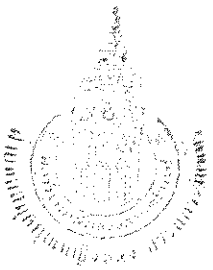

Speciation of Trihalomethanes (THMs) in Water by Headspace-Gas Chromatographic Technique (HS-GC)

Wanpen Rattanarungsi

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Analytical Chemistry Prince of Songkla University

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Thesis Title	Speciation of Trihalomethanes (THMs) in Water by Headspace-Gas Chromatographic Technique (HS-GC)
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Dean of Graduate School

ชื่อวิทยานิพนธ์ ศึกษาชนิดของไตรฮาโลมีเทนในน้ำโดยเทคนิคเฮดสเปซ/แก๊สโครมาโทกราฟี
ผู้เขียน นางสาววันเพ็ญ รัตนรังษี
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ปีการศึกษา 2550

บทคัดย่อ

ศึกษาการเตรียมตัวอย่าง ด้วยเทคนิคสเตติคเฮดสเปซ (Static headspace) และการสกัดด้วยตัวทำละลายหยดเดียวร่วมกับเฮดสเปซ (Headspace single-drop microextraction) สำหรับการวิเคราะห์ไตรฮาโลมีเทนในน้ำ ซึ่งได้แก่ คลอโรฟอร์ม ($CHCl_3$) ไดคลอโรโบรโมมีเทน ($CHCl_2Br$) ไดโบรโมคลอโรมีเทน ($CHClBr_2$) และโบรโมฟอร์ม ($CHBr_3$) ด้วยเทคนิคแก๊สโครมาโทกราฟี ใช้คอลัมน์แก้วขนาดเส้นผ่านศูนย์กลางภายใน 2 มิลลิเมตร ยาว 2 เมตร ภายในบรรจุด้วยสเตชันนารีเฟส คือ 1% SP-1000 เคลือบบนตัวซับพอร์ทชนิด Carbopack B ขนาด 60/80 mesh ร่วมกับตัวตรวจชนิดอิเลคตรอนแคปเจอร์

เทคนิคสเตติคเฮดสเปซที่สภาวะเหมาะสมมีขีดจำกัดการตรวจวัดของคลอโรฟอร์ม ไดคลอโรโบรโมมีเทน ไดโบรโมคลอโรมีเทน และโบรโมฟอร์ม 0.22 0.04 0.06 และ 0.35 ไมโครกรัมต่อลิตร ตามลำดับ และช่วงความเป็นเส้นตรงของคลอโรฟอร์ม ไดคลอโรโบรโมมีเทน ไดโบรโมคลอโรมีเทน และโบรโมฟอร์ม จาก 0.5 ถึง 75 ไมโครกรัมต่อลิตร 0.1 ถึง 60 ไมโครกรัมต่อลิตร 0.1 ถึง 70 ไมโครกรัมต่อลิตร และ 0.1 ถึง 180 ไมโครกรัมต่อลิตร ตามลำดับ มีค่าสหสัมพันธ์เชิงปริมาณ (coefficient of determination, R^2) มากกว่า 0.99 และค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ต่ำกว่า 4 เปอร์เซ็นต์ เทคนิคการสกัดด้วยตัวทำละลายหยดเดียวร่วมกับเฮดสเปซ ที่สภาวะเหมาะสมให้ขีดจำกัดการตรวจวัดของคลอโรฟอร์ม ไดคลอโรโบรโมมีเทน ไดโบรโมคลอโรมีเทน และโบรโมฟอร์ม 4.00×10^{-2} 2.85×10^{-3} 4.39×10^{-3} และ 1.34×10^{-2} ไมโครกรัมต่อลิตร ตามลำดับ และช่วงความเป็นเส้นตรงของคลอโรฟอร์ม ไดคลอโรโบรโมมีเทน ไดโบรโมคลอโรมีเทน และโบรโมฟอร์มในช่วง 0.05 ถึง 20 ไมโครกรัมต่อลิตร 0.05 ถึง 5 ไมโครกรัมต่อลิตร 0.01 ถึง 10 ไมโครกรัมต่อลิตร และ 0.01 ถึง 20 ไมโครกรัมต่อลิตร ตามลำดับ มีค่าสหสัมพันธ์เชิงปริมาณ (coefficient of determination, R^2) มากกว่า 0.99 และค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ต่ำกว่า 15 เปอร์เซ็นต์ มีการพิสูจน์การใช้ได้ของวิธีการเตรียมตัวอย่างทั้งสองและนำไปใช้วิเคราะห์ตัวอย่างจริง พบว่าความเข้มข้นของไตรฮาโลมีเทนในตัวอย่างน้ำบ่อ น้ำประปา และน้ำดื่มอยู่ในช่วงที่ไม่สามารถตรวจวัดได้ ถึง 25±2 ไมโครกรัมต่อลิตร ซึ่งไม่เกินค่ามาตรฐานขององค์การอนามัยโลก

องค์กรพิทักษ์สิ่งแวดล้อมแห่งสหรัฐอเมริกา และสหภาพยุโรป

เมื่อเปรียบเทียบวิธีการเตรียมตัวอย่างด้วยเทคนิคสเตติคเฮดสเปซและการสกัดด้วยตัวทำละลายหยดเดียวร่วมกับเฮดสเปซ พบว่าเทคนิคการสกัดด้วยตัวทำละลายหยดเดียวร่วมกับเฮดสเปซให้ความไววิเคราะห์สูงกว่าเทคนิคสเตติคเฮดสเปซ แต่เทคนิคสเตติคเฮดสเปซให้ความแม่นยำดีกว่าเทคนิคการสกัดด้วยตัวทำละลายหยดเดียวร่วมกับเฮดสเปซ ทั้งสองวิธีเป็นวิธีที่ง่าย รวดเร็ว ลดการใช้ตัวทำละลาย ราคาประหยัด มีความไววิเคราะห์ และ มีความจำเพาะเจาะจงสูง

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Major Program	Analytical Chemistry
Academic Year	2007

ABSTRACT

Headspace extraction techniques *i.e.,* static headspace (SHS) and headspace-single drop microextraction (HS-SDME) were used for sample preparation of trihalomethanes *i.e.,* chloroform ($CHCl_3$) dichlorobromomethane ($CHCl_2Br$) dibromochloromethane ($CHClBr_2$) and bromoform ($CHBr_3$) in water and simultaneously analyzed by gas chromatograph equipped with a 2 m × 2.0 mm i.d., glass column, 1% SP-1000 on Carbopack B 60/80 mesh and electron capture detector (GC-ECD).

At optimum conditions the SHS-GC-ECD system provided the limit of detections at 0.22, 0.04, 0.06, and 0.35 μg L^{-1}, for $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$, respectively. The linear dynamic range from 0.5 to 75 μg L^{-1} for $CHCl_3$, 0.1 to 60 μg L^{-1} for $CHCl_2Br$, 0.1 to 70 μg L^{-1} for $CHClBr_2$, and 0.1 to 180 μg L^{-1} for $CHBr_3$, with a coefficient of determination (R^2) more than 0.99 and the relative standard deviations (RSD) less than 4%. The HS-SDME-GC-ECD system at optimum conditions provided the limit of detections for $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$ at 4.00×10^{-2}, 2.85×10^{-3}, 4.39×10^{-3} and 1.34×10^{-2} μg L^{-1}, respectively. The linear dynamics range from 0.05 to 20 μg L^{-1} for $CHCl_3$, 0.05 to 5 μg L^{-1} for $CHCl_2Br$, 0.01 to 10 μg L^{-1} for $CHClBr_2$, and 0.01 to 20 μg L^{-1} for $CHBr_3$, with $R^2 > 0.99$ and RSD < 15%. Both systems were validated and applied to real water samples. The total trihalomethanes concentration in well water, tap water, and drinking water were in the range of ND to 25±2 μg L^{-1}, which do not exceed the WHO, US EPA, and EU guideline values.

Comparison between HS-SDME and SHS, HS-SDME gave a better sensitivity than SHS but the precision of SHS was better than HS-SDME. Both methods are very simple and rapid, less toxic solvent consumption, cost effective, and high sensitive and selective.

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My parents, my sisters, my brother and my best friends for their loves and attentions throughout my life;

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And finally, friendship of members in Analytical and Environmental Chemistry/Trace Analysis Research Unit and Biophysics Research Unit: Biosensors and Biocurrents who helped me in innumerable ways during these years.

Wanpen Rattanarungsi

THE RELEVANT OF THE RESEARCH WORK TO THAILAND

Speciation of Trihalomethanes (THMs) in Water by Headspace-Gas Chromatographic Technique (HS-GC) is Master of Science Thesis in Analytical Chemistry. It is a basic research which creates a new knowledge in analysis techniques for compound which is related to human health.

This technique can be applied for qualitative and quantitative analysis of trihalomethanes in water by several governmental and private organizations in Thailand which are the Ministry of Public Health, the Ministry of Industry and the Ministry of Education.

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CHAPTER 1

Introduction

1.1 Introduction

The production of water for human consumption is a major industry nowadays (Gibbons and Laha, 1999). Basically, water is collected from a source of supply, purified and distributed to the customers. Disinfection is the most important step in the treatment of drinking water supplies, because it removes or inactivates pathogenic organisms responsible for waterborne diseases such as cholera, typhoid fever, and dysentery (Golfinopoulos and Nikolaou, 2005; Uyak *et al.*, 2005). There are a variety of disinfection methods being utilized worldwide for treatment of water *e.g.*, chlorination, ozone addition and ultraviolet radiation.

Chlorination is the most common used disinfection method because it is extremely efficient and cost effective (Nazir and Khan, 2006). However, this method has become under scrutiny because of its potential to react with natural organic matter and form chlorinated disinfectant by-products such as trihalomethanes, haloacetic acids, haloacetonitriles, haloketones, chloral hydrate, and chloropicrin (Nikolaou *et al.*, 2005). The major categories of chlorinated disinfectant by-products detected in chlorinated drinking waters are trihalomethanes and haloacetic acids (Golfinopoulos and Nikolaou, 2005). Trihalomethanes are volatile chlorinated by-products in drinking water, formed when chlorine or bromine reacts with natural organic matter (*i.e.,* humic acid and fulvic acid). Generally, they are found in the forms of chloroform ($CHCl_3$), dichlorobromomethane ($CHCl_2Br$), dibromochloromethane ($CHClBr_2$), and bromoform ($CHBr_3$) (Garcia-Villanova *et al.*, 1997; Golfinopoulos and Arhonditsis, 2002; Golfinopoulos and Nikolaou, 2005). Trihalomethanes are considered by the United State Environmental Protection Agency (US EPA) to be possible carcinogens, where three of them, $CHCl_3$, $CHCl_2Br$ and $CHBr_3$, are classified as probable human carcinogens and one of them, $CHClBr_2$,

as a possible human carcinogen (Batterman *et al.*, 2002). In order to protect public health from the possible carcinogenic effects of these substances, US EPA and the European Union (EU) have set the maximum contaminant level (MCL) for total trihalomethanes in drinking water at 80 (US EPA, 2001) and 150 μg L^{-1} (EU, 1998), respectively. In Thailand, the Metropolitan Waterworks Authority (MWA) has adopted the World Health Organization (WHO) guideline values of trihalomethanes in drinking water where the maximum contaminant levels (MCLs) for $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$ are at 200, 60, 100, and 100 μg L^{-1}, respectively. In addition, the sum of ratio of concentration of each to its respective guideline value should not exceed 1 (WHO, 2004).

A technique for determination of trihalomethanes in water needs to be simple, rapid, high sensitive and selective because these compounds are volatile and present in water at very low concentration (ng L^{-1} to μg L^{-1}). A widely used technique is gas chromatography coupled with electron capture detector (ECD) or mass spectrometer detector (MS). Direct aqueous injection is the simplest approach since it requires no concentration or extraction step. However, this technique provides low sensitivity, possibly interfered by matrix interference and has problems with column stability and critical temperatures for column and injector (Cho *et al.*, 2003). Therefore, a sample preparation before analysis is necessary.

Conventional extraction technique like liquid–liquid extraction (LLE) is widely used for the extraction of trihalomethanes in water (Nikolaou *et al.*, 2005; Simpson and Hayes, 1998; Yu and Cheng, 1999); nevertheless, this technique has many disadvantages, for instance, laborious, time-consuming procedure and consuming large amount of toxic solvents which are carcinogenic and hazardous to the environment. These limitations can be reduced using headspace (HS) techniques such as static headspace (SHS), purge and trap (P&T), headspace solid-phase microextraction (HS-SPME) and headspace single-drop microextraction (HS-SDME). In headspace analysis only the vapour phase (gas) of the sample is used so, it is a very 'clean' technique avoiding the accumulation of non-volatile components in the GC system and decreasing the use of solvent which fits well with the idea of 'green' analytical chemistry.

