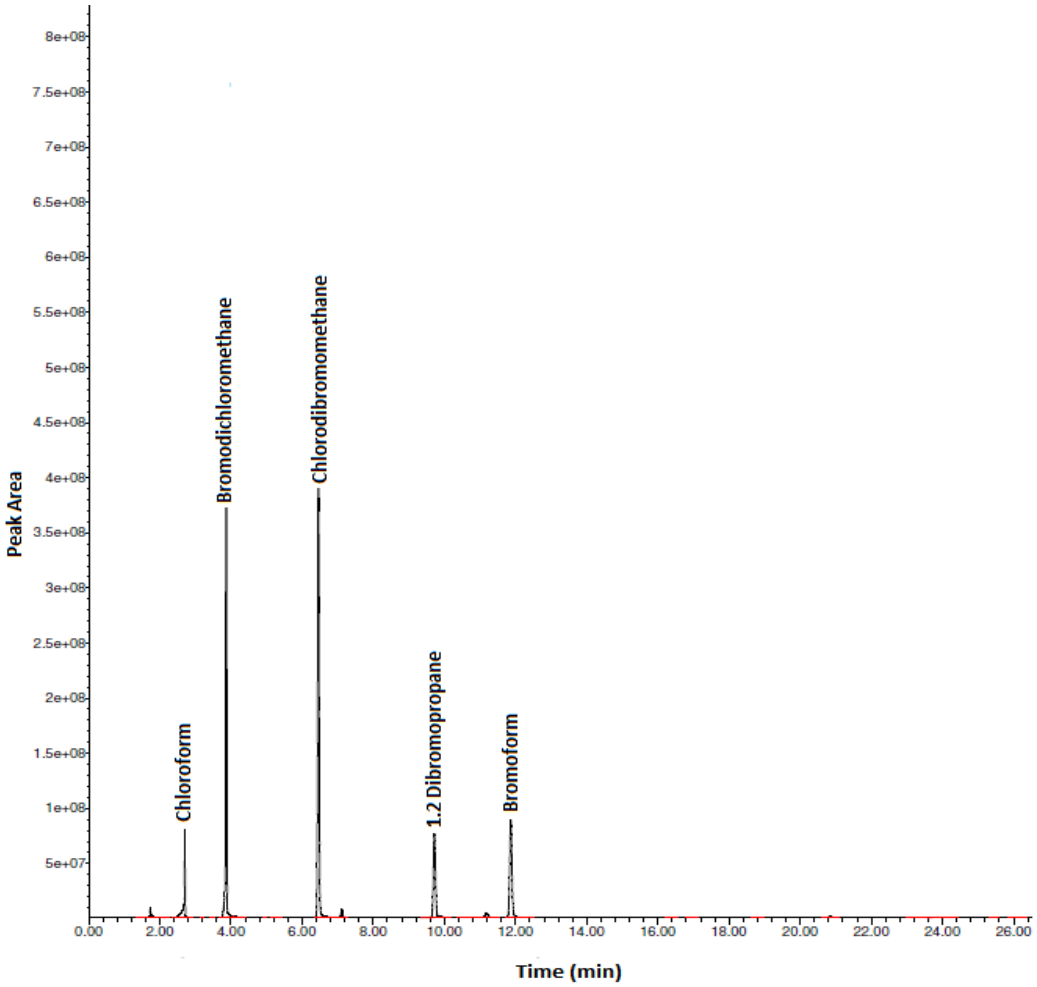


Figure 3.6 50µg/L spiked river water sample recoveries for the individual trihalomethanes GC-ECD HP 7683 auto-sampler series injector

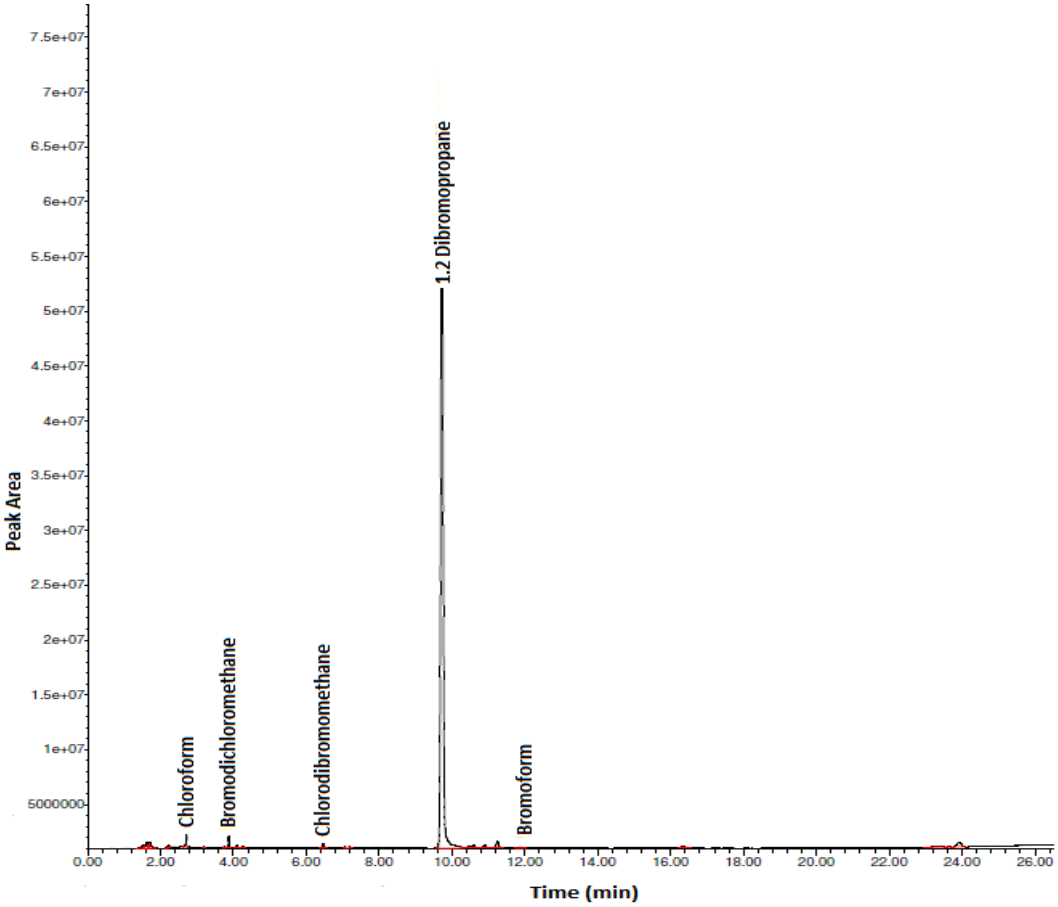


Figure 3.7 0 µg/L unspiked underground water sample recoveries for the individual trihalomethanes GC-ECD HP 7683 auto-sampler series injector.

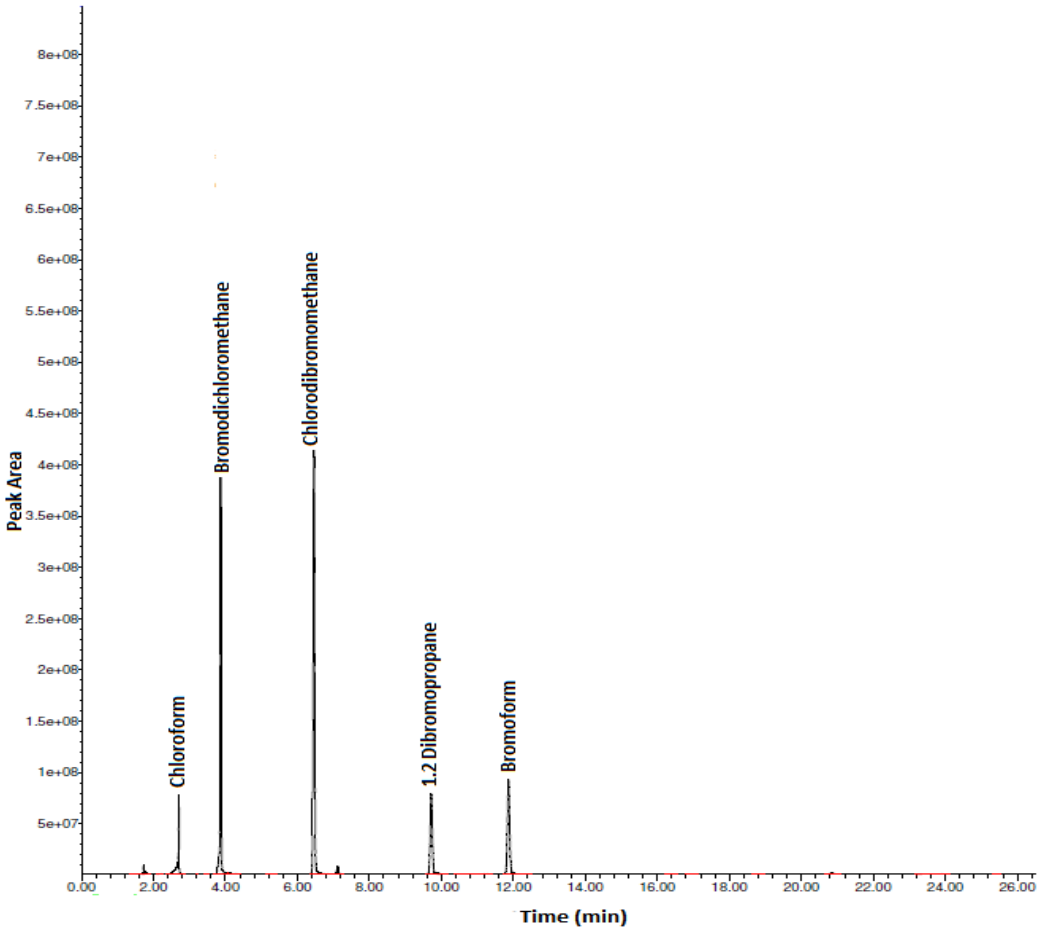


Figure 3.8 50 µg/L spiked underground water sample recoveries for the individual trihalomethanes GC-ECD HP 7683 auto-sampler series injector.

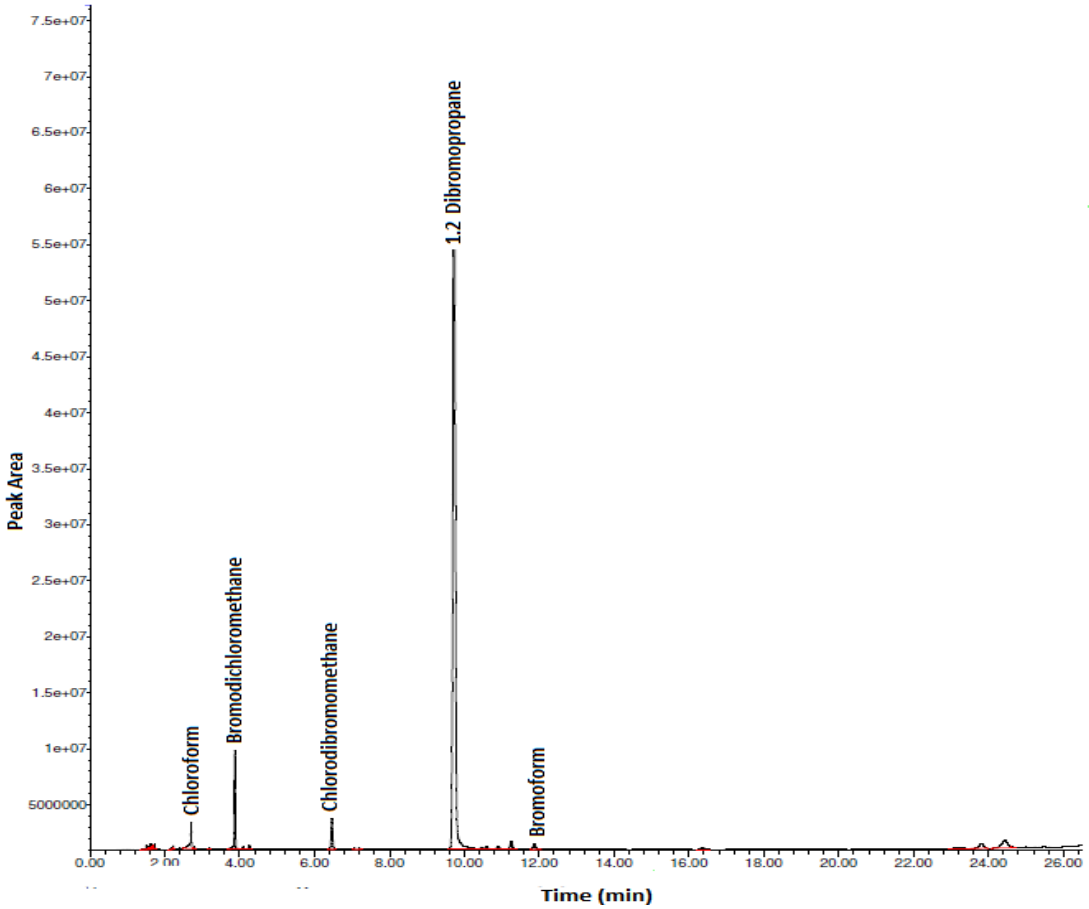


Figure 3.9 0 µg/L unspiked municipal water sample recoveries for the individual trihalomethanes GC-ECD HP 7683 auto-sampler series injector.