In this work, static headspace (SHS) and headspace single-drop microextraction (HS-SDME) are attempted and used to determine trihalomethanes concentrations in water using gas chromatographic technique with electron capture detector (GC-ECD). It is expected that this system will reduce toxic solvent, extremely simple, fast and inexpensive procedure and enhance sensitivity of method. These techniques will also be validated to obtain confidence of analysis.

1.2 Chlorination

Chlorination has been used for water disinfection since the last century, and it remains the most widely used technique in the water industry because it is extremely efficient and cost effective. Chlorine is usually added in sufficient quantity that a small residual will accompany treated water through the distribution system. This substance can protect consumers from bacterial contaminants later gain access to the supply (Gibbons and Laha, 1999). When chlorine is used for disinfection in water, the added chlorine hydrolyses to form hypochlorous acid (HOCl) (Reaction 1.1):

$$Cl_2 + H_2O \longrightarrow HOCl + H^+ + Cl^- \qquad (1.1)$$

Hypochlorous acid dissociates into hydrogen ions (H^+) and hypochlorite ions in the reversible reaction (Reaction 1.2):

$$HOCl \rightleftharpoons H^+ + OCl^- \qquad (1.2)$$

Hypochlorous acid, hydrogen ions (H^+) and hypochlorite ion can deactivate pathogenic microorganisms and protect them from regrowth in the water distribution system. Hypochlorous acid, the prime disinfecting agent, is a weak acid with p*K*a approximately 7.5 at 25 °C, therefore it is dominant as an effective disinfectant whereas hypochlorite ion dominates at pH above 7.5 (Yu and Cheng, 1999; IPCS, 2000).

Another reaction that occurs in waters containing bromide ion and hypochlorite is the production of hypobromous acid (Reaction 1.3). This reaction is irreversible and the product, hypobromous acid, is a stronger halogenating agent than hypochlorous acid (IPCS, 2000).

$$HOCl + Br^- \longrightarrow HOBr + Cl^- \qquad (1.3)$$

Chlorination lead to formation of chlorinated disinfectant by-products such as trihalomethanes, haloacetic acids, haloacetonitriles, haloketones, chloral hydrate, and chloropicrin (Nikolaou *et al.*, 2005). Thus, to minimize chlorinated disinfectant by-products formation, alternative disinfectants *e.g.*, ozone, chloramines, and chlorine dioxide have been tested. Although many of them help to minimize trihalomethanes, they also create potential new problems. Ozone cannot give a residual effect during distribution and it has higher cost. Chloramines are not as effective as chlorine and have toxicological properties. Chlorine dioxide may generate inorganic pollutants such as chlorite and chlorate. Hence, chlorine is still widely used over the world as a water disinfectant (Simpson and Hayes, 1998; Yu and Cheng, 1999).

1.3 Occurrence of trihalomethanes

Trihalomethanes in the form of chloroform ($CHCl_3$), dichlorobromo-methane ($CHCl_2Br$) dibromochloromethane ($CHClBr_2$), and bromoform ($CHBr_3$) are discovered in drinking water by Bellar *et al.* and Rook in 1974 (Simpson and Hayes, 1998), formed during chlorination of disinfection process by reaction between available chlorine and organic substances in water, mainly humic and fulvic acids (Reaction 1.4). Brominated trihalomethanes are formed when bromide presented because it is oxidized by hypochlorous acid to yield hypobromous acid which, along with the residual hypochlorous acid, reacts with the organic precursors to produce mixed chloro-bromosubtituted products (San Juan *et al.*, 2007). After that, iodinated trihalomethanes have been reported in 1975. They are formed when iodide (from

natural sources, sea-water intrusion or brines) is present in raw water (Cancho *et al.*, 1999).

Free chlorine + Humic substances → Trihalomethanes + Other by-products (1.4)

Trihalomethanes are the major categories of disinfection by-products detected in chlorinated drinking waters (Simpson and Hayes, 1998). The formation of trihalomethanes in chlorinated water depends on the composition of the raw water such as inorganic compounds and natural organic matter, on the operational parameters such as temperature, pH, dose, and contact time, on the occurrence of residual chlorine in the distribution system and on the seasonal variation (Golfinopoulos and Nikolaou, 2005). In chlorinated water, trihalomethanes are usually found in the forms of $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$ exist together. The formation potential of trihalomethanes followed the order $CHCl_3$ > $CHCl_2Br$ > $CHClBr_2$ > $CHBr_3$ (Nikolaou *et al.*, 2005) that is, chloroform is a dominated species of trihalomethanes in water which speciations of trihalomethanes depend on the nature of the water source; when the increasing levels of bromide in sources waters cause a shift in the distribution of trihalomethanes to the more highly brominated species (Golfinopoulos and Nikolaou, 2005).

Trihalomethanes have often been studied in the drinking water, tap water and well water. In Australia, the total trihalomethanes concentrations from water samples in 16 different locations across Australia are ranged between 25 to 191 $\mu g\ L^{-1}$. These do not exceed the National Health and Medical Research Council (NHMRC) trihalomethanes guideline value which are 250 $\mu g\ L^{-1}$ but these exceed the US EPA and EU guideline values of 80 $\mu g\ L^{-1}$ and 150 $\mu g\ L^{-1}$, respectively (Simpson and Hayes, 1998). The quantity of total trihalomethanes in drinking water in the 19 districts of Hong Kong are also studied and found in the range 15.8 to 131.0 $\mu g\ L^{-1}$. These are below the WHO and EU guideline values but the total concentrations of trihalomethanes in tap water samples from 4 out of the 19 districts are above the US EPA guideline values (Yu and Cheng, 1999). In Greece and Italy, the concentrations of total trihalomethanes were investigated in water samples from two of the water

resources supplying drinking water treatment plants, *i.e.,* Galatsi in Athens, Greece and Carmine in Italy. The result indicated that total trihalomethanes concentrations in Greece and Italy were 203.9 μg L^{-1} and 120.3 μg L^{-1}, respectively which exceeds the US EPA guideline values (Nikolaou *et al.*, 2005). In addition, Golfinopoulos and Nikolaou (2005) investigated the occurrence of disinfection by-produces in the drinking water sample collected from four water treatment plants and from the distribution system of Athens, Greece for a period of two years (2001-2002). They found that the total trihalomethanes concentrations are in the range of 18.9 to 63.8 μg L^{-1} which lower than US EPA and EU guideline. In Sweden, the survey of 209 Swedish drinking water samples found that 3% exceeded the Swedish limit value of 50 μg L^{-1} of total trihalomethanes (Kuivinen and Johnsson, 1999).

In addition, total trihalomethanes have been investigated in swimming pool, beer and juice sample. In swimming pool, the total trihalomethanes are ranged from 105 to 134 μg L^{-1} which do not exceed the EU guideline value of 150 μg L^{-1} (Stack *et al.*, 2000). For beer and juice are ranged from 1.5 to 5.0 μg L^{-1} and 2.1 to 7.3 μg L^{-1}, respectively which also below the US EPA guideline values (Campillo *et al.*, 2004).

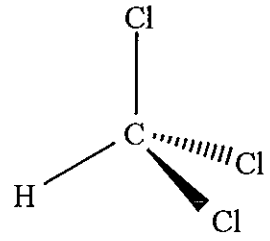
1.4 Chemical identification

Trihalomethanes are halogen-substituted single carbon compounds with general chemical formular CHX_3, where X is halogen compound *i.e.,* Cl, Br, I, and F or a combination of halogens (Nazir and Khan, 2006). Several trihalomethanes have been reported were present in chlorinated water such as

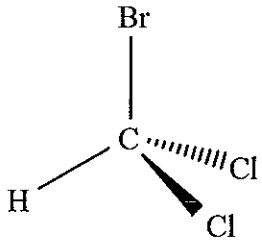
- Chloroform ($CHCl_3$)
- Dichlorobromomethane ($CHCl_2Br$)
- Bromoform ($CHBr_3$)
- Dibromochloromethane ($CHClBr_2$)
- Dibromoiodomethane ($CHBrI_2$)
- Dibromoiodomethane ($CHBr_2I$)
- Dichloroiodomethane ($CHCl_2I$)

(Cancho *et al.*, 1999; Golfinopoulos and Nikolaou, 2005)

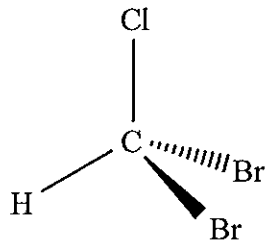
Generally, trihalomethanes are found in the forms of $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$. In addition, these four compounds are classified as possible carcinogens. Thus, they will be the target analytes throughout this study. Further, trihalomethanes will mean only these four compounds. Figure 1.1 and Table 1.1 shows their chemical structures and chemical identifications, respectively.



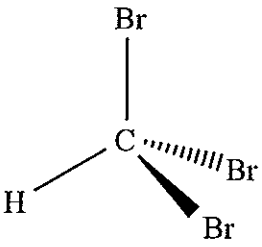
Chloroform ($CHCl_3$)



Dichlorobromomethane ($CHCl_2Br$)



Dibromochloromethane ($CHClBr_2$)



Bromoform ($CHBr_3$)

Figure 1.1 Chemical structures of trihalomethanes.

Table 1.1 Chemical identifications of trihalomethanes (Lewis, 1997).

Molecular formula	IUPAC name	Other names	CAS registry number
$CHCl_3$	trichloromethane	chloroform, methyl trichloride, formyl trichloride, methane trichloride, trichloroform, methenyl trichloride	67-66-3
$CHBrCl_2$	bromodichloromethane	dichlorobromomethane, dichloromonobromomethane, monodibromomethane	75-27-4
$CHBr_2Cl$	dibromochloromethane	chlorodibromomethane, dibromomonochloromethane, monochlorodibromomethane	124-48-1
$CHBr_3$	tribromomethane	bromoform, methyl tribromide, methenyl tribromide, Formyl tribromide	75-25-2

1.5 Physical properties

Trihalomethanes are volatile liquids at room temperature and the water solubility of trihalomethanes is poor. The basic physical and chemical properties of trihalomethanes, for instance, color, molecular weight, boiling point, melting point, vapor pressure, *etc.*, are summarized in Table 1.2.

Table 1.2. Physical properties of trihalomethanes (Batterman *et al.*, 2002; Lewis, 1997).

Properties	Compounds			
	Chloroform ($CHCl_3$)	Dichlorobromomethane ($CHBrCl_2$)	Dibromochloromethane ($CHBr_2Cl$)	Bromoform ($CHBr_3$)
Appearance	colorless liquid with a sweet odor	colorless liquid	colorless to yellow-orange liquid	colorless to yellow liquid
Molecular weight (g mol^{-1})	119.38	163.83	208.28	252.73
Boiling point (°C)	61-62	87-90	119-120	149-150
Melting point (°C)	-63.5	-55	-22	5-8
Vapor pressure at 25 °C (kPa)	26.30	2.27	0.74	0.72
Density (g cm^{-3})	1.48	1.98	2.38	2.89
Octanol-water partition coefficient, log K_{ow}	1.97	2.10	2.24	2.38

1.6 Heath effects

The addition of chlorine (as oxidants) into water generates a group of trihalomethanes, which have been found to be associated with adverse health effect (Gopal *et al.*, 2007).

1.6.1 Exposure pathways

The principal source of human exposure to trihalomethanes is chlorinated water supplied to home, work and public places through difference exposure pathway *i.e.*, ingestion, inhalation and dermal absorption by the ingestion of tap water and contaminated food or drinks, the inhalation of vapor trihalomethanes resulting from showering, dish washing and swimming, and the dermal exposure during bathing, showering, washing and swimming. Infants may also be exposure to these compounds by lactation transfer from mother to infant (Batterman *et al.*, 2002).

Nazir and Khan (2006) studied the human health risk modeling for various exposure routes of trihalomethanes in potable water supply and found that the inhalation route has the higher cancer risk, due to shower activity, than the ingestion and dermal absorption route. On the other hand, from the study of Uyak (2006) who conducted a multi-pathway exposure assessment of the drinking water of Istanbul based on the concentrations of trihalomethanes within water distribution systems in European side of Istanbul city. The results indicated that Istanbul residents had a higher cancer risk through oral ingestion than the inhalation and dermal absorption pathways. The total cancer risk analysis due to the trihalomethanes exposure through the multi-pathways concluded that each year approximately 5 of the 8 million Istanbul residents could get cancer from the daily intake of tap water (Uyak, 2006). In addition, Wang and coworker (2007) reported the risk assessment of trihalomethanes in drinking water of Beijing, China and three treatment plants using different disinfection processed of Canada. The results also showed that people had a higher cancer risk through oral ingestion (Wang *et al.*, 2007).

1.6.2 Toxicity

Several studies show that exposure to trihalomethanes increase the risk of cancer. The exposure to trihalomethanes has been associated with increased risks of bladder, colon, kidney, stomach and pancreatic cancers (Tokmak *et al.*, 2004). A number of epidemiology studies indicate that exposure to trihalomethanes result in reproductive effect such as intrauterine growth retardation, low birth weight, preterm birth, congenital malformations, and stillbirth (Graves *et al.*, 2001). Studies conducted on mammals have revealed that trihalomethanes induce neurotoxicity, hepatotoxicity, reproductive toxicity and nephrotoxicity (Gopal *et al.*, 2007). The health effects from these compounds are listed in Table 1.3.

Table 1.3 Trihalomethanes and its health effects (Gopal *et al.*, 2007).

Compounds	Health effects
Chloroform ($CHCl_3$)	Cancer, liver, kidney and reproductive effects
Dichlorobromomethane ($CHCl_2Br$)	Cancer, liver, kidney and reproductive effects
Dibromochloromethane ($CHClBr_2$)	Nervous system, liver, kidney and reproductive effects
Bromoform ($CHBr_3$)	Cancer, liver, kidney and reproductive effects

The toxicity is dependent on a reactive compound actually reaching a sensitive target site. Perhaps to a greater extent than with other chemicals in this class, $CHCl_2Br$ appears to reach a variety of target tissues where it can be readily metabolized to several intermediates, leading to adverse effects in experimental animals. As a result $CHCl_2Br$ represents the most serious cancer risk, followed by $CHBr_3$, $CHCl_3$ and $CHClBr_2$ (IPCS, 2000).

The United State Environmental Protection Agency (US EPA) has assigned, in a weight-of-evidence classification, $CHCl_3$, $CHCl_2Br$ and $CHBr_3$ as probable human carcinogens, group B2, and $CHClBr_2$ as a possible human carcinogen, group C (US EPA, 1993) as shown in Table 1.4.

Table 1.4 US EPA classifications of trihalomethanes (US EPA, 1993).

Compounds	Classification
Chloroform ($CHCl_3$)	Probable human carcinogens (group B2)
Dichlrobromomethane ($CHCl_2Br$)	Probable human carcinogens (group B2)
Dibromochloromethane ($CHClBr_2$)	Possible human carcinogens (group C)
Bromoform ($CHBr_3$)	Probable human carcinogen (group B2)

The International Agency for Research on Cancer (IARC) reviews research conducted on potential carcinogens and classifies the compound. As can be seen from Table 1.5, $CHCl_3$ and $CHCl_2Br$ are classified as possibly carcinogenic to humans whereas, $CHClBr_2$ and $CHBr_3$ are not classifiable, indicating there is no evidence supporting these two compounds as carcinogens, but there is not enough research to classify them as non-carcinogenic (WHO, 1993).

Table 1.5 IARC classifications of trihalomethanes (WHO, 1993).

Compounds	Classification
Chloroform ($CHCl_3$)	Possibly carcinogenic to humans (group 2B)
Dichlorobromomethane ($CHCl_2Br$)	Possibly carcinogenic to humans (group 2B)
Dibromochloromethane ($CHClBr_2$)	Not classifiable as to its carcinogenicity in humans (group 3)
Bromoform ($CHBr_3$)	Not classifiable as to its carcinogenicity in humans (group 3)

1.7 Guideline values

A guideline value represents the concentration of a constituent that does not result in any significant risk to health over a lifetime of consumption (Gopal *et al.*, 2007). Identification of trihalomethanes and concern over the possible adverse health effects of these compounds has promoted considerable research activity in many countries in order to minimize the risk of cancers. The United State Environmental Protection Agency (US EPA), the World Health Organization (WHO) and the European Union (EU) introduced regulations for trihalomethanes in drinking water. Because these compounds usually exist together, they have been regulated as a total trihalomethanes. In 1979, US EPA initiated a regulatory standard of 100 $\mu g\ L^{-1}$ for total trihalomethanes under the "safe drinking water act" and has considering extensive revisions to regulate covering disinfectant by-products, including a two-stage reduction in total trihalomethanes limits. The proposed maximum contaminant level (MCL) for total trihalomethanes is 80 $\mu g\ L^{-1}$ in stage 1 and 40 $\mu g\ L^{-1}$ in stage 2 (US EPA, 1998). The European Union (EU) set limit values for total trihalomethanes at 150 $\mu g\ L^{-1}$ until December 2008; thereafter, it will be 100 $\mu g\ L^{-1}$ (EU, 1998) and chloroform at 30 $\mu g\ L^{-1}$ (EU, 1995). In addition, Germany set the guideline value for total trihalomethanes at 10 $\mu g\ L^{-1}$. For Sweden regulation of total trihalomethanes is divided into two parts: guide level of 20 $\mu g\ L^{-1}$ and a limit value of 50 $\mu g\ L^{-1}$ (Kuivinen and Johnsson, 1999).

In Thailand, the Metropolitan Waterworks Authority (MWA) has adopted the guideline values provided by the World Health Organization (WHO) of trihalomethanes in drinking water by setting the maximum contaminant level (MCL) for $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$ at 200, 60, 100, and 100 $\mu g\ L^{-1}$, respectively. In addition, the sum of ratio of concentration of each to its respective guideline value should not exceed 1 (WHO, 2004).

1.8 Analytical methods

The determination of trihalomethanes in water usually consists of in 2 steps, (i) sample preparation, in which analytes must be separated from other interference in sample matrix and (ii) analysis, in which analytes are measured using a suitable technique (Cano *et al.*, 2002). There are numerous methods in the literatures for measuring trihalomethanes concentration in water and these are summarized in Table 1.6. Many of these methods focused on developing or improving sample extraction or sample introduction to GC.

Table 1.6 Application of GC to analyze trihalomethanes in water samples.

Sample preparation	Detection	Column	LOD ($\mu g\ L^{-1}$)	References
Laboratory-built purge and trap	GC-ECD	DB-1 (30 m × 0.32 mm × 5.0 μm)	0.001	Zygmunt, 1996
Static headspace (SHS)	GC-ECD	Chrompack CP-SIL 13 CB (25 m× 0.32 × mm × 1.2 μm)	0.03-0.06	Kuivinen and Johnsson, 1999
Headspace solid-phase microextraction (HS-SPME)	GC-MS	HP1 (50 m × 0.20 mm × 0.5 μm)	1.0-2.8	Stack *et al.*, 2000
Continuous-flow purge and trap	GC-MS	DB-5MS (5 m × 0.25 mm × 0.25 μm)	0.01-0.05	Chen and Her, 2001
Liquid-liquid extraction (LLE)	GC-ECD	HP-1 (10 m × 0.53 mm × 2.65 μm)	0.8-1.0	Golfinopoulos *et al.*, 2001
Direct aqueous injection (DAI)	GC-ECD	Chrompack CP-sil 13 CB (25 m × 0.53 mm × 2.0 μm)	3.0-5.0	Golfinopoulos *et al.*, 2001
Static headspace (SHS)	GC-MS	HP-VOC (60 m × 0.32 mm × 1.8 μm)	0.1	Golfinopoulos *et al.*, 2001
Purge and trap	GC-MS	HP-VOC (60 m × 0.32 mm × 1.8 μm)	0.05-0.1	Golfinopoulos *et al.*, 2001
Purge and trap	GC-ECD	HP-1 (10 m × 0.53 mm × 2.65 μm)	0.025-0.05	Golfinopoulos *et al.*, 2001
Liquid-liquid extraction (LLE)	GC-ECD	DB-1 (30 m × 0.32 mm × 0.25μm)	0.005-0.010	Nikolaou *et al.*, 2002
Liquid-liquid extraction (LLE)	GC-MS	HP-VOC (60 m × 0.32 mm × 1.8 μm)	0.01-0.03	Nikolaou *et al.*, 2002
Purge and trap	GC-MS	HP-VOC (60 m × 0.32 mm × 1.8 μm)	0.01-0.05	Nikolaou *et al.*, 2002
Static headspace (SHS)	GC-MS	HP-VOC (60 m × 0.32 mm × 1.8 μm)	0.05-0.20	Nikolaou *et al.*, 2002
Headspace solid-phase microextraction (HS-SPME)	GC-ECD	HP-5 (30m × 0.32 mm × 1.0 μm)	0.005-0.010	Cho *et al.*, 2003
Headspace single-drop microextraction (HS-SDME)	GC-ECD	HP-5 (30m × 0.32 mm × 1.0 μm)	0.15-0.4	Zhao *et al.*, 2004
Supported capillary membrane sampling (SCMS)	GC-ECD	VB-624 (30 m × 0.32 mm × 1.8 μm)	0.4-0.9	Emmert *et al*, 2004

Table 1.6 Application of GC to analyze trihalomethanes in water samples (continued).

Sample preparation	Detection	Column	LOD ($\mu g\ L^{-1}$)	References
Capillary membrane sampler (CMS)	GC-ECD	VB-5 (15 m × 0.53 mm × 1.0 μm)	0.3-0.5	Emmert *et al.*, 2006
Direct single-drop microextraction (DI-SDME)	GC-ECD	DB-624 (30 m × 0.32 mm × 1.0 μm)	0.23-0.45	Tor and Aydin, 2006
Supported liquid hollow fiber membrane microextraction	GC-ECD	DB-35MS (30 m × 0.25 mm × 0.25 μm)	0.01-0.2	Vora-adisak and Varanusupakul, 2006
Headspace solid-phase microextraction (HS-SPME)	GC-MS	HP-5 (30 m × 0.25 mm ×0.25 μm)	0.078 - 0.52	San Juan *et al.*, 2007

1.9 Sample preparation

Before analysis, the sample must go through sample preparation in order to (i) remove of potential interferences from the sample, which resulting in the increasing the method selectivity, (ii) increase the concentration of the analyte and hence the increase of sensitivity of the assay, (iii) if needed, convert the analyte into a more suitable form for detection or separation and (iv) provide a robust and reproducible method (Smith, 2003). Trihalomethanes concentration levels found in drinking water present at very low concentration, typically in the order of ppt (ng L^{-1}) to ppb (μg L^{-1}), and sample was contaminated with interfering compounds so the appropriate sample preparation techniques are necessary (Zygmunt, 1996).

Several sample preparation techniques have been reported for determination of trihalomethanes such as direct aqueous injection (DAI) (Biziuk *et al.*, 1996), liquid-liquid extraction (LLE) (Golfinopoulos and Nikolaou, 2005; Nikolaou *et al.*, 2005; Simpson and Hayes, 1998), static headspace (SHS) (Kuivinen *et al.*, 1999), purge and trap (P&T) (Allonier *et al.*, 2000; Campillo *et al.*, 2004; Chen and Her, 2001), headspace solid-phase microextraction (HS-SPME) (Cho *et al.*, 2003; Stack *et al.*, 2000), single-drop microextraction (SDME) (Tor and Aydin, 2006), and headspace single-drop microextraction (HS-SDME) (Zhao *et al.*, 2004).

1.9.1 Direct aqueous injection (DAI)

Direct aqueous injection (DAI) developed by Grob and coworkers (Golfinopoulos *et al.*, 2001) is a convenient sample introduction method, in which an aqueous sample is directly introduced into a GC column. This has been used for the determination of trihalomethanes in drinking water where 1-2 μL of aqueous real sample is directly injected into a column coated with a non-polar stationary phase, with on column injector. Trihalomethanes are detected with electron capture detector, a detection system that is very sensitive towards halogen compounds (Wolska *et al.*, 1998).

Using the method, Biziuk and coworker (1996) reported the determination of volatile organohalogen compounds in tap and surface waters of the

Gdan'sk district. The 2 μL of aqueous sample was injected to GC by the injector system; cold on column with secondary cooling which the cold on column injection can improve efficiency of analysis. The analytes were separated with a 30 m × 0.32 mm i.d., fused-silica capillary column, coated with bonded 5 μm apolar DB-1 phase and precolumn was a 2 m × 0.32 mm i.d., fused-silica capillary column and then detected with ECD. Although, this method provided good detection limits of trihalomethanes at 0.01 μg L^{-1} and the standard deviation varied between 1.74 % and 3.02 %, it required special device of cold on column injection system (Grob and Barry, 1985). In 2001, Golfinopoulos and coworker reported comparison of four methods including liquid-liquid extraction (LLE), direct aqueous injection (DAI), static headspace (SHS), and purge and trap (P&T) for determination of volatile organic compounds in drinking water. For DIA method, the 1 μL of aqueous sample was injected into GC. The sample passes directly from the syringe into inlet where it vaporizes. The analytes were separated with a 25 m × 0.53 mm i.d., fused-silica capillary column, coated with 2 μm Chrompack CP-Sil 13 CB, and precolumn was a 4 m × 0.53 mm i.d., uncoated silica capillary column and then detected with ECD. This method provided limit of detection of trihalomethanes between 3 to 5 μg L^{-1} with lower sensitivity than the method reported by Biziuk and coworker (1996) and recovery ranged between 84-109 %. From comparison of the four methods the sensitivity of DAI was lower than LLE, SHS, and P&T.