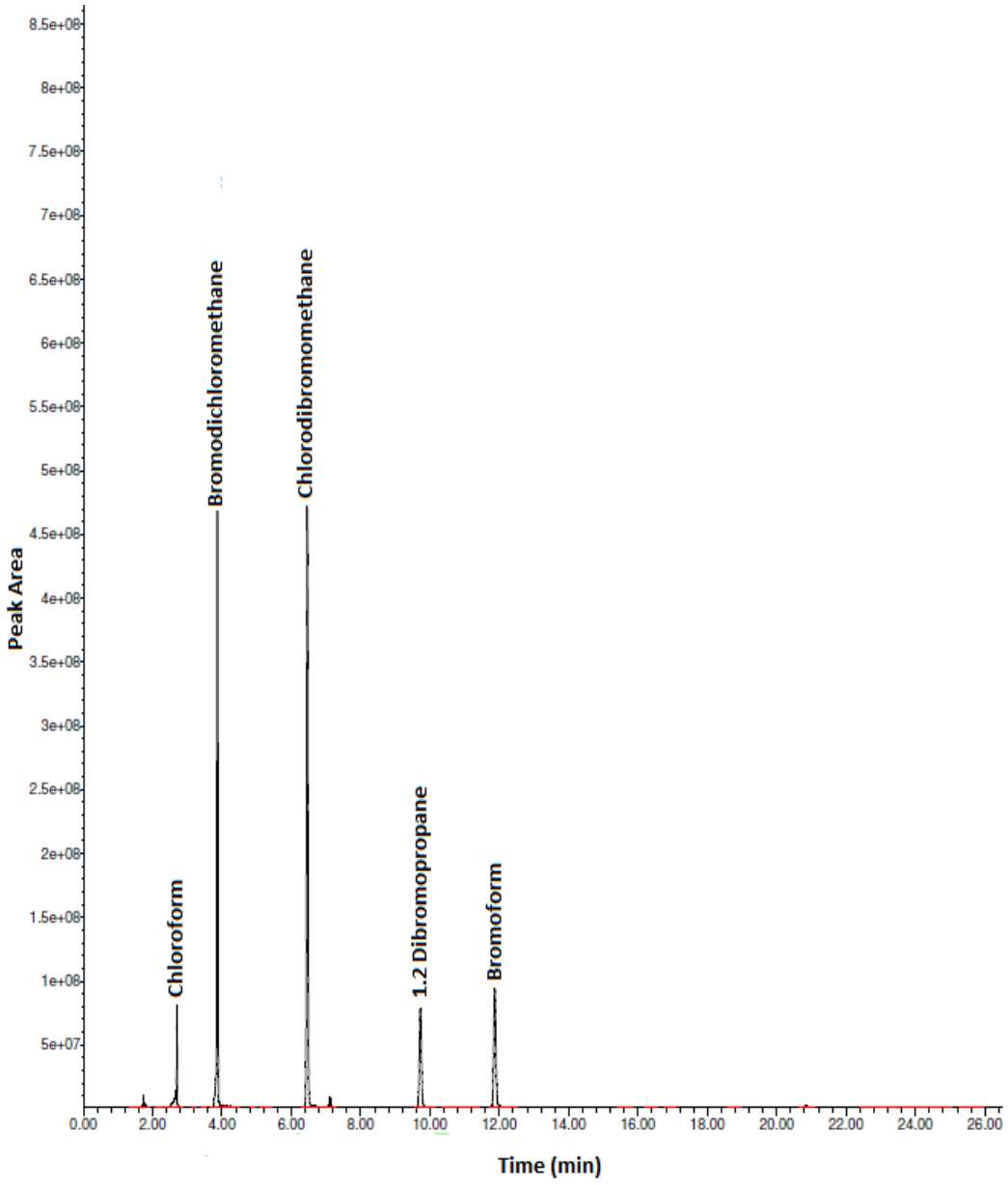


Figure 3.10 50 µg/L spiked municipal water sample recoveries for the individual trihalomethanes GC-ECD HP 7683 auto-sampler series injector.

3.2.9 Selectivity based on Interferences

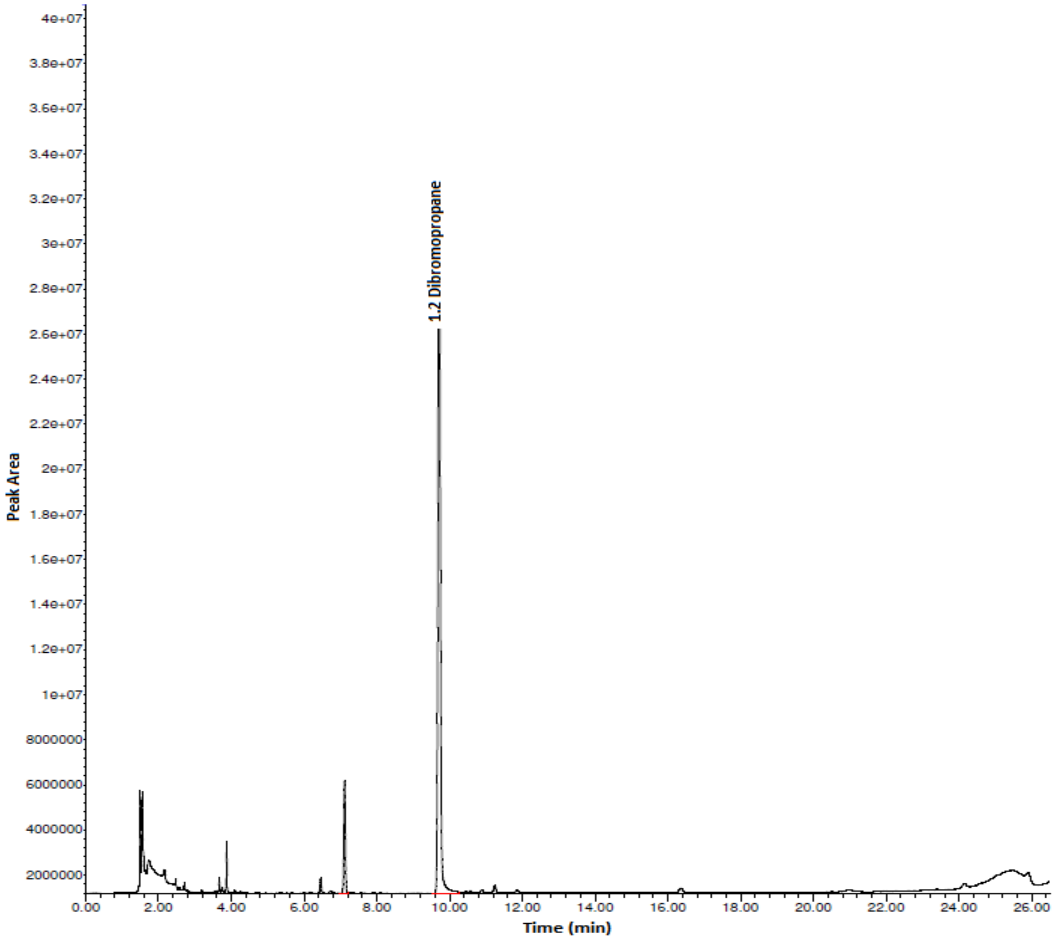


Figure 3.11 0µg/L unspiked chloride standard sample recoveries for the individual trihalomethanes GC ECD HP 7683 auto-sampler series injector

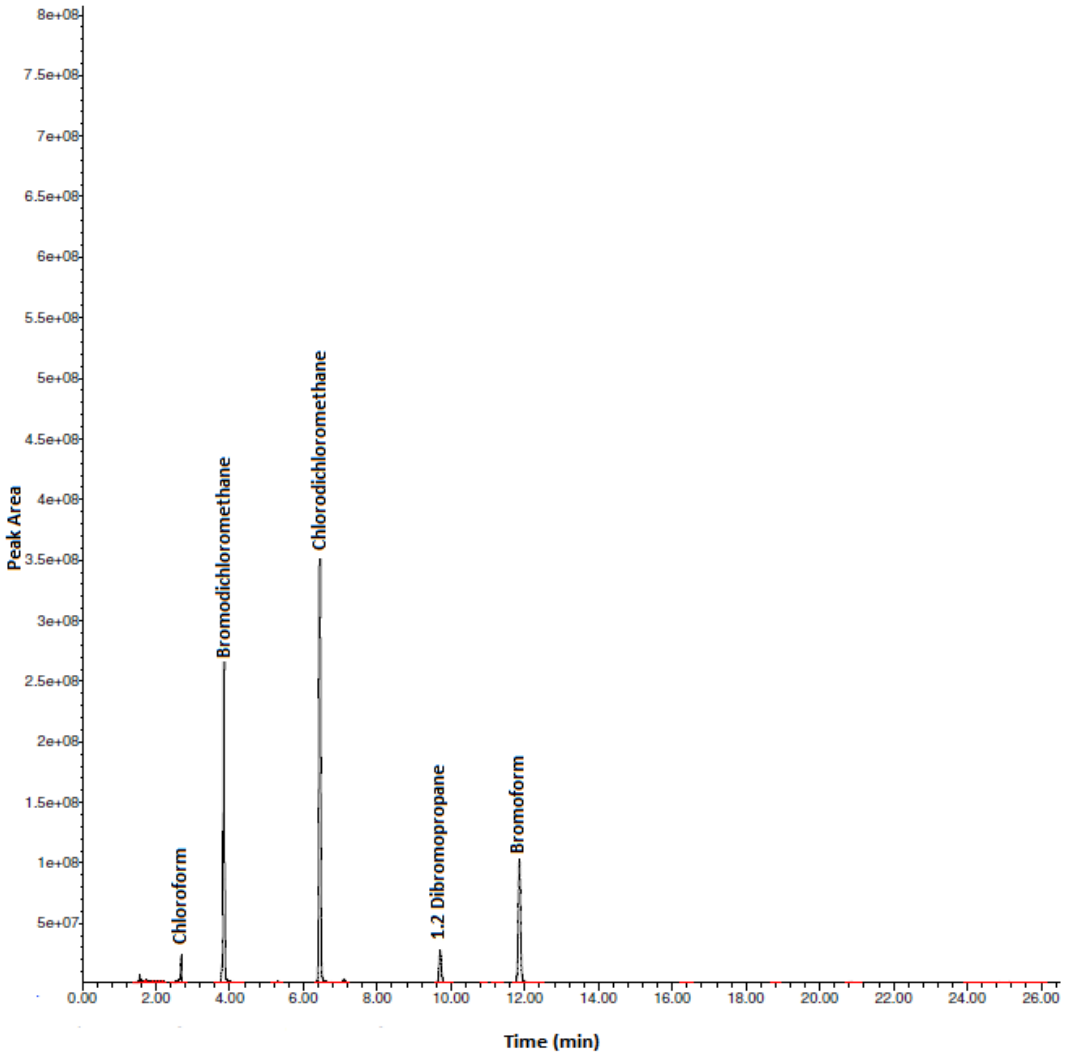


Figure 3.12 50µg/L spiked chloride standard sample recoveries for the individual trihalomethanes from GC-ECD HP 7683 auto-sampler series injector

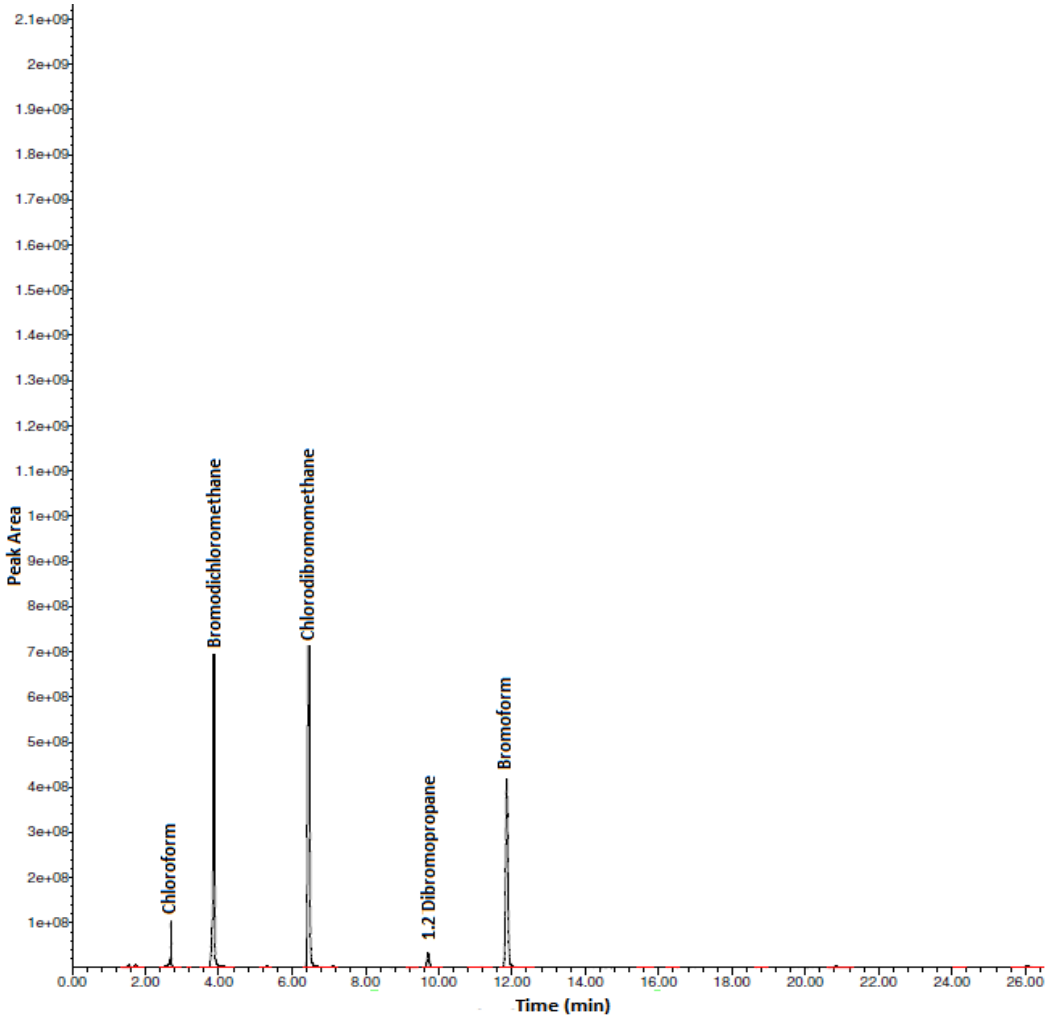


Figure 3.13 150µg/L spiked chloride standard sample recoveries for the individual trihalomethanes from GC-ECD HP 7683 auto-sampler series injector

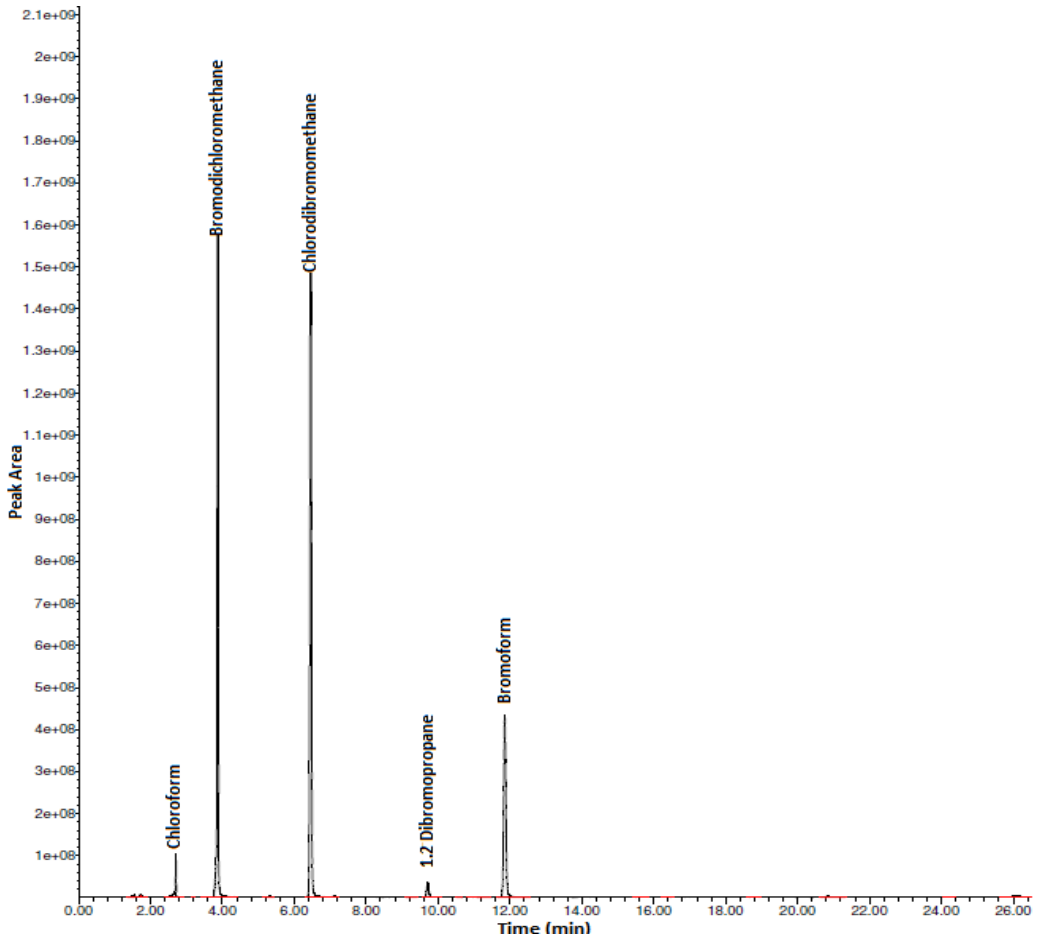


Figure 3.14 350µg/L spiked chloride standard sample recoveries for the individual trihalomethanes from GC-ECD HP 7683 auto-sampler series injector

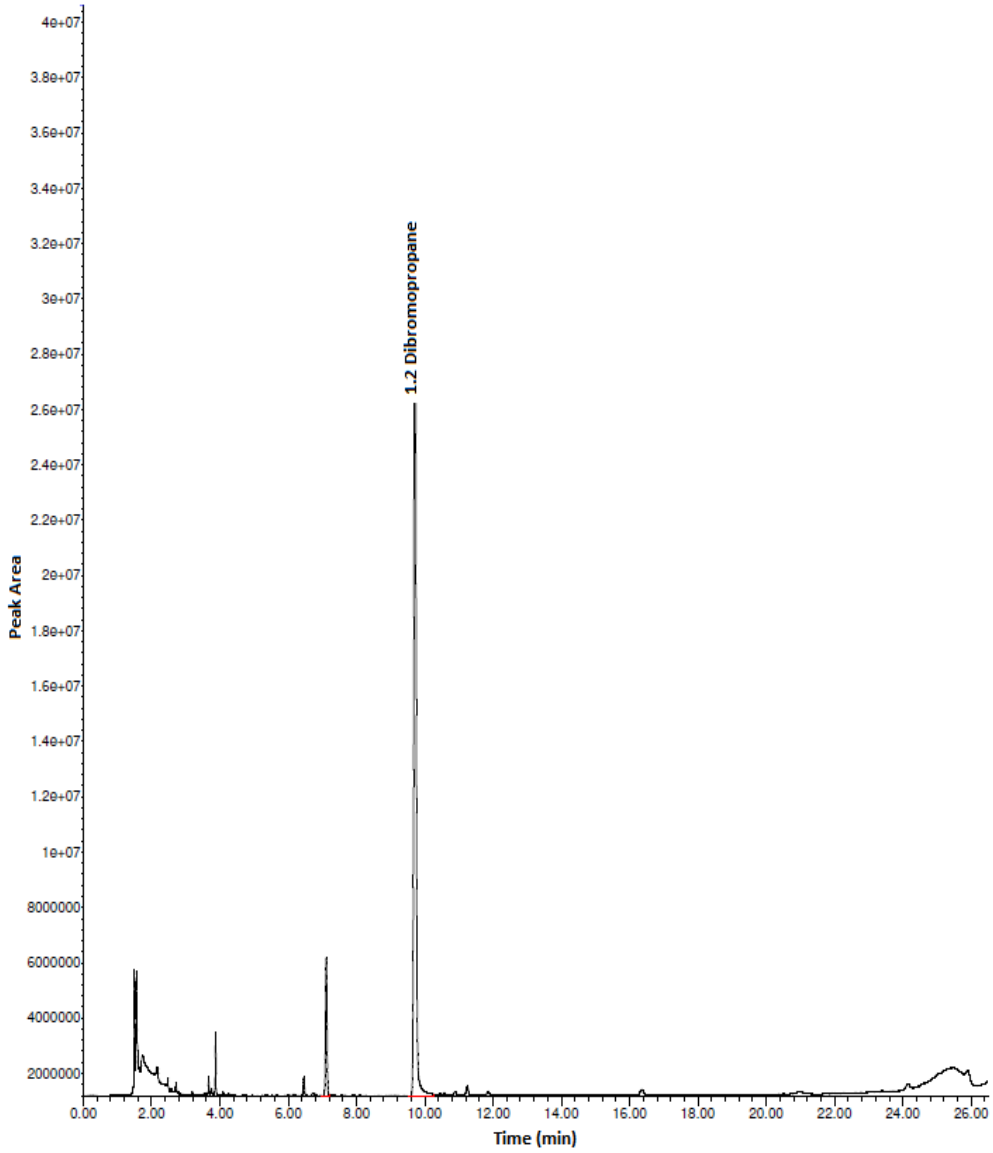


Figure 3.15 0µg/L unspiked fluoride standard sample recoveries for the individual trihalomethanes from GC-ECD HP 7683 auto-sampler series injector

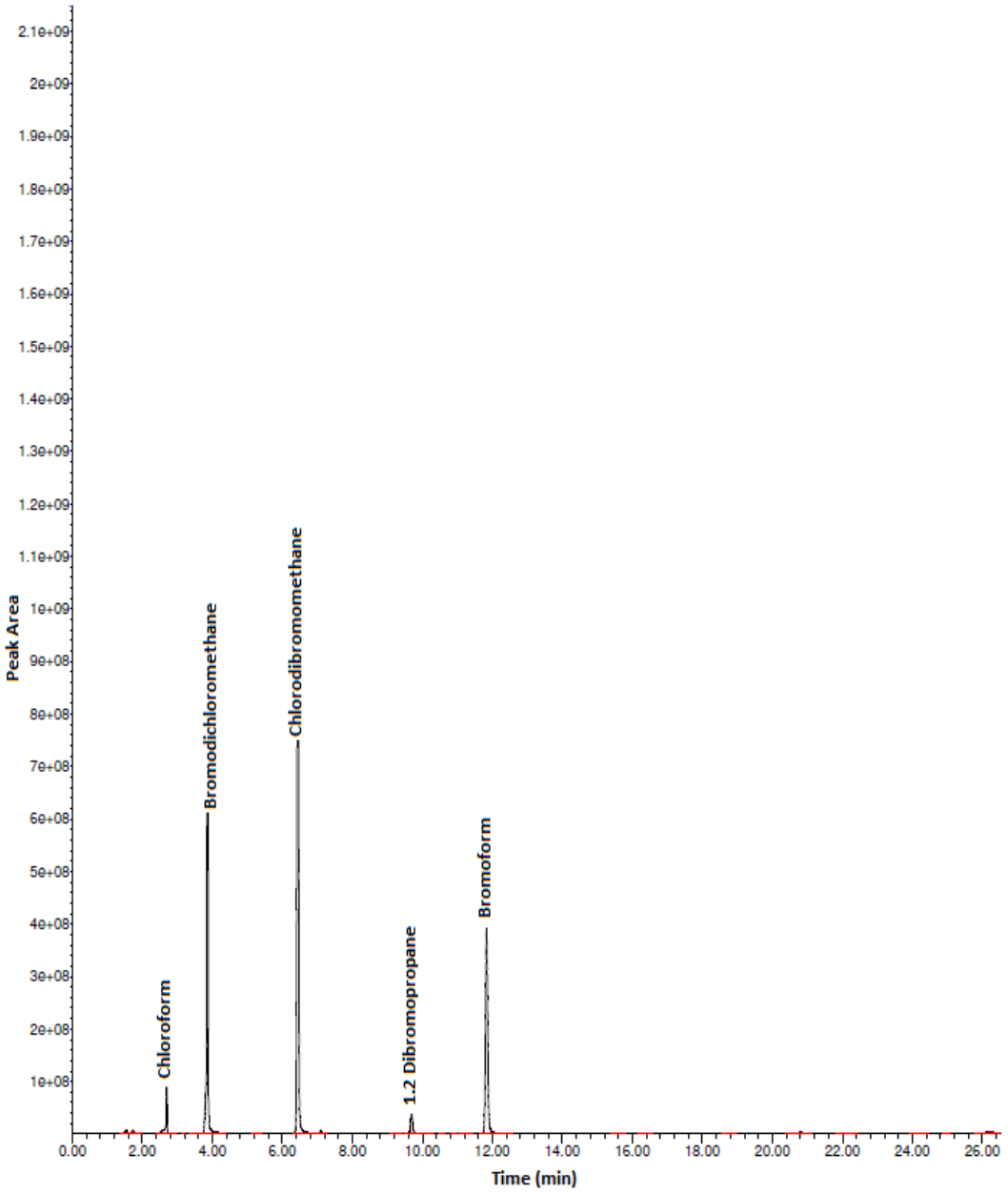


Figure 3.16 50µg/L spiked fluoride standard sample recoveries for the individual trihalomethanes from GC-ECD HP 7683 auto-sampler series injector