The advantages of direct injection analysis combined with gas chromatography are simple and rapid, easy automation, and no sample pre-treatment and preconcentration. However, main disadvantages of this technique are possible interferences due to matrix interferences, problems with the column stability and critical temperatures for column and injector.

1.9.2 Liquid-liquid extraction (LLE)

Conventional liquid-liquid extraction or solvent extraction is one of the most commonly used sample preparation in water analysis. It is based on the partition of analytes between an aqueous sample phase and an immiscible organic solvent (Christian, 1994), which is non-or just slightly polar. The liquid-liquid extraction method may be carried out manually by shaking the water sample with an organic solvent in a separatory funnel or automatically by using a continuous liquid-liquid extractor (Golfinopoulos *et al.*, 2001).

Several applications of liquid-liquid extraction in trihalomethanes determination in drinking water have been reported. The organic solvent including *n*-hexane (Golfinopoulos *et al.*, 2001), pentane (Yu and Cheng, 1999) and methyl-*tert*-butyl ether (MTBE) (Nikolaou *et al.*, 2002) are frequently used to extract trihalomethanes from water sample. EPA methods 551 and 551.1 are standard methods base on liquid-liquid extraction for determination of trihalomethanes in drinking water. In method 551, a 35 mL sample aliquot is extracted with 2 mL MTBE and analyzed with GC-ECD. The method provides detection limits of trihalomethanes in the range of 0.002 to 0.012 $\mu g\ L^{-1}$ (US EPA method 551, 1990). For method 551.1, 3 mL MTBE or 5 mL pentane was used to extract analytes from 50 mL sample aliquot and inject the extract into GC-ECD for analysis. This provides detection limits in the range of 0.002 to 0.080 $\mu g\ L^{-1}$ and good recovery (US EPA method 551.1, 1995).

In 1997, Simpson and Heyes used EPA method 551 for a survey of drinking water to assess the occurrence of disinfection by-products in Australian water. The result indicated that the total trihalomethanes concentration ranged between 25 to 191 $\mu g\ L^{-1}$. These do not exceed the National Health and Medical Research Council (NHMRC) trihalomethanes guideline value which are 250 $\mu g\ L^{-1}$. However, the waters containing relatively high levels of trihalomethanes, in excess of the US EPA and EU guideline values. Yu and Cheng (1999) used liquid-liquid extraction (pentene) coupled with GC-MS to investigate the concentration of trihalomethanes in drinking water in the 19 districts of Hong Kong. The detection limits for this method were in the range 0.03 to 0.05 $\mu g\ L^{-1}$. The measurements show

the total trihalomethanes concentrations are in the range 15.8-131.0 μg L^{-1} which below the WHO and EU guideline values but the total concentrations of trihalomethanes in tap water samples from 4 out of the 19 districts is above the US EPA guideline value.

In order to increase the efficiency and improve procedure of the extraction, Nikolaou and coworker (2002) modified EPA method 551.1 for determination of volatile chlorination by products in drinking water by adding 6 g of sodium sulphate anhydrous and 2 mL of MTBE to 35 mL of disinfectant by-products solution in a 40 mL glass vial capped with PTFE-faced silica septum. The vial was sealed and shaken by hand for 1 minutes and left undisturbed for 2 minutes. Sodium sulphate was added to increase the ionic strength of the solution, therefore enhancing the extraction of volatile disinfectant by-products. One microliter of the ether phase was then injected into GC-ECD and GC-MS. For trihalomethanes, these methods provided very low detection limit and good recoveries, the detection method of LLE-GC-ECD method, ranged from 0.005 μg L^{-1} to 0.010 μg L^{-1} which is lower than LLE-GC-MS method, ranged from 0.01 μg L^{-1} to 0.03 μg L^{-1} and the recoveries, ranged from 87.6-112.8% for LLE-GC-ECD and 78.0-138.4% for LLE-GC-MS (Nikolaou *et al.*, 2002). Later, the LLE-GC-ECD as a modification of EPA 551.1 method was applied by Nikolaou and coworkers (2002) who optimized the amounts of sodium sulphate anhydrous and from 6 g to 2 g. The performances of this method for the determination of trihalomethanes in drinking water has given in most cases comparable recoveries and in some cases better recoveries than the previous method (Golfinopoulos and coworkers, 2005).

In another work, Golfinopoulos and Nikolaou (2005) used a modification of EPA method 551.1, which includes liquid-liquid extraction (LLE) with MTBE and analyzed by GC-MS method for investigation of the occurrence of disinfection by-produces in the drinking water of Athens, Greece. For trihalomethanes, these methods provided the detection limits in the range 0.04 to 0.9 μg L^{-1}, which are higher than the method reported by Nikolaou and coworkers (2002).

Liquid-liquid extraction technique provides high sensitivity of analysis; nonetheless, it has several main disadvantages such as requires large amounts of solvents, which are expensive, toxic and result in the production of

hazardous waste, considered to be time consuming procedure. Moreover, it can occur of the solvent peak in the chromatogram which possible limited the range of analyte being measured (Cho *et al.*, 2003; Demeestere *et al.*, 2007; Kuivinen and Johnsson, 1999).

1.9.3 Static headspace techniques (SHS)

Static headspace extraction is also known as equilibrium headspace extraction or simply as headspace. It is one of the most common techniques for quantitative and qualitative analysis of volatile organic compound from aqueous matrices. The method of extraction involves heating an aliquot of a liquid or solid sample in sealed vial at optimum time and temperature. After equilibrium, a portion of the headspace is injected into a GC for analysis. This can be done by either manual injection or by use of autosampler (Mitra, 2003). Headspace sensitivity depends on the combined effect of partition coefficient (K) and phase ratio (β) of vial and the parameter must be carefully considered as reproducible analysis requires exact replication of analytical conditions (Kolb and Ettre, 1997).

Kuivinen and Johnsson (1999) used static headspace technique with capillary column gas chromatography equipped with electron capture detector (ECD) for the determination of trihalomethanes and some chlorinated solvents in drinking water, in which sample solution vials were thermostated at 50 °C in a heating block for 30 minutes and shaken further for 1 minutes and then 0.5 mL for headspace was injected by the headspace syringe into GC. The limits of quantification are 0.1 μg L^{-1} for three of the trihalomethanes analytes and 0.2 μg L^{-1} for bromoform. Good recoveries were obtained in the range between 89-110%.

In 2001, Golfinopoulos and coworkers reported comparison of four methods including liquid-liquid extraction (LLE), direct aqueous injection (DAI), purge and trap (P&T) and static headspace (SHS) to determinate volatile organic compounds in drinking water. For static headspace (SHS), sample solutions in sealed vial are heated in 45 °C water bath for 40-50 minutes. A 0.5 mL of the headspace in the vials is removed with a gas tight syringe and then injected to GC-MS. This

method provided the detection limit of trihalomethanes at 0.1 μg L^{-1}, which is better than LLE and DAI.

This method is very simple and rapid, no use of solvent, and no need for sample preparation. Furthermore, the extraction phase (air or an inert gas) is compatible with the analytical instruments and matrix effects are minimized.

1.9.4 Dynamic headspace technique (purge and trap)

Dynamic headspace or purge and trap technique was introduced by Bellar and Lientenberg in 1974 (Chen and Her, 2001). The method has been widely used for the analysis of volatile organic pollutants in water, including trihalomethanes in various aqueous matrices (Zygmunt, 1996), in which an inert gas is flown through the water sample, causing the purgeable organics to move from the aqueous to the vapour phases. The analytes are then trapped on an adsorbent trap containing the sorbent material, generally built in a desorption chamber equipped with a powerful heating machine which when activated, permits desorption of the trapped compounds (Golfinopoulos, 2001). Commonly used adsorbent trap were Tenax GC (Zygmunt, 1996), Tenax TA (Chen and Her, 2001), and Vocarb 3000 (Nikolaou *et al.*, 2002).

US EPA has developed standard methods based on the purge and trap technique for analyzing trihalomethanes in water such as EPA method 502.2 and 524.2. Method 502.2 is the determination of volatile organic compounds in water by the purge and traps capillary column gas chromatography with photoionization and electrolytic conductivity detectors. This provides the method detection limits (MDLs) from approximately 0.01 to 3.0 μg L^{-1} (US EPA method 502.2, 1995). Another method is 524.2 which used purge and trap technique combined with gas chromatography-mass spectrometry for measurement of purgeable organic compounds in water. Method detection limits (MDLs) vary from approximately 0.02 to 1.6 μg L^{-1} (US EPA method 524.2, 1995).

To develop purge and trap method for the determination of trihalomethanes in aqueous samples, Zygmunt (1996) used a laboratory-built purge-and-trap device coupled to gas chromatography with electron capture detection (GC-ECD). Analytes from a primary trap (macrotrap) were desorbed and then focused

in a microtrap (also with sorbent). After that, the microtrap was desorbed and analytes were transferred into a gas chromatography for analysis. The sorbents were packed with Tenax GC and Carbosieve S III and moisture was removed from purge gases by a Nation tube (walls selectively permeable to water vapor). The procedure was applied to determine trihalomethanes in real aqueous samples, *i.e.*, tap water, orange drinks and an infusion fluid. Sodium sulphate was added to improved recovery. This method provided good detection limit (concentration equivalent to a peak height three times the baseline noise) at 1 ng L^{-1}, the relative standard deviations (RSD) were in the range 2.9-4.1% for seven measurements and recoveries are in the range from 92 to 101%.

In order to reduce the analysis time, Chen and Her (2001) used a continuous-flow purge and trap-GC-MS system for on-line monitoring of trihalomethanes in drinking water. Tenax-TA is used as an adsorbent since it is a hydrophobic polymeric adsorbent so can prevent the adsorption of purged water vapor. Trihalomethanes are trapped and concentrated on Tenax-TA by using cryofocusing trap system. The detection limits of the system could be lowered to 10 ppt, 25 ppt, 40 ppt, and 50 ppt (w/w) for $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$, respectively. The cycle time can be reduced to less than 5 minutes, resulting in a maximum frequency of 15 samples per hour.

Atomic emission detection (AED) was also reported as a detection method in purge and trap-GC for the determination of ten volatile halogenated organic compounds in water, beer and juice samples. Trapping materials were Tenax GC, silica gel and activated carbon. The elements were analyzed and their emission lines, in nanometers, were: chlorine 479, bromine 478 and iodine 193. For trihalomethanes, detection limits were in the range between 0.05 to 0.18 μg L^{-1}. The repeatability was calculated using the relative standard deviation for 10 successive injections of a standard mixture and was in the range of 3.1 to 10.0% (R.S.D.). The recoveries of the VOCs from a spiked beer varied from 96.5 to 103.4% and from a spiked juice from 96.4 to 104.2% (Campillo *et al.*, 2004).

In another work, Zoccolillo and coworker (2005) reported using a cold trap to improve the sensitivity of purge and trap injection system to analyse volatile chlorinated hydrocarbons in water. Samples were purged and trapped in a cold trap

(−100 °C) fed with liquid nitrogen (cryo-concentration), a cold trap would allow even small amounts of analyte to be concentrated and recovered. The modification entails sample introduction into the purge and trap injector, in order to avoid any air intake into the system. This method provide very low detection limit that allows analysis of samples whose concentrations range from μg L^{-1} to ng L^{-1} but the recovery is poor, for $CHCl_3$ the value was about 75%; for $CHBrCl_2$, $CHBr_2Cl$ and $CHBr_3$ the values were approximately 60, 40 and 30%, respectively.

Purge and trap technique has the distinct merit of providing a high sensitivity method, a clean sample free from its often very matrix; nonetheless, the disadvantages of technique are more time consuming and the instrument is quite complex for on-line and real-time monitoring.

1.9.5 Headspase solid-phase microextraction (HS-SPME)

Solid-phase microextraction (SPME), developed by Pawliszyn and co-workers (Mitra, 2003), has become popular for the analysis of organic compounds from water samples because it is a solvent-free process used for simultaneous extraction and pre-concentration of analytes from the samples. The SPME extraction is based on the partitioning of analytes between sample matrixes and the polymer-coated fibre and the subsequent desorption in the injection port of a chromatograph. There are two modes of SPME sanpling: direct SPME and headspace SPME. In HS-SPME mode, fibre was held above the sample giving a lower background than DI-SPME mode which the fibre is immersed in the sample. Selectivity of the SPME method towards classes of compounds depends on the polarity and the film thickness of the coating phase and the parameter affecting efficiency of the extraction are kind of SPME fibers, phase ratio, salting-out effect, stirring rate, extraction temperature, extraction time and desorption time *etc.*, (Cho *et al.*, 2003).

Headspace solid-phase microextraction has been successfully applied to the analysis of trihalomethanes in water by Stack and coworkers (2000), which reported using headspace solid-phase microextraction with polydimethylsiloxane (PDMS) and gas chromatography-mass spectrometry (GC-MS) for measurement of trihalomethanes in potable and recreational waters. Limits of detection (LOD) were

1.0 to 2.8 μg L^{-1}. The highest total trihalomethanes concentration in drinking water supplies was 61.8 μg L^{-1} which was well within the proposed European Union directive of 100 μg L^{-1}. The total trihalomethanes determined in swimming pool waters ranged from 105 to 134 μg L^{-1}, with chloroform accounting for 84 to 86% of total trihalomethanes.

To increase sensitivity of the HS-SPME, Cho and coworker (2003) evaluated the extraction efficiency of six different fibers *i.e.*, polydimethylsiloxane (PDMS), polyacrylate (PA), polydimethylsiloxane/divinylbenzene (PDMS/DVB), carbowax/divinylbenzene (CW/DVB), divinylbenzene/carboxen/polydimethyl siloxane (DVB/CAR/PDMS) and carboxen/polydimethylsiloxane (CAR/PDMS) by comparison of peak areas obtained for each trihalomethane with different coatings. Higher responses were obtained with the CAR-PDMS fibre, followed by DVB-CAR-PDMS and PDMS-DVB (Cho, *et al.*, 2003). The headspace-solid phase microextraction (HS-SPME) with CAR-PDMS fibre was then applied to extract trihalomethanes in drinking water and the analyte was separated and detected with gas chromatograph equipped with electron capture detector (GC-ECD). Good detection limits were obtained between 0.005 and 0.01 μg L^{-1}. The repeatability of HS-SPME was investigated by analyzing the fortified reagent water. The relative standard deviation (%RSD) for the repeatability ranges from 2.3-6.2, 1.0-3.2 and 0.8-2.9 at standard concentrations of 1, 10 and 40 μg L^{-1}, respectively. Subsequently, Juan and coworker (2007) described the optimization of three different SPME fibres for the determination of trihalomethanes in water *i.e.,* Carboxen-polydimethylsiloxane (CAR-PDMS), divinylbenzene-Carboxen-polydimethylsiloxane (DVB-CAR-PDMS) and polydimethylsiloxane-divinylbenzene (PDMS-DVB). The PDMS-DVB fibre was selected because it provided a broader linear range, better repeatability and lower detection and quantification limits than the others and no detectable carryover. The reproducibility (expressed as a percentage of relative standard deviation) was 6-11% and the detection limits were between 0.078 and 0.52 μg L^{-1} for bromoform and chloroform, respectively, which higher than the method reported by Cho and coworker (2003).

The HS-SPME technique has some advantages such as fast, solvent-free and portable; nevertheless, this technique has several disadvantages that are SPME fibres are rather expensive, have a limited lifetime and degraded with increasing use. Furthermore, because the length and the coating character of each SPME fibre may differ from lot to lot, variations in analyte enrichment may be observed from fibre to fibre. Before using a fibre for the first time, a thermal conditioning step is required. Even when this step is carefully done, partial loss of the coating may occur resulting in extra peaks during the chromatographic analysis, consequently affecting the performance of the method. In addition, sample carry-over between runs has often been reported with SPME (Prosen and Zupancic-Kralj, 1999; Psillakis and Kalogerakis, 2001).

1.9.6 Single-drop microextraction (SDME)

Single-drop microextraction (SDME) or liquid phase microextraction (LPME) or solvent microextraction was developed as a solvent-minimized extraction technique, which combines the classic extraction by liquid and microextraction in a stationary phase. It is based on the distribution of the analytes between a microdrop of organic solvent hanging on the tip of a Teflon rod or a microsyringe needle and the aqueous solution or headspace of the solution. The organic solvent drop is first exposed to the aqueous solution or headspace of the solution and target analytes are extracted from the sample matrix into the drop. After the equilibrium is reached, the drop with concentrated analytes is then transferred to the injection port of a GC for analysis (Zhao *et al.*, 2004). Similar to SPME, there are two modes of SDME sampling: direct SDME (Figure 1.2) and headspace SDME (Figure 1.3) (Jeannot and Cantwell, 1996).

Selectivity of this technique can be achieved through the choice of solvent, which is dependent on the chemical nature of the target analytes. As in liquid-liquid extraction, the selection of extraction solvent is based on the principle of "like dissolves like" (Cao *et al.*, 2006). The extraction solvent has to meet three requirements: to have low volatility in order to be stable at the extraction period, to extract analytes well that it should provide high solubility for target analyte and to be

separate from the analyte peaks in the chromatogram (Tankeviciute *et al.*, 2001). Sensitivity of this technique can be enriched by affecting parameters such as organic solvent, salting-out effect, stirring rate, extraction time, phase ratio, organic volume and extraction temperature *etc.*, (Zhao *et al.*, 2004).

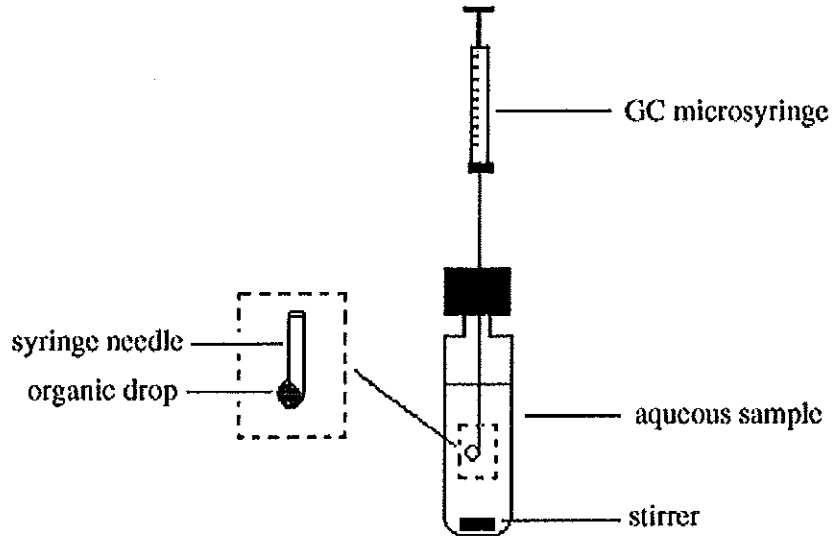


Figure 1.2 Direct single-drop microextractions (DI-SDME) (Xu *et al.*, 2007).

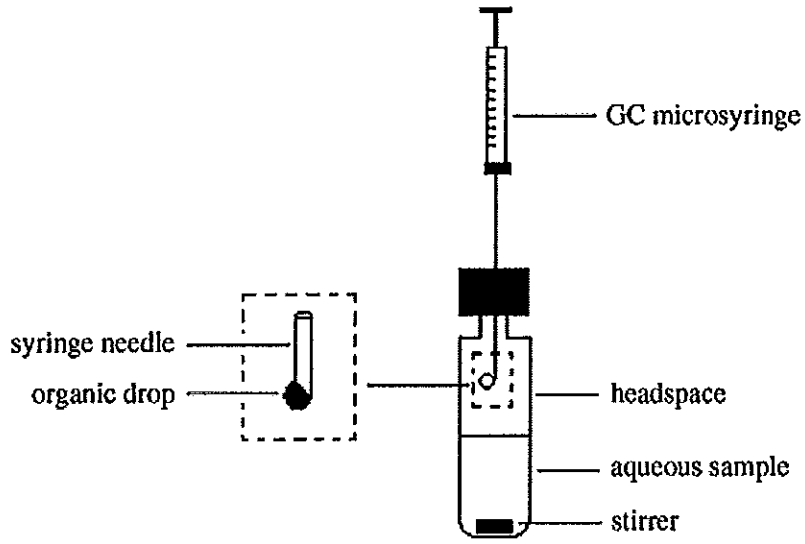


Figure 1.3 Headspace single-drop microextraction (HS-SDME) (Xu *et al.*, 2007).

The applications of SDME, either DI-SDME or HS-SDME for analysis of trihalomethanes in water have been reported. In headspace single-drop microextraction (HS-SDME) mode, trihalomethanes was extracted with 1 μL of 1-octanol that was placed about 1 cm above the surface of sample solution with the extraction time for 10 minutes and then the extractant was transferred into gas chromatograph-electron capture detector for analysis. The LODs are in the range of 0.15 μg L^{-1} (for $CHCl_2Br$ and $CHClBr_2$) to 0.4 μg L^{-1} (for $CHCl_3$), relative standard deviations (RSD) below 10 % for most of trihalomethanes at the 10 μg L^{-1} level and good recoveries of spiked water samples was obtained from 101 to 112% (Zhao *et al.*, 2004).

Another mode, direct single-drop microextraction (DI-SDME) used a 2 μL *n*-hexane as organic solvent to extract trihalomethanes from water sample, in which organic solvent was hanged on the tip of microsyringe and immersed in a water sample. After the extraction time of 5.0 minutes, the extractant was analyzed by gas chromatography-electron capture detection (GC-ECD). The limit of detection (LODs) ranged from 0.23 μg L^{-1} (for $CHBr_2Cl$) to 0.45 μg L^{-1} (for $CHCl_3$) and recovery of spiked water samples was from 73% to 78% with relative standard deviations below 7% (Tor and Aydin, 2006). These applications indicate that the HS-SDME technique provided higher sensitivity and better recovery than DI-SDME technique.

Single-drop microextraction has the advantages over LLE, P&T and SPME methods because only one microsyringe and very little organic solvent used to perform the extraction. It combines extraction, concentration and sample introduction in one step, which is extremely simple, short analysis time and inexpensive method compare to time consuming operation of P&T method, using a large among of toxic solvent of LLE and a fiber of SPME. In addition, it can eliminate sample carry-over between runs of SPME.

1.10 Analysis method

After sample preparation is completed, the analysis is carried out by an instrument of choice. For trihalomethanes compound, gas chromatography is the most common analysis method (Chen and Her, 2001; Golfinopoulos *et al.*, 2001; Nikolaou *et al.*, 2002; Stack *et al.*, 2000) due to these compounds have appreciable vapor pressures in the temperature range typically suit for GC.

Gas chromatography is a separation technique in which the mobile phase is gas. It is base on the differences in partitioning and adsorption behavior between a mobile phase which is gas and a stationary phase to separate the compounds in a mixture. The stationary phase, a material in the chromatographic column, will retard the passage of the components of the sample. The technique is carried out by passing of sample, in a form of either the gas or the liquid phase which contains the analyte, in the mobile phase through the stationary phase. During elution, constituents migrate through the system at varying rates, depending upon their relative affinity for the two different phases. Components that interact strongly with the stationary phase are retarded, therefore, taking longer to pass through the system than analytes that preferentially stay in the mobile phase (Harrison and Mora, 1996) and the information obtained from analysis is contained in the chromatogram that indicated the type, concentration or mass flow of the sample components (Poole and Schuette, 1985). The classification of GC is based on the physical nature of stationary phase, when the stationary phase is a liquid they are classified as gas-liquid chromatography (GLC) and as gas-solid chromatography (GSC) when the stationary phase is a solid (Grob and Barry, 2004).

Most GC methods for separation of trihalomethannes including use of columns both packed and capillary columns based on a non-polar or slightly-polar liquid phases. The separation of trihalomethanes by GC requires a relatively wide range of temperature programming. Detectors used to identify and qualify trihalomethanes are electron capture detector (ECD), mass spectrometer detector (MS) electrolytic conductivity detector (ELCD, Hall) and atomic emission detector (AED).

1.10.1 Electron capture detector (ECD)

Electron capture detector is a sensitive, selective detector and widely used for detection of trihalomethanes as they are halogen containing compounds. Nickel^{63} is the most common radiation source for this detector. The β-ray from Nickel^{63} collide with the carrier gas molecules to produce electrons, the halogen species which were eluted from the column could capture an electron to form a negatively charged species which causes a reduction in the standing or background current and the decrease of this current is registered. The LOD is in the femtogram level (Grob and Barry, 2004).