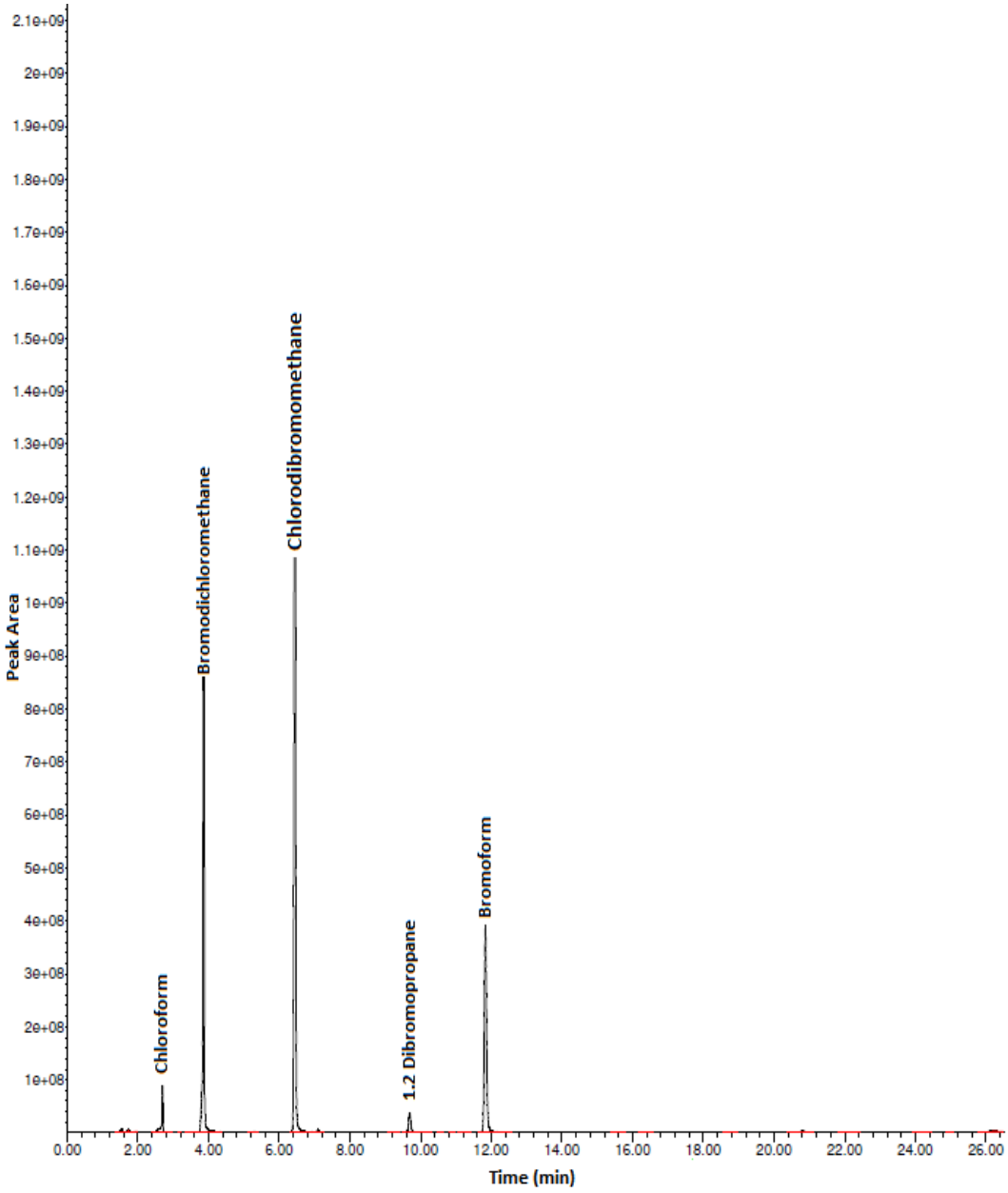


Figure 3.17 150 µg/L spiked fluoride standard sample recoveries for the individual trihalomethanes from GC-ECD HP 7683 auto-sampler series injector

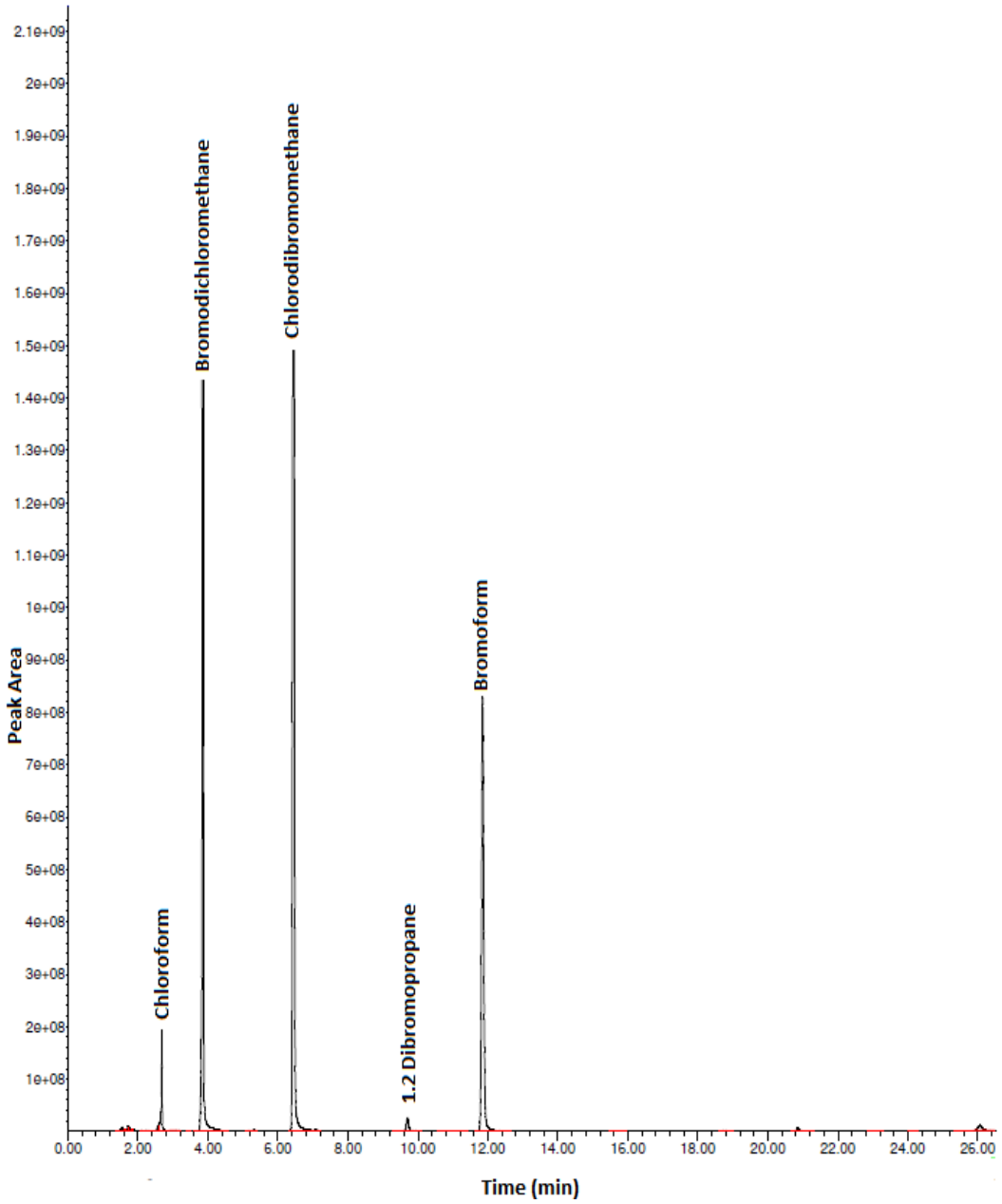


Figure 3.18 350µg/L spiked fluoride standard sample recoveries for the individual trihalomethanes from GC-ECD HP 7683 auto-sampler series injector

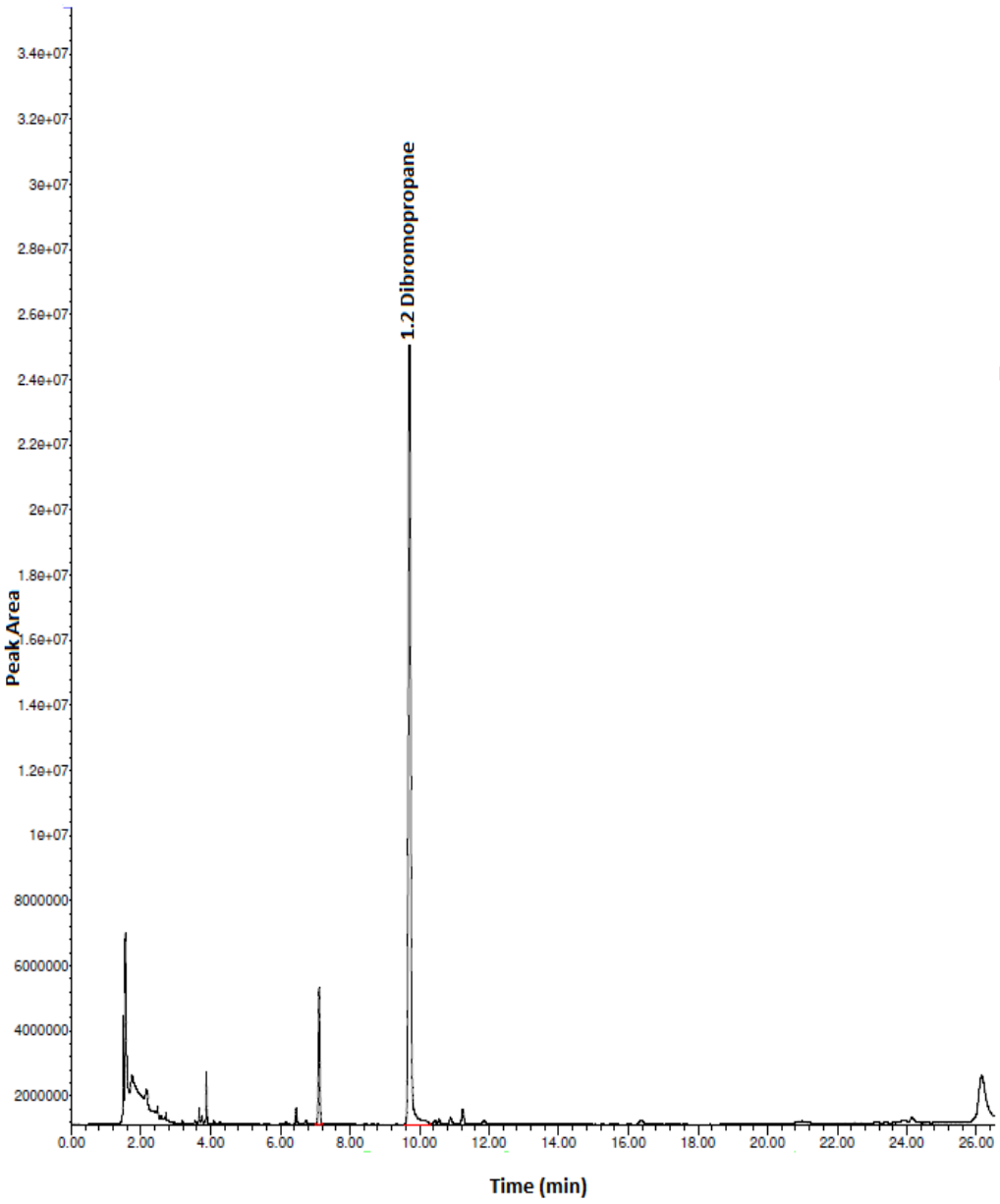


Figure 3.19 0µg/L unspiked heavy metal standard sample recoveries for the individual trihalomethanes from GC-ECD HP 7683 auto-sampler series injector

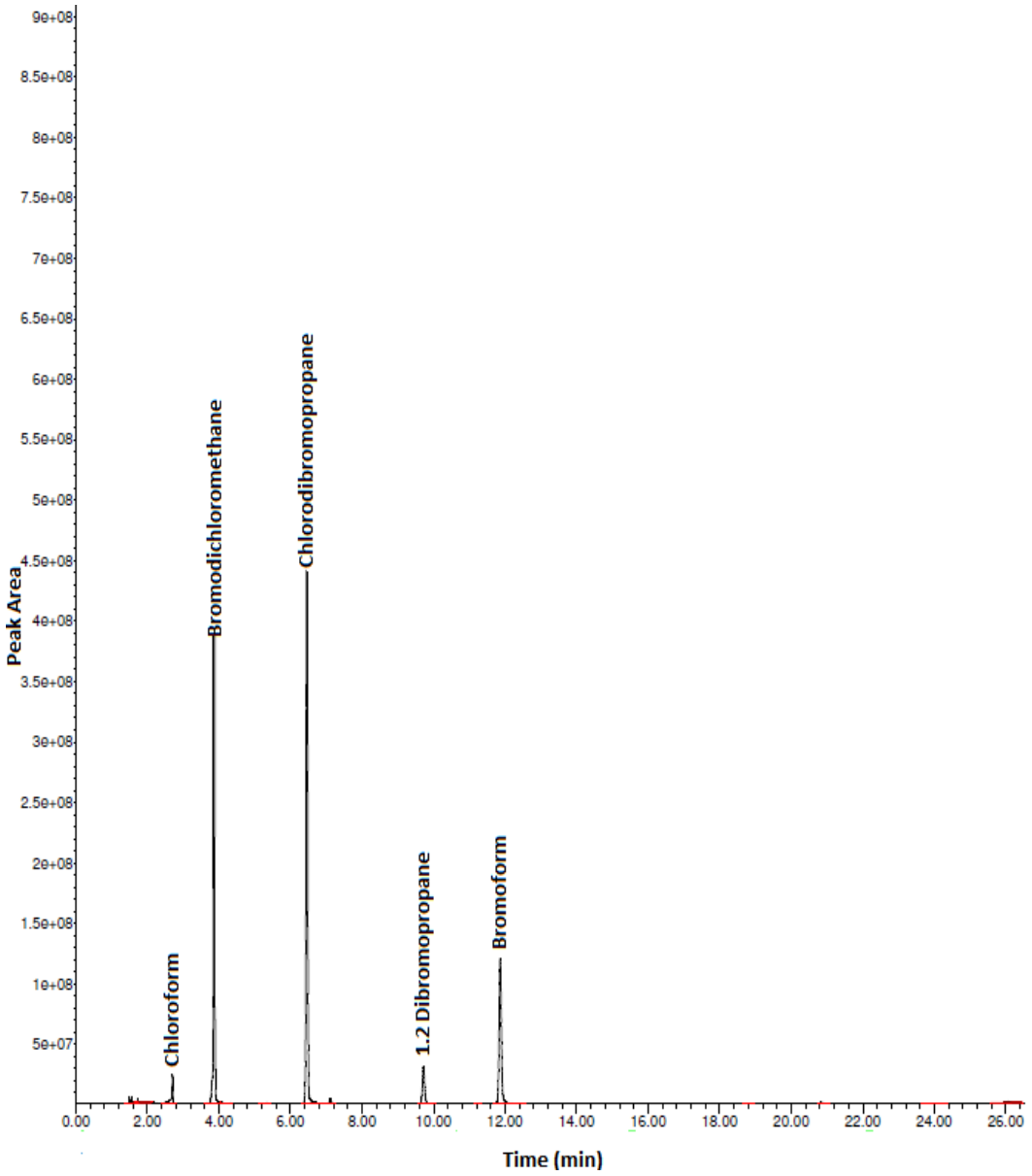


Figure 3.20 50µg/L spiked heavy metal standard sample recoveries for the individual trihalomethanes from GC-ECD HP 7683 auto-sampler series injector

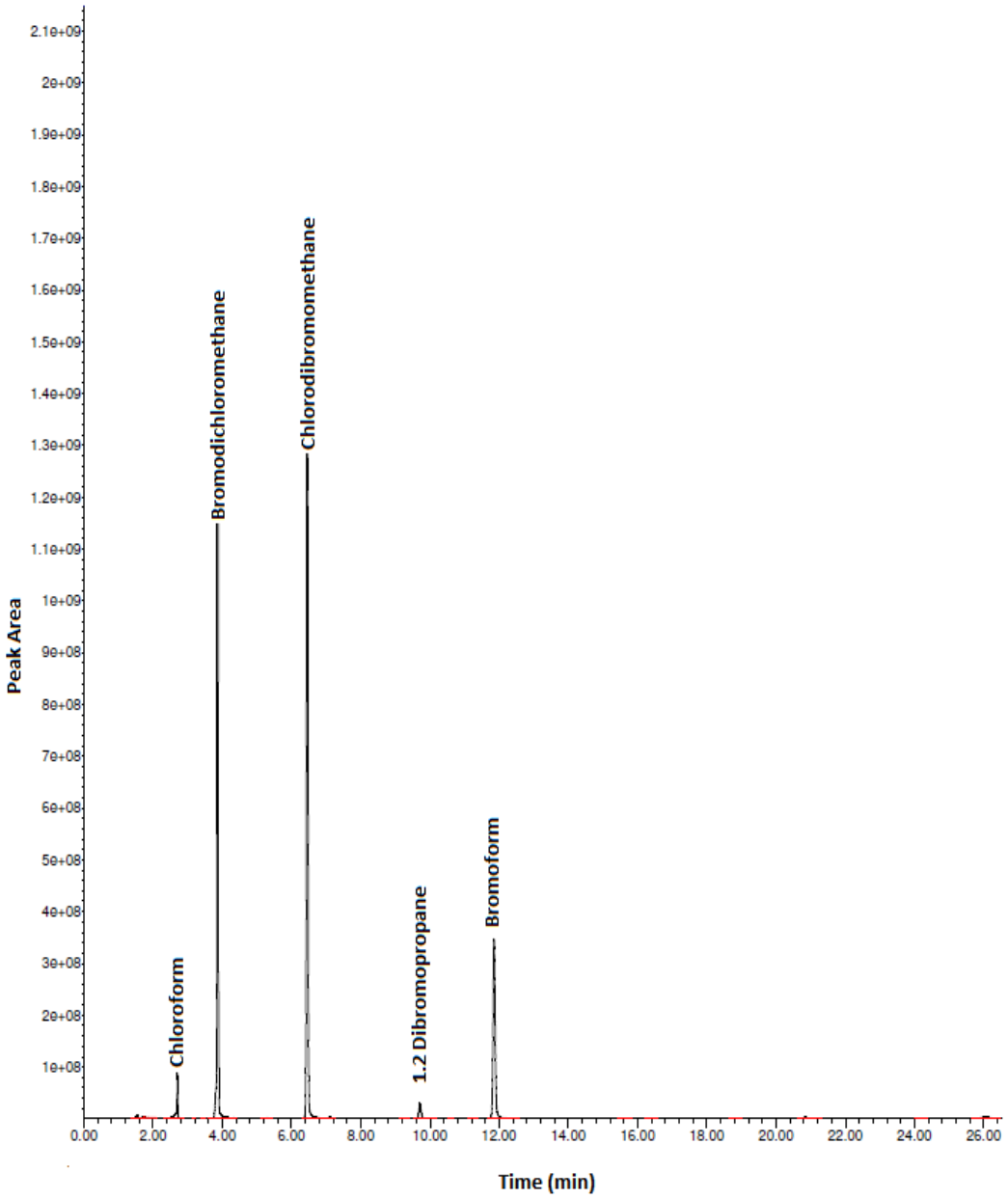


Figure 3.21 150µg/L spiked heavy metal standard sample recoveries for the individual trihalomethanes from GC-ECD HP 7683 auto-sampler series injector

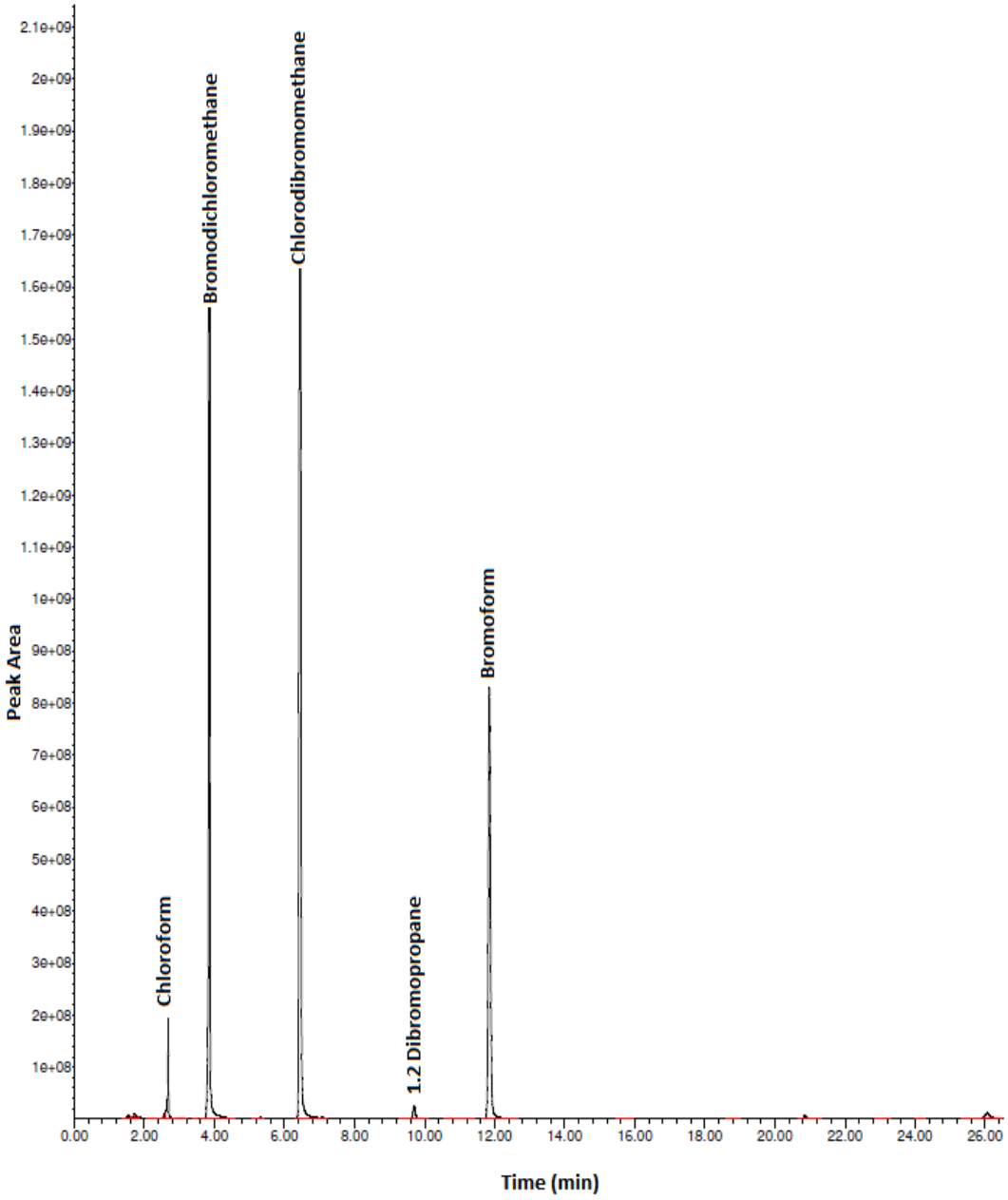


Figure 3.22 350µg/L spiked heavy metal standard sample recoveries for the individual trihalomethanes from GC-ECD HP 7683 auto-sampler series injector

3.2.12 Robustness

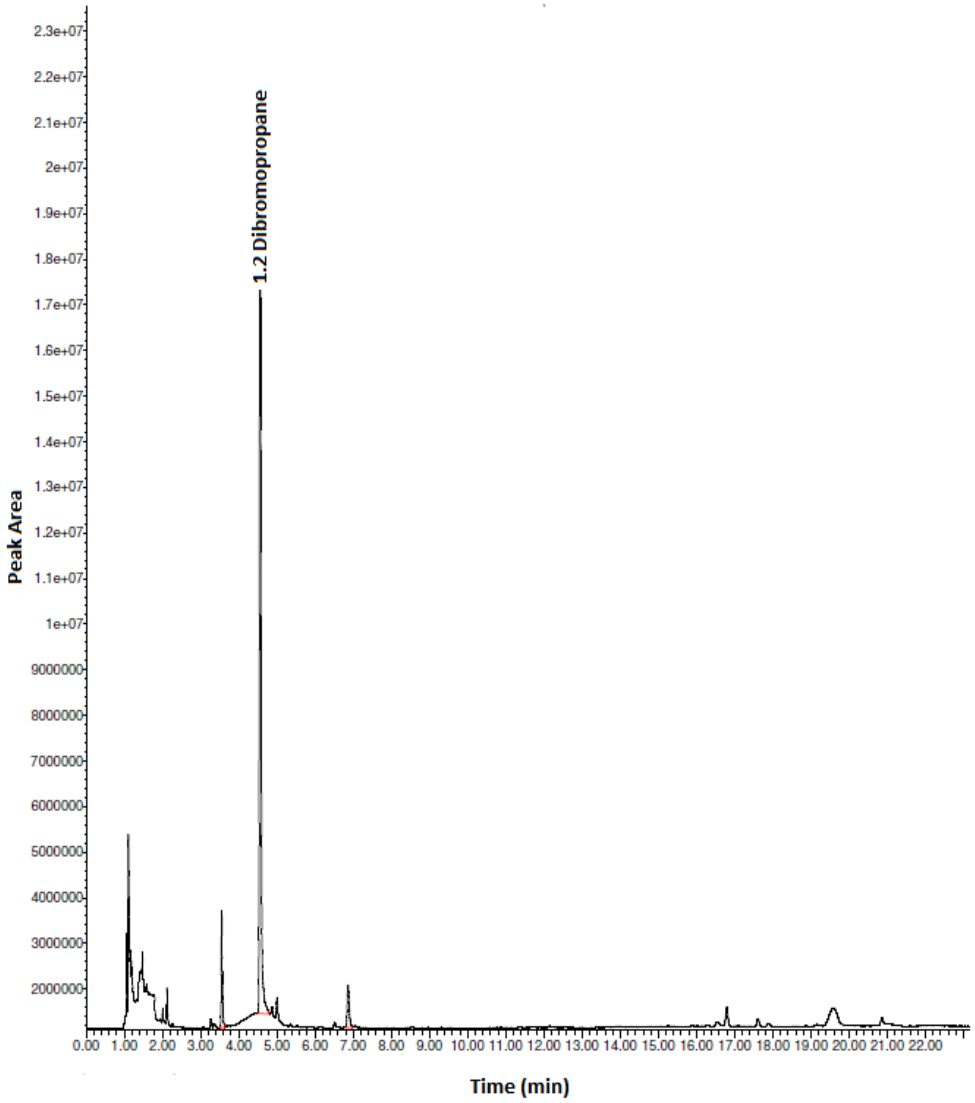


Figure 3.23 0µg/L unspiked municipal water sample chromatogram for modified method. GC-ECD HP 7683 auto-sampler series injector