Several applications of ECD as the detector of GC to analyze trihalomethanes in water have been reported. In 1996, a laboratory-built purge and trap-GC-ECD method provided the detection limit at the level of 1 ng L^{-1} (Zygmunt, 1996). The detection limit between 5 ng L^{-1} and 10 ng L^{-1} was obtained using LLE-GC-ECD (Nikolaou and coworker, 2002) and HS-SPME-GC-ECD method (Cho and coworker, 2003). There is reported comparison of LLE-GC-ECD and LLE-GC-MS for the analysis of volatile chlorination by-products in drinking water. The result found that ECD detector provide low detection limit than MS as regards halogenated organic compounds (Nikolaou and coworker, 2002). The main advantage of ECD is a high sensitivity and selectivity for trihalomethanes as halogen containing compounds which generally more sensitive than mass spectrometer detector (MS) (Zygmunt, 1996).

1.10.2 Mass spectrometer detector (MS)

Mass spectrometer detector (MS) is also widely used to detect trihalomethanes due to its ability to certify the detected compounds. Interfacing GC with MS via electron impact ionization (EI) was performed at 70 eV; the trihalomethanes molecule is volatilized and bombarded by electron to produce a protonated molecular ion (M^+) and then ions are separated according to their *m/z* ratio in the quadrupole analyzer (Stack *et al.*, 2000; Chen and Her, 2001; Nikolaou *et al.*, 2002) or the ion trap analyzer (San Juan *et al.*, 2007). The spectrum is then scanned

for the molecular and fragment ions. The quantitative analysis of trihalomethanes were performed in selected ion monitoring (SIM) mode, selecting the appropriate ions for each compound (*m/z*) *i.e.*, chloroform 83 and 85, dichlorobromomethane 83 and 85, dibromochloromethane 127 and 129, bromoform 173 and 175 with a dwell time of 100 ms (Stack *et al.*, 2000; Chen and Her, 2001; Nikolaou *et al.*, 2002).

Gas chromatography-mass spectrometry coupled with HS-SPME used for the analysis of trihalomethanes in water was reported by Stack and coworker (2000). It provided detection limit at the range of 1.0-2.8 μg L^{-1}. In 2007, Juan and coworker reported better detection limit at the range of 0.078-0.52 μg L^{-1}. The GC-MS coupled with the continuous-flow purge and trap gave good detection limit at the range of 0.01-0.05 μg L^{-1} (Chen and Her, 2001).

The advantage of MS detection is the ability to certify the compounds detected in the samples (Nikolaou *et al.*, 2002). However, it provided lower sensitivity of trihalomethanes than ECD (Nikolaou and coworker, 2002).

1.10.3 Electrolytic conductivity detector (ELCD, Hall)

Electrolytic conductivity detector (ELCD, Hall) has also been reported as the detection principle of GC for halogenated compound in water by EPA methods 502.2. It is based on the electrolytic conductivity of ionic species in water. Analytes that eluted from the column were oxidized or reduced catalytically to from an ionic species that was transferred to a stream of deionized water for detection (Grob and Barry, 2004). For trihalomethanes, the method detection limits (MDLs) vary from 0.01-0.06 μg L^{-1} (US EPA method 502.2, 1995).

1.10.4 Atomic emission detector (AED)

For atomic emission detector (AED), the solutes eluting from the GC column are atomized in a microwave-induced plasma (MIP), while the resulting excited atoms and ions emit light as they return to the ground state. The polychromatic light is dispersed in a spectrometer and the emission intensity of the characteristic wavelengths is measured by a photodiode array. AED is a sensitive

detection system for gas chromatography, providing selective information which cannot be obtained with other commonly used element-selective detectors (Grob and Barry, 2004). Campillo and coworker (2004) employed AED in a purge and trap-GC to determine ten volatile halogenated organic compounds, including trihalomethanes, in water, beer and juice samples. Gas chromatograph was directly coupled by a transfer line to a microwave-induced plasma atomic emission detector. The elements analyzed and their emission lines, in nanometers, were: chlorine 479, bromine 478 and iodine 193. Detection limits were calculated using a signal-to-noise ratio of 3 for all the investigated compounds and were found between 0.05-0.40 $\mu g\ L^{-1}$.

The analysis of trihalomethanes in water is very important because of its possible adverse health effects and human are expose to these compound everyday through multiple routes. The appropriate sample preparation method for investigation and evaluation of these compounds is necessary. Thus, a simple and rapid, inexpensive, and solventless technique using static headspace (SHS) and headspace single-drop microextraction (HS-SDME) followed by gas chromatography equipped with electron capture detector as a sensitive and selective detector for trihalomethanes were chosen as sample preparation technique. This work will attempt to develop the sample preparation to obtain high sensitivity method for the determination of trihalomethanes at very low concentration in water. SHS, HS-SDME and GC-ECD conditions will be optimized for the best analytical performance. These methods will also validate and applies to the real samples. Finally, there are comparison between SHS and HS-SDME.

1.2 Objective

The objectives of this work are to develop and evaluate sample preparation techniques for qualitative and quantitative analysis of trihalomethanes in water using gas chromatography with electron capture detector (GC-ECD).

CHAPTER 2

Experimental

2.1 Chemicals and materials

2.1.1 Standard chemicals

- Chloroform (99.0 %, $CHCl_3$, density: 1.48, Merck, England)
- Dichlorobromomethane (98.0 %, $CHCl_2Br$, density: 1.98, Fluka,Switzerland)
- Dibromochloromethane (97.0 %, $CHClBr_2$, density: 2.38, Fluka, Switzerland)
- Bromoform (99.0 %, $CHBr_3$, density: 2.89, Fluka, Switzerland)

2.1.2 General chemicals and solvents

- Methanol (CH_3OH, AR grade, Merck, USA)
- L-ascorbic acid ($C_6H_8O_6$, AR grade, Merck, Germany)
- 1-octanol ($CH_3 (CH_2)_7OH$, 98.0 %, Fluka, Switzerland)
- Anhydrous sodium carbonate (Na_2CO_3, AR grade, Merck, Germany)
- Anhydrous sodium sulphate (Na_2SO_4, AR grade, Merck, Germany)
- Sodium chloride (NaCl, AR grade, Merck, Germany)
- Potassium chloride (KCl, AR grade, Merck, Germany)
- Acetone (CH_3COOH, AR grade, Merck, Germany)
- Ultra pure water (H_2O, water was de-ionized with reverse osmosis system and purified with a Maxima ultra pure water instrument to obtain the resisitivity of 18.2 M Ω, ELGA, England)

2.1.3 Samples

Water samples were drinking water, tap water and well water. Bottled drinking water samples were sampling from local stores. Tap water samples were collected from the outlet in our laboratories and nearby sources and well water samples were collected from a local soft drink factory.

2.2 Instruments and apparatus

2.2.1 Gas chromatography-electron capture detector (GC-ECD)

- Gas chromatograph model Shimadzu GC-14B equipped with electron capture detector and data processor modal C-R7A chromatopac (Shimadzu, Japan)
- Empty clean glass column, 2 m x 2.0 mm i.d., (Shimadzu,Japan)
- Stationary phase, 1% SP-1000 on carbopack B, 60/80 mesh (Supelco, USA)
- High purity nitrogen carrier gas (purity 99.995%, TIG,Thailand)
- Flow meter

2.2.2 Apparatus

- Headspace vial, 30 and 10 mL (Shimadzu, Japan)
- Silicone septa (GM, Thailand)
- Aluminum crimp cap (GM, Thailand)
- Gastight syringe (0.5 mL, Hamilton, USA)
- Liquid microsyringe 10 μL (Hamilton, USA)
- Stirrer (Heidolph, type: MR 3001, Germany)
- Magnetic stir bar (0.8 cm)
- Analytical balance (Sartorius, USA)
- Amber vial 2 mL with septum and silver aluminum cap (Agilent Technogies, USA)

- 11-mm crimper and 11-mm decrimper (Agilent Technogies, USA)
- 20-mm crimper and 20-mm decrimper (Shimadzu, Japan)
- Stand and base
- Syringe cleaner (Hamilton, USA)
- Microliter pipette: 10, 100, 200 and 1000 μL
- Measurement pipette: 2, 5 and 10 mL (Pyrex, USA)
- Oven (Fisher Scientific, UK)
- Refrigerator (Hitachi, Japan)
- Ultrasonic bath (Atomatic Science, Thailand)
- Vacuum pump (Gast manufacturing, Inc., USA)
- Volumetric flask 10, 100, and 250 mL (Pyrex, USA)
- Beaker 50, 100, and 500 mL

2.3 Analysis system

The determination of trihalomethanes in water samples consists of two parts, sample preparation and analysis steps. Sample preparation was carried out using two different extraction methods *i.e.,* static headspace (SHS) and headspace single-drop microextraction (HS-SDME). The subsequent analysis step was carried out by using gas chromatograph equipped with electron capture detector (GC-ECD) (Gas chromatograph model Shimadzu GC-14B and data processor modal C-R7A Chromatopac). The analytical column was a packed column: 2 m x 2.0 mm i.d., 1% SP-1000 on Carbopack B, 60/80 mesh. The diagram of the analysis system was shown in Figure 2.1.

The highest efficiency and performance obtained by optimization and validation of both conditions. Finally, the techniques at optimum conditions were applied to real samples.

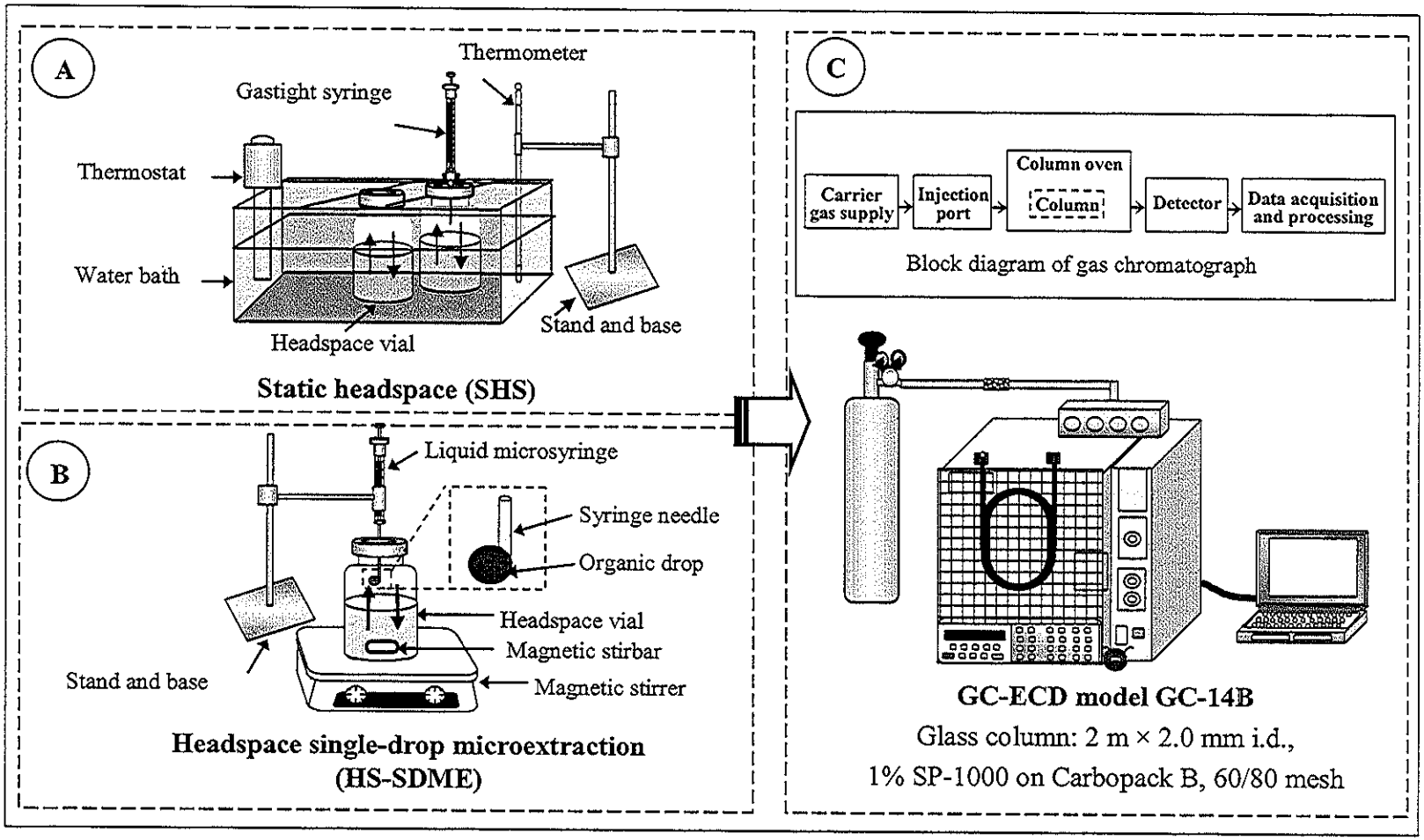


Figure 2.1 The analysis system for trihalomethanes analysis: A and B represent the two sample preparation methods, C represents the analysis part.