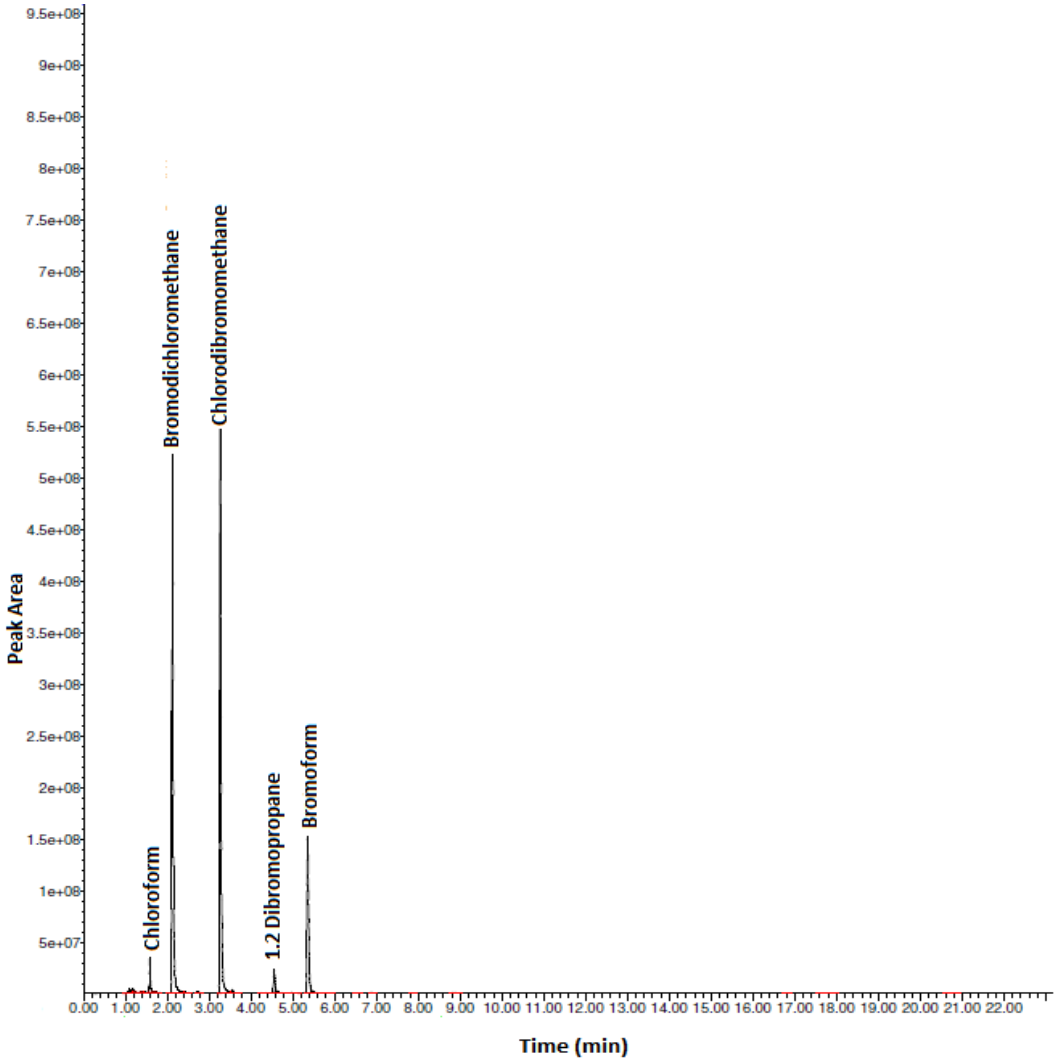


Figure 3.24 100 µg/L spiked municipal water sample chromatogram for modified method. GC-ECD HP 7683 auto-sampler series injector

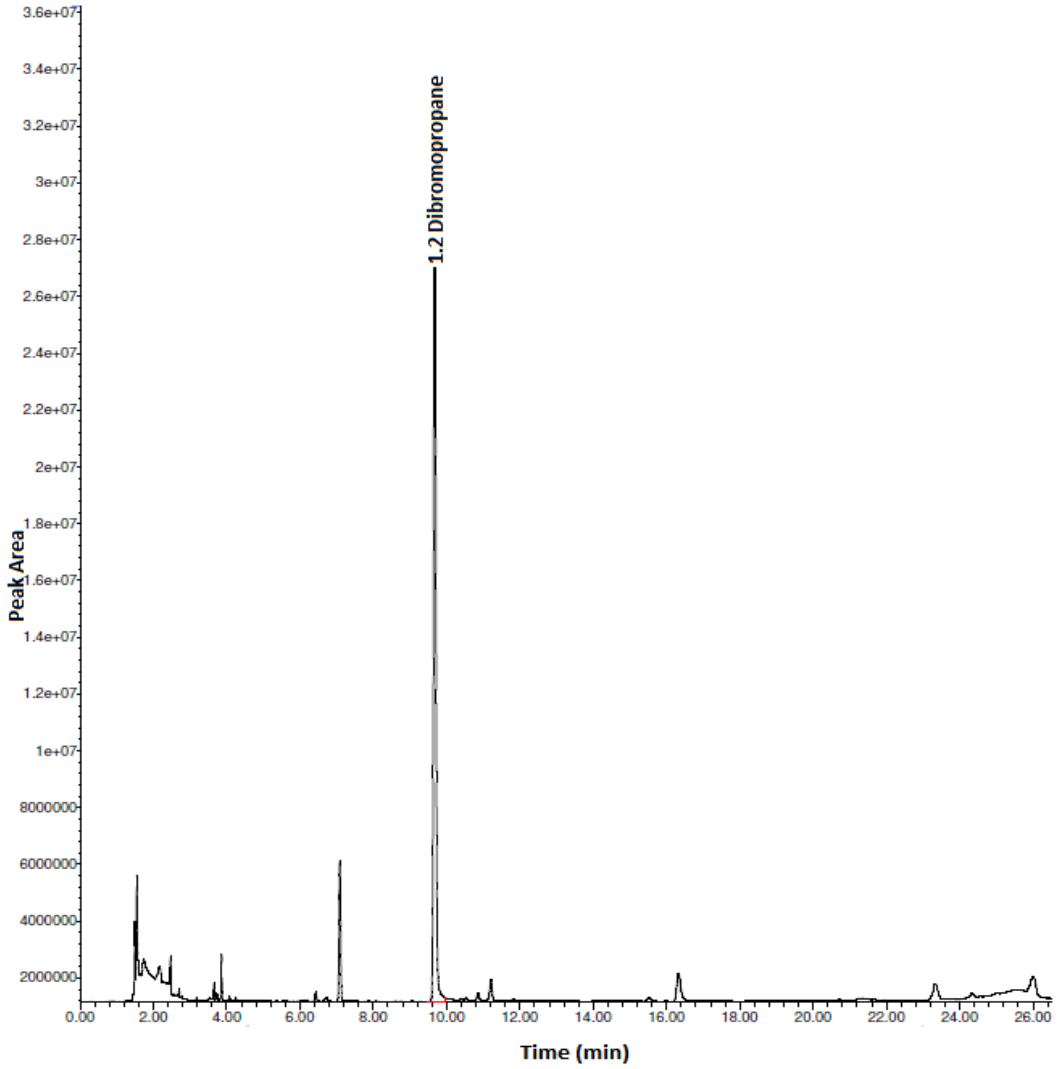


Figure 3.25 0 µg/L unspiked municipal water sample chromatogram for present method. GC-ECD HP 7683 auto-sampler series injector

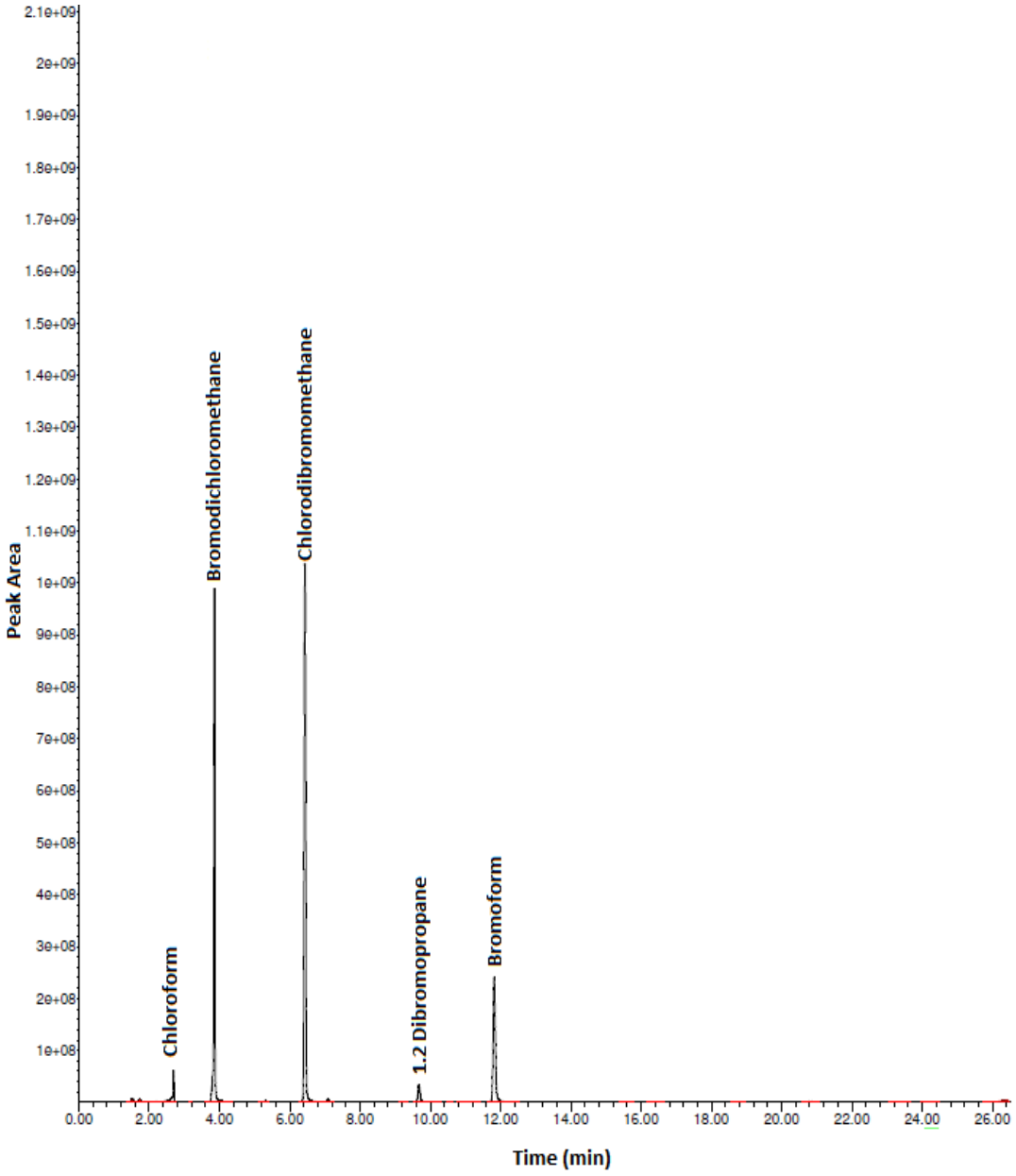


Figure 3.26 100 µg/L spiked municipal water sample chromatogram for present method GC-ECD HP 7683 auto-sampler series injector

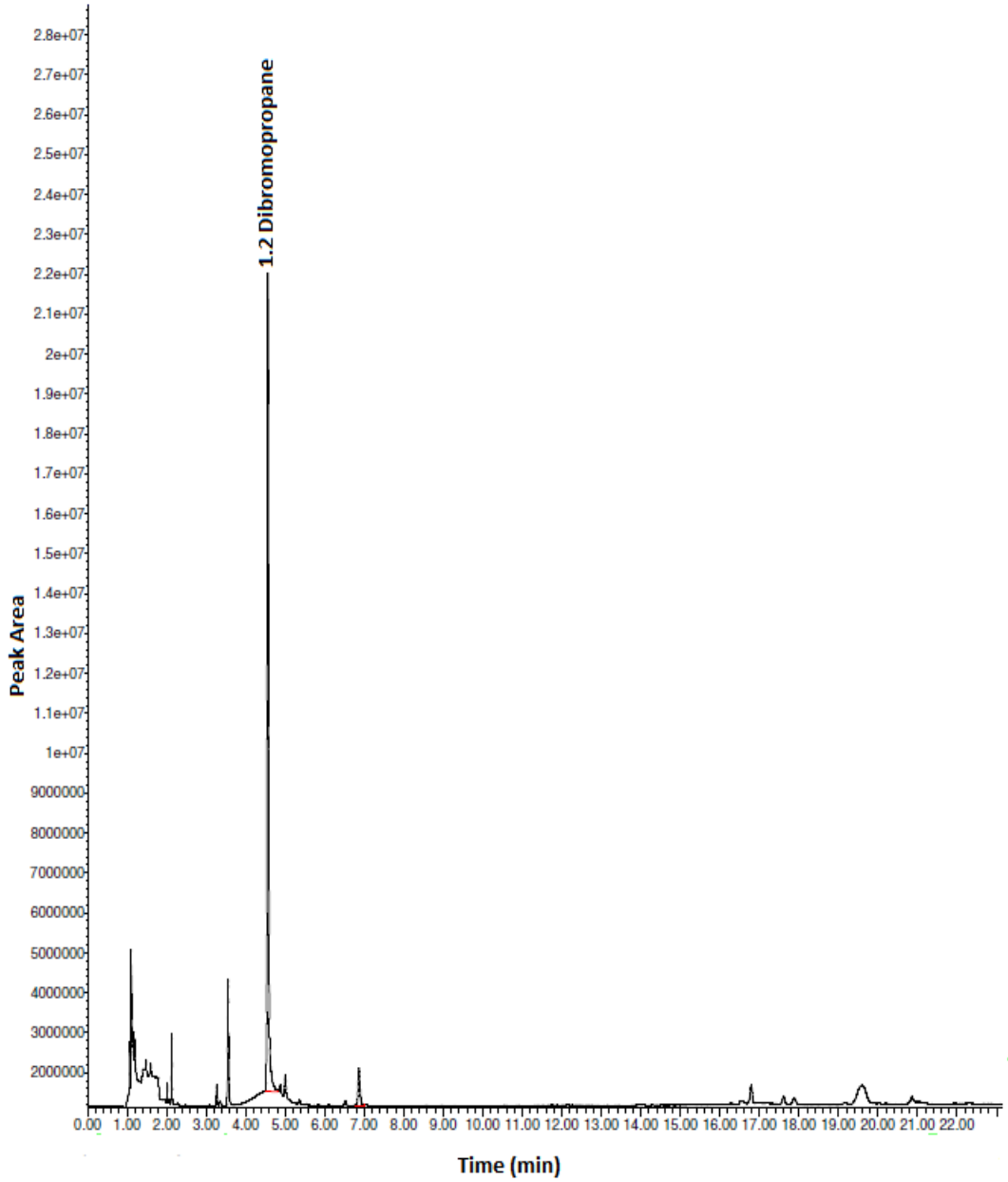


Figure 3.27 0 µg/L spiked borehole water sample chromatogram for modified method. GC-ECD HP 7683 auto-sampler series injector