2.4 Preparation of standard solutions

In the analysis of trihalomethanes, high blank signals are often encountered due to the present of trihalomethanes in the laboratory. To avoid all contamination, all glassware and septa were washed with detergent solution, rinsed with ultra pure water, and then dried at 150 °C for at least 4 hours. After that, the items were then cooled to room temperature in a location remained from the laboratory (Nikolaou *et al.*, 2002).

When opening vials or tubes containing standards or samples they must be sealed as soon as possible with screw-caps to avoid evaporation or contamination, wrapped with aluminum foil to protect from light and stored at 4 °C (Kuivinen and Johnsson, 1999).

2.4.1 Trihalomethanes standard stock solutions

Standard stock solution of trihalomethanes (1,000 mg L^{-1}) was prepared by pipetting 6.87 µL of $CHCl_3$, 5.15 µL of $CHCl_2Br$, 4.21 µL of $CHClBr_2$, and 3.50 µL of $CHBr_3$ added into the 0.5 mL degassed methanol in 10 mL volumetric flask and adjusted the volume with ultra pure water (Kuivinen and Johnsson, 1999). The intermediate standard solutions in the range 0.01-400 mg L^{-1} were obtained by diluting stock standard solutions with degassed methanol. All standard solutions were transferred to vials 2 mL (completely filled (no headspace) and sealed with polytretrafluoroethylene (PTFE)-line screw-caps and stored in the dark at 4 °C.

2.4.2 Trihalomethanes working standard solutions

The working standard solutions, in the range 0.001-200 µg L^{-1}, were diluted from the intermediate standard solutions with ultra pure water in the headspace vials.

2.5 Preparation of the chromatographic packed column

A packed column used to separate trihalomethanes compound was prepared by packing the stationary phase, 1% SP-1000 on Carbopack B, 60/80 mesh, into the empty clean glass column, 2 m × 2.0 mm i.d., the preparation procedures are as follows:

Preparation of glass column: an empty glass column was thoroughly cleaned with 0.5 % sulfuric acid and rinsed with distilled water to eliminate acid residues and flushed with methanol. The column was then purged with dry nitrogen (Supina, 1974).

Preparation of column: A plug of glass wool was inserted into the inlet end of the column that would be connected to a detector. A small funnel was connected to the inlet end of the column and the packing (stationary phase) was slowly added while vacuum was applied at the other end of the column to optimize the filling. The funnel was filled with an appropriate amount of 1% SP-1000 on Carbopack B. While filling it was moderately vibrated, starting at the exit and slowly moved towards the inlet until the packing ceased to flow. The vacuum pump hose was removed and the column was vibrated until the packing level dropped at the exit end. The column was then reconnected to the vacuum pump. The inlet was capped and again vibrated, from the exit to the inlet until the packing stop to flow. Stationary phase was added until the volume of the packing material reached a proper inlet level and then the column was slightly vibrated to settle the packing. The vacuum hose was finally removed and the glass wool plug was inserted.

Column condition: The packed column must be condition to purge all volatile compound components that would foul the detector and produce unsteady baseline and noise. Generally, heating the column was done by flowing carrier gas at a temperature slightly above the proposed operating temperature overnight for the specific kind of packing. The packed column, 2 m × 2.0 mm i.d., 1% SP-1000 on Carbopack B, 60/80 mesh was conditioned by connecting the inlet end of the column to the injection side of a gas chromatograph while the detector side was disconnected. The column connections and septa were checked for leaks, set the flow at 20 mL min^{-1} N_2. The column was purged with carrier gas at ambient temperature for

30 minutes to remove air. After that, the temperature program was set at 2 °C min^{-1} to 210 °C, hold at 210 °C for 15 hours (overnight) with the injection temperature at 100 °C during conditioning.

2.6 Optimization of GC-ECD conditions

Gas chromatograph consists of carrier gas supply, injection port, column, detector and data processing (Figure 2.1 (C). The investigated conditions of the GC-ECD system for analysis of trihalomethanes were carrier gas flow rate, column temperature programming, injector temperature and detector temperature. Optimization of conditions was carried out by varying one parameter at a time while the others were kept constant. The optimum conditions were obtained by considering the best resolution, highest response and less analysis time.

In these studies, 20 mL of mixed working standard solution (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$) in the sealed 30 mL headspace vials were placed in the laboratory-built water bath at optimum time and temperature. After equilibrium, a gastight syringe was used to transfer the 0.5 mL vapor phase to gas chromatographic analysis. Five replications were performed for each tested value. After every injection the syringe was placed on a cleaned heating plate (50-60 °C). A syringe cleaner was used to remove suspected residuals for confirmation the cleanliness of syringe at the beginning of the day and after injections of high concentrations of standards or samples. The starting operating conditions of GC-ECD system were shown in Table 2.1. When an optimize value of one parameter was obtained it was used in the optimization of the next parameter following the sequence in this section.

Table 2.1 Starting operating conditions of GC-ECD technique (US EPA Method 501.3)

Column : Packed column	Glass column, 2 m × 2.0 mm i.d., 1% SP-1000 on Carbopack B, 60/80 mesh.
Carrier gas flow rate	10 mL min^{-1}
Column temperature program	
- Initial temperature	50 °C
- Initial holding time	6 minutes
- Ramp rate temperature	10 °C min^{-1}
- Final temperature	200 °C
Injector temperature	250 °C
Detector temperature	250 °C

2.6.1 Carrier gas (N_2) flow rate

To optimize the carrier gas flow rate, a 0.5 mL gas phase of trihalomethanes working standard solution was injected to the GC-ECD system. The carrier gas flow rate was obtained by varying the nitrogen flow rate at 10, 20, 30, and 40 mL min^{-1}. The retention time and peak area from chromatograms of trihalomethanes were used to calculate the plate number (N) and height equivalent to a theoretical plate (HETP). From the van deemter plot, the optimum flow rate was obtained at the lowest HETP.

2.6.2 Column temperature

Column temperature programming consists of the following steps: initial temperature, initial holding time, ramp rate, final temperature and final holding time. To optimize the column temperature programming parameters, a 0.5 mL gas phase was injected into the GC-ECD system, by varying one parameter at a time as follows.

***Step I*- Initial temperature:** The optimum initial temperature was investigated by varying the temperature at 50, 70, 80, 90 and 100 °C.

***Step II*- Initial holding time:** Initial holding time was varied at 0, 1, 2 and 3 minutes.

***Step II*- Ramp rate of temperature:** The ramp rate was varied at 5, 10, 15 and 20 °C min^{-1}.

***Step IV*- Final temperature:** Optimum final temperature was investigated by varying the temperature at 170, 180, 190 and 200 °C.

***Step V*- Final holding time:** Optimum final holding time was obtained by considering the retention time of the bromoform which was retained longer than the others.

2.6.3 Injector temperature

The optimization of injection temperature was investigated by varying the temperature at 150, 160, 170, 180, 190 and 200 °C. The flow rates of carrier gas and column temperature were at the optimum, obtained from 2.6.1-2.6.2.

2.6.4 Detector temperature

The optimization of detector temperature was investigated at the optimum conditions obtained from 2.6.1-2.6.3 and varied the temperature at 200, 225, 230, 240, 250, 260 and 270 °C. Responses obtained from the different temperatures were compared. The temperature that provided the highest response was the optimum detector temperature.

2.7 Optimization of static headspace (SHS) conditions

The response of headspace technique depended on equilibration temperature, equilibration time and phase ratio. To optimize headspace conditions, a 20 mL of mixed working standard solution (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$) in the sealed 30 mL headspace vials were placed in the laboratory-built water bath at optimum time and temperature. After equilibrium, a gastight syringe was used to transfer the 0.5 mL vapor phase and injected into GC-ECD system which was set at optimum conditions from 2.61-2.64. Five replications were performed for each tested value. The optimum headspace conditions were obtained by considering high response and short analysis time. The starting operating conditions of HS technique were as shown in Table 2.2. Schematic diagram of the SHS system was shown in Figure 2.1 (A).

Table 2.2 Starting operating conditions of SHS technique (Kanatharana, 2003).

Equilibration temperature	70 °C
Equilibration time	20 minutes
Phase ratio	0.5
Vial volume	30 mL
Injection volume (gas phase)	0.5 mL

2.7.1 Equilibration temperature

The optimization of equilibration temperature was investigated by varying the temperature at 50, 60, 70, 80, and 90 °C. The temperature that gave the highest response and the best % RSD was chosen.

2.7.2 Phase ratio

The phase ratio is the rate constant of the sample phase and gas phase above the sample. The sample and gas phase of the working standard solution in headspace vial is shown in the Figure 2.2. The optimization of phase ratio was investigated by varying the volume of mixed working standard solution at 5, 10, 15, 20 and 25 mL for the 30 mL headspace vial at the optimum equilibrate temperature obtained from 2.7.1. The phase ratio is calculated as follow:

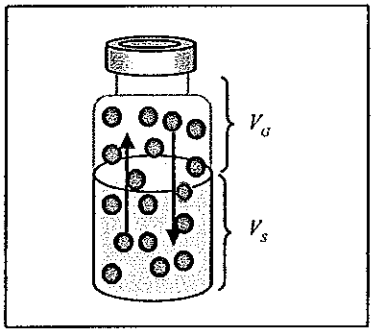


Figure 2.2 A headspace vial containing a working standard solution of trihalomethanes: V_G = volume of the gas phase, V_S = volume of the sample phase.

$$V_T = V_G + V_S \tag{2.1}$$

$$\beta = \frac{V_G}{V_S} \tag{2.2}$$

Where β is the phase ratio

V_T is the volume of the total

V_G is the volume of the gas phase

V_S is the volume of the sample phase

2.7.2 Equilibration time

To optimize of equilibration time a 20 mL of mixed working standard (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$) was placed in the laboratory-built water bath at optimum temperature and phase ratio (2.7.1-2.72) and varied the time at 15, 20, 25 and 30 minutes. The time that gave the highest response and the shortest analysis time was selected as the optimum equilibration time.

2.8 Optimization of headspace single-drop microextraction (HS-SDME) conditions

To achieve the optimum extraction efficiency of HS-SDME for analysis of trihalomethanes, the investigated conditions were type of organic solvent, phase ratio, organic drop volume, stirring rate, salting-out-effect, extraction temperature, and extraction time. Optimization of these conditions was carried out by varying one parameter at a time while the others were kept constant.

In these studies 5 mL of 10 μg L^{-1} mixed standard solution containing 0.3 g mL^{-1} NaCl in a 10 mL headspace vial was placed on a magnetic stirrer. A 10 μL microsyringe was inserted through the silicone septum where the tip of needle was located above the surface of the stirred solution (Figure 2.1 (B)). Then a drop of 1 μL of 1-octanol was hung at the needle tip. After extraction, the drop was retracted and injected into the GC-ECD system at the optimum conditions. Before each extraction, the microsyringe was rinsed at least 10 times with acetone, dried and rinsed 5 times with 1-octanol to avoid the formation of air bubbles and the carry over of compounds between extractions. The optimum conditions were obtained by considering high response and short analysis time. The starting operating conditions of HS-SDME technique are as shown in Table 2.3. When an optimized value of one parameter was obtained it was used in the optimization of next parameter following the sequence in this section.

Table 2.3 Starting operating conditions of HS-SDME technique (Zhao *et al.*, 2004).

Type of organic solvent	1-octanol
Phase ratio	0.6
Organic drop volume	1 μL
Stirring rate	800 rpm.
Salting-out effect	
- Type of salt	NaCl
- Concentration of salt	0.3 g mL^{-1}
Extraction time	10 minutes
Extraction temperature	26 °C (room temperature)

2.8.1 Type of organic solvent

In order to obtain high extraction efficiency, first step for HS-SDME is the selection of an appropriate extraction solvent. Four solvents, *n*-hexane, *p*-xylene, 1-octanol and ethylene-glycol, were selected according to the analytes polarity and tested for the best extraction of trihalomethanes from water samples. The solvent that gave the best extraction efficiency was selected.

2.8.2 Phase ratio

Phase ratio plays an important role in headspace microextraction analysis. An increase in sample volume and consequently a decrease in headspace volume enhance the extracted amount of analyte, which improves the sensitivity.

To optimize the phase ratio, the sample and headspace volumes were investigated by varying the volumes of 10 μg L^{-1} working standard at 3, 5, 7, and 9 mL for the 10 mL vial. The phase ratio is calculated as shown in 2.7.3. The optimum phase ratio was the one that gave the highest response.