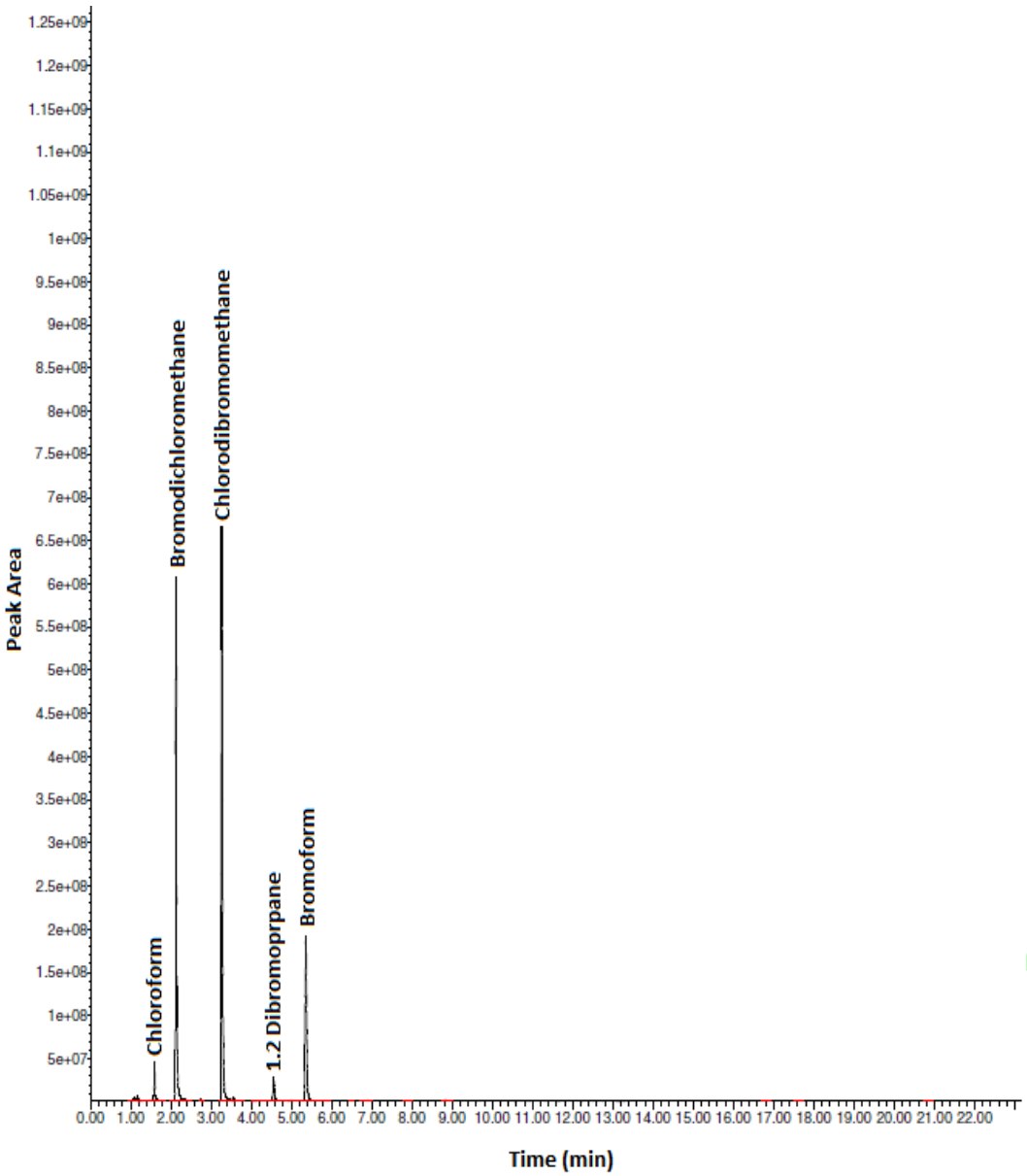


Figure 3.28 100 µg/L spiked borehole water sample chromatogram for modified method. GC-ECD HP 7683 auto-sampler series injector

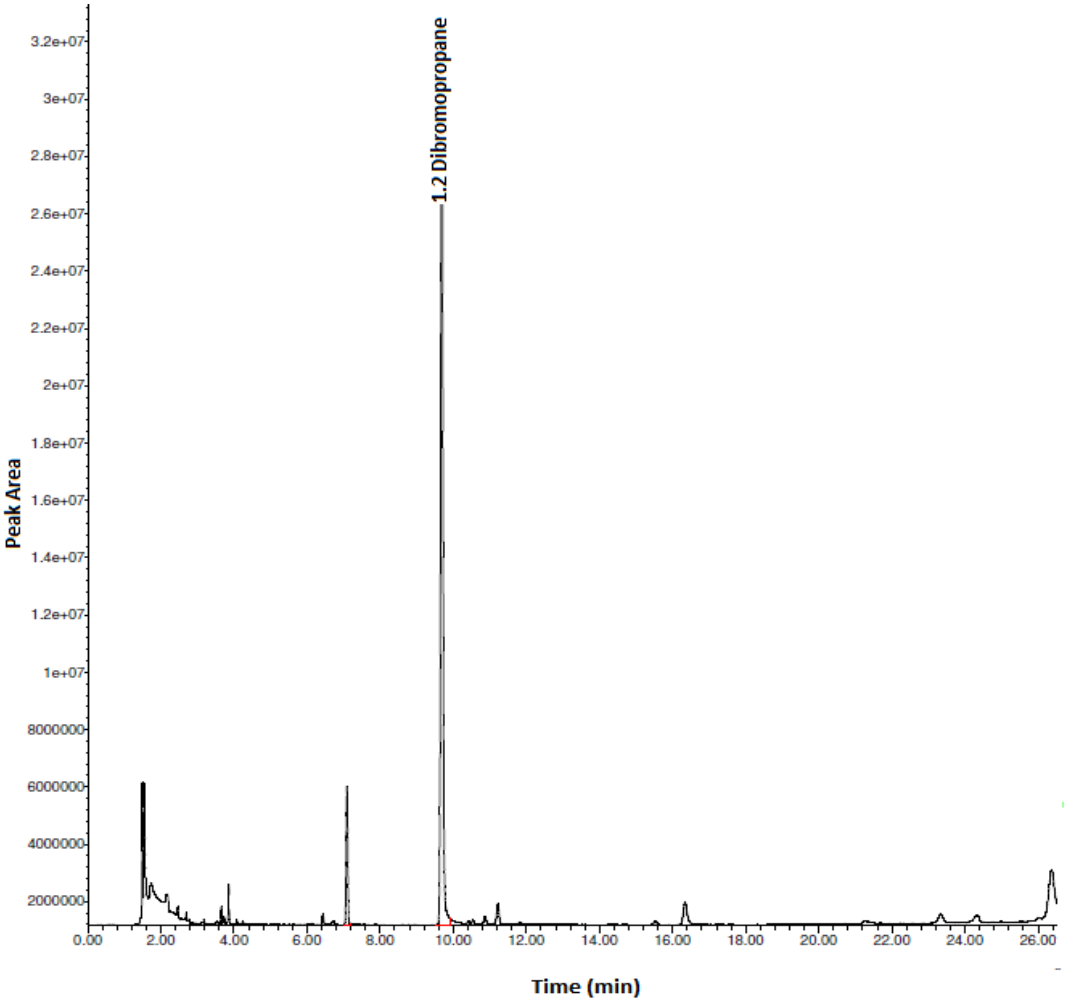


Figure 3.29 0 µg/L unspiked sample borehole water chromatogram for present method. GC-ECD HP 7683 auto-sampler series injector

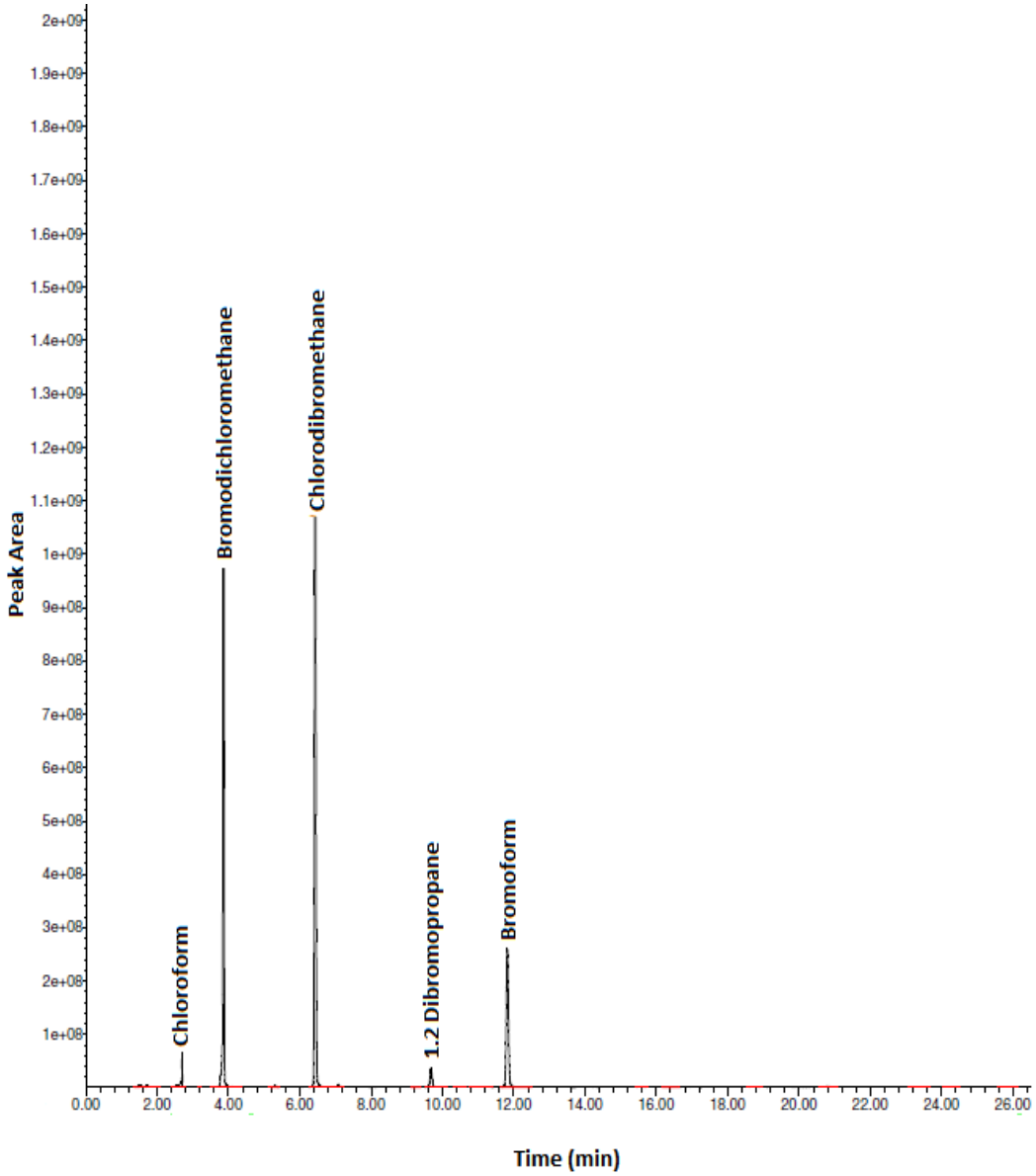


Figure 3.30 100 µg/L spiked borehole water sample chromatogram for present method GC-ECD HP 7683 auto-sampler series injector

Appendix C Chapter 3

A.L. ABBOTT AND ASSOCIATES (PTY) LTD

(Reg. No. 1982/004379/07)

Consulting Analytical & Industrial Chemists
Specialists in Water & Waste Water Treatment
Telephone (021)448 6340/1
After Hours (021)712 0940
Telefax (021)448 6342
e-Mail Address :
info@alabbott.co.za

T0276

No. 1, Vine Park
Vine Road
7925
P.O. Box 483
WOODSTOCK, CAPE
7915

Certificate of Analysis

MICROCHEM LAB SERVICES (PTY) LTD

ANALYSIS

AG60080

DATE SAMPLED : 2017/02/14

DATE RECEIVED : 2017/02/14

DATE ANALYSIS COMMENCED : 2017/02/14

ORDER:PO100432

OUR REF. : 2017/02/14/4152

REPORT NO . : 716

	Sample Number	4152	
Mthd ALA No.	**Analyses**	**Results**	**SANS 241-1:2015**
N/A	Total microcystin (µg/l)	<0.15	≤1
N/A	Combined Trihalomethane	0.09	≤1.0
N/A	Trihalomethane (Bromodichloromethane) (µg/l)	<1.0	≤60 Chronic Health
N/A	Trihalomethane (Bromoform) (µg/l)	<1.0	≤100 Chronic Health
N/A	Trihalomethane (Chloroform) (µg/l)	16.0	≤300 Chronic Health
N/A	Trihalomethane (Dibromochloromethane) (µg/l)	<1.0	≤100 Chronic Health

JOSE DA SILVA (Cert.Sci.Nat.)
TECHNICAL MANAGER
23 February 2017

TO: MICROCHEM
176 Sir Lowry Road
WOODSTOCK
CAPE TOWN
7925

Att: Salwa Banien<Chem@microchem.co.za>

Sampler : Unknown

A.L. ABBOTT AND ASSOCIATES (PTY) LTD

(Reg. No. 1982/004379/07)

Consulting Analytical & Industrial Chemists
Specialists in Water & Waste Water Treatment
Telephone (021)448 6340/1
After Hours (021)712 0940
Telefax (021)448 6342
e-Mail Address :
info@alabbott.co.za

T0276

No. 1, Vine Park
Vine Road
7925
P.O. Box 483
WOODSTOCK, CAPE
7915

Certificate of Analysis

MICROCHEM LAB SERVICES (PTY) LTD

ANALYSIS

AG 85690

DATE SAMPLED : 2017/05/10

DATE RECEIVED : 2017/05/10

DATE ANALYSIS COMMENCED : 2017/05/10

ORDER: PO.100931

OUR REF. : 2017/05/10/11094

REPORT NO . : 2101

	Sample Number	11094	
Mthd ALA No.	**Analyses**	**Results**	**SANS 241-1:2015**
N/A	Total microcystin (µg/l)	<0.15	≤1
N/A	Combined Trihalomethane	0.04	≤1.0
N/A	Trihalomethane (Bromodichloromethane) (µg/l)	<1.0	≤60 Chronic Health
N/A	Trihalomethane (Bromoform) (µg/l)	<1.0	≤100 Chronic Health
N/A	Trihalomethane (Chloroform) (µg/l)	<1.0	≤300 Chronic Health
N/A	Trihalomethane (Dibromochloromethane) (µg/l)	<1.0	≤100 Chronic Health

N. VAN BINSBERGEN (Pr.Sci.Nat.)
DIRECTOR
22 May 2017

TO: MICROCHEM
176 Sir Lowry Road
WOODSTOCK
CAPE TOWN
7925

Att: SALWA BADIEN <Chem@microchem.co.za>

Sampler : Unknown

A.L. ABBOTT AND ASSOCIATES (PTY) LTD

(Reg. No. 1982/004379/07)

Consulting Analytical & Industrial Chemists
Specialists in Water & Waste Water Treatment
Telephone (021)448 6340/1
After Hours (021)712 0940
Telefax (021)448 6342
e-Mail Address :
info@alabbott.co.za

T0276

No. 1, Vine Park
Vine Road
7925
P.O. Box 483
WOODSTOCK, CAPE
7915

Certificate of Analysis

MICROCHEM LAB SERVICES (PTY) LTD

ANALYSIS

AG 92981

DATE SAMPLED : 2017/06/07 **PO:101098**

DATE RECEIVED : 2017/06/07 **OUR REF. :** 2017/06/07/13121

DATE ANALYSIS COMMENCED : 2017/06/07 **REPORT NO . :** 2538

	Sample Number	13121	
Mthd ALA No.	**Analyses**	**Results**	**SANS 241-1:2015**
N/A	Total microcystin (µg/l)	<0.15	≤1
N/A	Combined Trihalomethane	0.04	≤1.0
N/A	Trihalomethane (Bromodichloromethane) (µg/l)	<1.0	≤60 Chronic Health
N/A	Trihalomethane (Bromoform) (µg/l)	<1.0	≤100 Chronic Health
N/A	Trihalomethane (Chloroform) (µg/l)	<1.0	≤300 Chronic Health
N/A	Trihalomethane (Dibromochloromethane) (µg/l)	<1.0	≤100 Chronic Health

JOSE DA SILVA (Cert.Sci.Nat.)
TECHNICAL MANAGER
26 June 2017

TO: MICROCHEM
176 Sir Lowry Road
WOODSTOCK
CAPE TOWN
7925

Att: SALWA BADIEN <Chem@microchem.co.za>

Sampler : Unknown

A.L. ABBOTT AND ASSOCIATES (PTY) LTD

(Reg. No. 1982/004379/07)

Consulting Analytical & Industrial Chemists
Specialists in Water & Waste Water Treatment
Telephone (021)448 6340/1
After Hours (021)712 0940
Telefax (021)448 6342
e-Mail Address :
info@alabbott.co.za

T0276

No. 1, Vine Park
Vine Road
7925
P.O. Box 483
WOODSTOCK, CAPE
7915

Doc.No. 5.10/1 Rev.3

Certificate of Analysis

MICROCHEM LAB SERVICES (PTY) LTD

ANALYSIS

AG 92982

DATE SAMPLED : 2017/06/07 **PO:101096**

DATE RECEIVED : 2017/06/07 **OUR REF. :** 2017/06/07/13122

DATE ANALYSIS COMMENCED : 2017/06/07 **REPORT NO . :** 2540

	Sample Number	13122	
Mthd ALA No.	**Analyses**	**Results**	**SANS 241-1:2015**
N/A	Total microcystin (µg/l)	<0.15	≤1
N/A	Combined Trihalomethane	0.04	≤1.0
N/A	Trihalomethane (Bromodichloromethane) (µg/l)	<1.0	≤60 Chronic Health
N/A	Trihalomethane (Bromoform) (µg/l)	<1.0	≤100 Chronic Health
N/A	Trihalomethane (Chloroform) (µg/l)	<1.0	≤300 Chronic Health
N/A	Trihalomethane (Dibromochloromethane) (µg/l)	<1.0	≤100 Chronic Health

JOSE DA SILVA (Cert.Sci.Nat.)
TECHNICAL MANAGER
26 June 2017

TO: MICROCHEM
176 Sir Lowry Road
WOODSTOCK
CAPE TOWN
7925

Att: SALWA BADIEN <Chem@microchem.co.za>

Sampler : Unknown

TraceCERT®
Traceable Certified Reference Materials

SUPELCO
Solutions within.™
595 North Harrison Road · Bellefonte, PA
16823-0048 USA · Phone(814)359-3441

Certificate of Analysis

Description: Trihalomethanes Calibration Mix, 1x1ml, 2000µg/ml in methanol

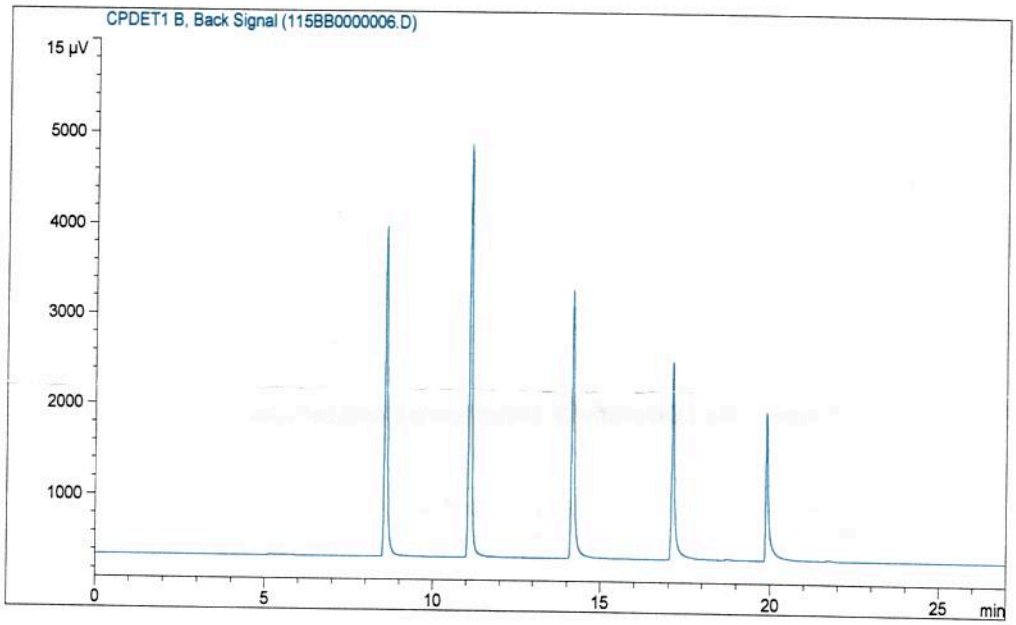
Part Number: CRM48140

Lot Number: XA16018V

Expiration Date: August 2018

Storage: Refrigerate

Analytical Method Parameters:
Column: VOCOL 105m x 0.53mm x 3.0µm df
40°C for 4 min to 200°C at 8°C/min hold 3 min
Detector: HALL, 900°C
Injection Volume: 1µl



Elution	Analyte	Lot Number	CAS Number	Certified Purity %	Certified Gravimetric Conc. µg/ml	Expanded Uncertainty µg/ml	Analytical Conc. µg/ml
	Internal Standard	N/A	N/A	N/A	N/A	N/A	N/A
2.	Chloroform, CRM	LB97902	67-66-3	98.3	1966.0	±14	1959.1
3.	Bromodichloromethane, CRM	LB97899	75-27-4	97.5	1999.9	±16	1951.9
4.	Dibromochloromethane, CRM	LB98625	124-48-1	97.1	2000.3	±20	1990.3
5.	Bromoform, CRM	LB98624	75-25-2	99.7	1994.4	±23	1923.1

ANAB ACCREDITED TESTING LABORATORY
Cert# AT-1607

ANAB ACCREDITED REFERENCE MATERIAL PRODUCER
Cert# AR-1606

Produced in double accredited laboratory fulfulling
ISO/IEC 17025 and
ISO Guide 34

SIGMA-ALDRICH®

Notes:

• All starting materials are Certified Reference Materials.

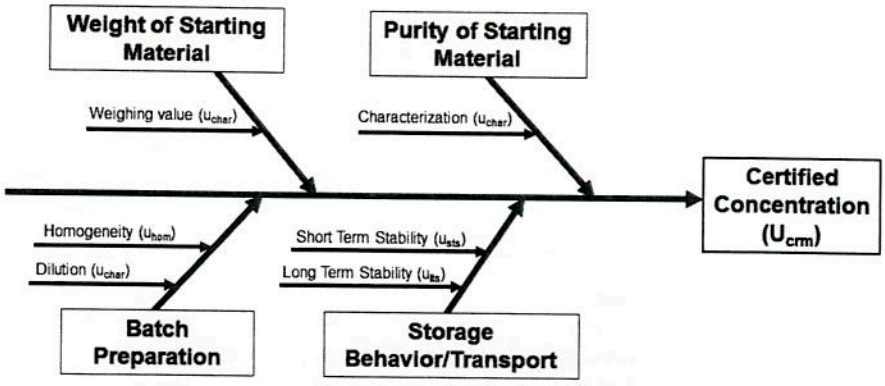
• NIST traceable weights are used to verify balance calibration with the preparation of each lot. Concentration of analyte in solution is µg/ml.

• Certified value is the gravimetric concentration weighed at room temperature. Uncertainty of the gravimetric concentration in this document is expressed as Expanded Uncertainty (Ucrm) corresponding to the 95% confidence interval. Ucrm is derived from the combined standard uncertainty multiplied by the coverage factor k = 2. The components of combined standard uncertainty include the uncertainties due to characterization, homogeneity, long-term stability and short-term stability. The components due to stability are generally considered to be negligible unless otherwise indicated by stability studies.

• Homogeneity was assessed in accordance with ISO Guide 35. Completed units were sampled using a random stratified sampling protocol. The results of chemical analysis were then compared by Single Factor Analysis of Variance (ANOVA). The uncertainty due to homogeneity was derived from the ANOVA. Heterogeneity was not detected under the conditions of the ANOVA.

• Product intended for laboratory use only. Supelco warrants that its products conform to the information contained in this publication. Purchaser must determine the suitability of the product for its particular use. Please see the latest catalog or order invoice and packing slip for additional terms and conditions of sale.

Cause-Effect Diagram for Uncertainty Contributions



$$U_{crm} = k\sqrt{u_{char}^2 + u_{hom}^2 + u_{sts}^2 + u_{lts}^2}$$

Test Date: August 26, 2015
Form: CRM48140

Duane Funk

Duane Funk
QC Manager