2.8.3 Stirring rate

Agitation of the sample solution is one parameter that enhances the mass transfer in the aqueous phase, induces convection in the headspace, and consequently reduces the time for reaching a thermodynamic equilibrium. Thus, the equilibrium between the aqueous phase and the headspace can be achieved more rapidly by stirring the aqueous sample (Deng *et al.*, 2006). To evaluate the effect of different stirring rate a 10 μg L^{-1} working solution was extracted in five replicates with a microdrop of 1 μL 1-octanol with stirring rates in the range of 100-1,250 rpm.

2.8.4 Salting-out effect

Addition of salt to the solution sample has important effects on the extraction efficiency. Thus in this work, type and concentration of salts were optimized.

2.8.4.1 Type of salt

Four types of salt were investigated *i.e.,* sodium chloride, sodium sulphate, sodium carbonate and potassium chloride. To optimize the effect of salt addition, 0.3 g mL^{-1} of each salt was added to a 5 μg L^{-1} working solutions and extracted with a microdrop of 1 μL 1-octanol. The type of salt that gave the highest response was selected.

2.8.4.2 Concentration of salt

Concentrations of salt were evaluated by adding salt from 0 to 0.4 g mL^{-1} into a 5 μg L^{-1} working solution. The least amount of salt that provided the highest response was selected.

2.8.3 Organic drop volume

To increase the sensitivity of the HS-SDME procedure, the organic drop volume was optimized. Different volumes of microdrop were evaluated. The best solvent from 2.8.1, 1-octanol, with drop volumes of 1.0, 2.0, 3.0 and 4.0 µl were exposed separately for 10 minutes to the 7 mL working solutions (phase ratio 0.4), 0.3 g mL^{-1} NaCl, and stirring at 1,000 rpm.

2.8.6 Extraction time

Extraction time is one important factor in HS-SDME method to determine when the amount of analyte in organic solvent drop is at its optimum (Gupta *et al.*, 2007). It is a compromise between analysis sensitivity and chromatographic running time. Generally, the amount of extracted analyte should increase with extraction time before equilibrium is reached. The extraction time was tested at 1, 3, 5, 7.5, and 10 minutes. The time that gave the highest response with short analysis time was selected as the optimum equilibration time.

2.8.7 Extraction temperature

Figure 2.3 shows a heating block designed to control the extraction temperature. An aluminum block was drilled to obtain a hole that was a close fitted to a 10 mL headspace vial (2.1 cm inner diameter and 3.3 cm deep). A soldering iron was used as a heater and was fitted into another hole on the side of the aluminum block. Heating was controlled through a heating cord connected to the heating sensor and the temperature control unit.

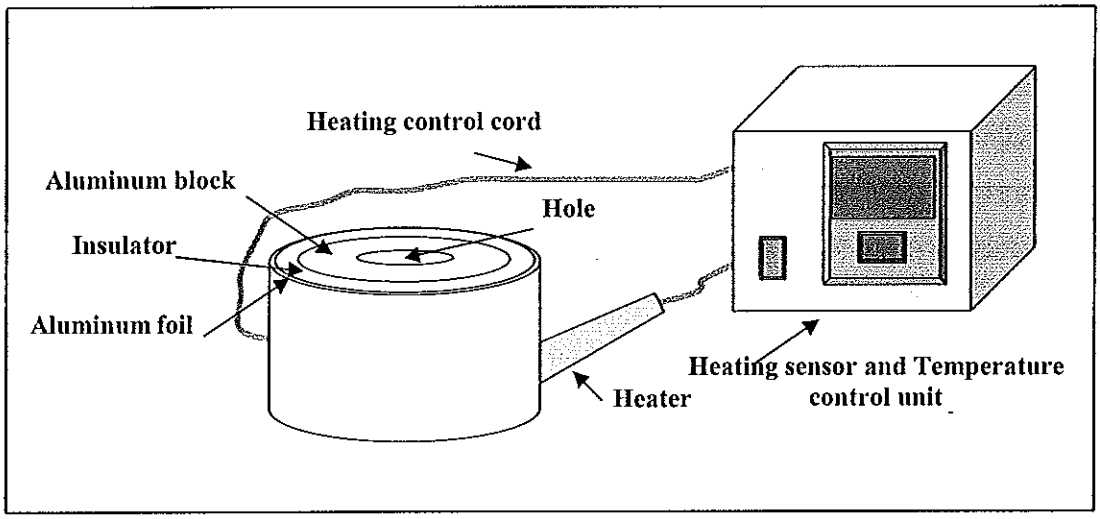


Figure 2.3 Laboratory-built heating block

The temperature was calibrated as follows. Temperature of the laboratory built heating block was set at the temperature control unit. A 10 mL headspace vial filled with solution blank was then placed in the hole of the aluminum block. A thermometer was inserted inside the solution blank and held with a clamp. The temperature of the solution blank was read from the thermometer every 5 minutes for 50 minutes for each tested temperature. The obtained temperatures were matched with the values on the temperature control unit to use in later studies.

The effect of extraction temperature on extraction efficiency was investigated by varying the temperatures at 26, 30, 35, 45, and 50 °C. Before the extraction step, 5 μg L^{-1} working standard solution in headspace vial was placed in a laboratory-built heating block at desirable temperature for 20 minutes. The extraction temperature that gave the highest response was chosen.

2.9 Linear dynamic range (LDR)

To investigate linear dynamic range of SHS-GC-ECD technique, standard mixture solutions of trihalomethanes were prepared at concentrations between 0.05 to 200 μg L^{-1}. A 0.5 mL gas phase of each standard solution was injected into the GC-ECD system set at the optimum conditions. Linear dynamic

range of HS-SDME-GC-ECD technique was obtained by preparing the standard mixture solutions of trihalomethanes at concentrations between 0.005 to 30 μg L^{-1}. Each concentration of standard mixture solution of trihalomethanes was extracted with 2 μL of 1-octanol and injected into the GC-ECD system set at the optimum conditions. Linear dynamic range was determined from the calibration curve by considering the linear regression coefficient.

2.10 Limit of detection (LOD)

The limit of detection was determined based on IUPAC method. Blank response was measured 20 times and the value for standard deviation of the blank maximum response was obtained. The limit of detection (LOD) is calculated by

$$C_L = \frac{kS_B}{m} \tag{2.3}$$

Where C_L is the limit of detection.

S_B is the standard deviation of the blank response: 20 times.

m is the analytical sensitivity (slope of the analytical curve).

k is the numerical factor chosen according to the confidence level where; $k = 3$ or 3σ limits of detection, corresponds to a confidence level of about 99.7%.

2.11 Limit of quantitation (LOQ)

Limit of quantitation of the method can be defined as the smallest amount of analyte that can be reliably quantified with a certain degree of reliability quantified with a certain degree of reliability within a particular matrix. It can be applied to analytical procedures that exhibit baseline noise. The limit of quantitation is generally agreed to begin at a concentration equal to 10 standard deviations of the blank (ICH Q2B, 1996).

2.12 Sampling

Water samples were drinking water, tap water and well water. Bottled drinking water samples were sampling from local stores in Hat Yai, Songkhla. Tap water samples were collected from the outlet in our laboratories and nearby sources and well water were collected from a local soft drink factory in plastic bottles with screw caps. Before sampling, the tap water and well water were allowed to run for 3 minutes. L-ascorbic acid as dechlorination agent was first added into the sampling bottles, 10 mg for 10 mL sample in order to stop trihalomethanes formation after sample collection (Cho *et al.*, 2003). The sampling bottles were completely filled and sealed with no headspace.

2.13 Matrix interference

The matrix effects of SHS-GC-ECD technique was studied by spiking known amount of trihalomethanes standard solution in the concentration range of 0.5-50 $\mu g\ L^{-1}$ into 20 mL of tap water and well water in a 30 mL headspace vial. The HS-SDME-GC-ECD technique was investigated by spiking known amount of trihalomethanes standard solution in the concentration range of 0.1-4 $\mu g\ L^{-1}$ into 7 mL of drinking water, tap water and well water in a 10 mL headspace vial. A sample blank was analyzed before each water samples. Each set was analyzed by GC-ECD at optimum conditions. The responses, peak areas, were plotted against the known concentrations of trihalomethanes standard. The slope of the standard and the spiked sample were compared for matrix interference and confirmed/evaluated by using the statistic test.

2.14 Method validation

To create confidence that an analytical method generated reliable and interpretable information about the sample, the methods for trihalomethanes analysis need to be validated. The objective of the method validation is to demonstrate that the steps in the analytical procedure, and may be valid for a restricted matrix, produce acceptably accurate and precise results for a given analyte.

2.14.1 Recovery

Recovery of the method was performed by spiking well water and tap water with trihalomethanes standard solutions, 0.5 and 10 μg L^{-1} for SHS and spiking drinking water and tap water with trihalomethanes standard solutions, 0.1, 0.5 and 3.0 μg L^{-1} for HS-SDME. The results from the spiked sample were compared with trihalomethanes standard solution prepared at the same concentrations. Percentage recovery (%R) is calculated as follows:

$$\%R = \left[\frac{(CF - CU)}{CA}\right] \times 100 \qquad (2.4)$$

Where *CF* is the concentration of analyte measured in the fortified sample or spiked sample.

CU is the concentration of analyte measured in the unfortified sample or blank.

CA is the concentration of analyte added in the sample (Eurachem, 1998).

2.14.2 Precision and accuracy

Precision and accuracy of method were studied at the same time by analyzing well water and tap water with trihalomethanes at 0.5 and 10 μg L^{-1} for SHS and analyzing drinking water and tap water with trihalomethanes 0.1, 0.5 and 3.0 μg L^{-1} for HS-SDME. A sample blank was also run in parallel. Five replicates

were performed at each concentration. The relative standard deviations (RSD) were then calculated for each type of samples by the following equations (Miller and Miller, 2000).

$$\%RSD = \frac{s}{\bar{x}} \times 100 \tag{2.5}$$

$$s = \sqrt{\frac{\sum \left(x_i - \bar{x}\right)^2}{n-1}} \tag{2.6}$$

Where s is the standard deviation

n is the total number of measurements

$\bar{x}$ is the mean of n measurements

2.15 Qualitative and quantitative analysis of trihalomethanes in water samples

2.15.1 Qualitative analysis

Qualitative analysis was carried out by comparing the retention time (t_R) of the chromatogram of trihalomethanes standard to unknown samples. The retention time is the time elapsed from injection of the sample component to the recording of the peak maximum (Grob and Barry, 2004).

2.15.2 Quantitative analysis

Quantitation of trihalomethanes in water was based on the response of chromatographic peak that was proportional to the amount of analyte. Two analytical techniques, external standard method and the matrix-matched calibration curve were implemented in this work.

2.15.2.1 External standard

External standard was carried out by preparing the working standard solution of trihalomethanes at concentration closed to the unknown samples concentration. The samples were analyzed under the optimum conditions. The calibration curve was obtained by plotting the peak areas *versus* concentration of trihalomethanes. Concentrations of trihalomethanes in sample were obtained by calculating from the calibration curve (Grob and Barry, 1985).

2.15.2.2 Matrix-matched calibration curve

In the case where the analysis of sample has matrix interference, the technique of matrix-matched calibration curve was used. This technique was carried out by spiking all standard trihalomethanes into drinking water and tap water to the final concentration at 0.1, 0.5, 1.0, 3.0 and 4.0 $\mu g\ L^{-1}$. Then the spiked and unspiked samples were analyzed chromatographically under the optimum conditions. The matrix-matched calibration curves were obtained by plotting the peak area, after subtracting the concentration of the analytes in the unspiked sample, versus concentration of trihalomethanes. The response from the chromatogram per unit of concentration of trihalomethanes in the unknown samples is then calculated mathematically from the matrix-matched calibration curve.

2.15 Quality assurance and quality control

In the laboratory, at any stage of sample preparation and analysis, contamination can occur. Therefore, it must be demonstrated that the sample preparation procedure is essentially free of trace trihalomethanes residue. This was assessed by preparing reagent blanks and method blank.

Reagent blanks are reagents used during the analytical process (including solvents used for extracting and dissolution). These are analyzed in order to see whether they contribute to measure signal. The signal arising from the analyte

can then be corrected accordingly (Eurachem, 1998). Reagent blanks were prepared and analyzed for trihalomethanes prior to the sample preparation steps.

Method blank is performed by carried through all the steps of sample preparation and analyses as if it was an actual sample. It is used to evaluate the laboratory contamination for the targeted analytes and corrected systematic errors due to impurities that could arise from the reagents, the glasswares, or the laboratory environment (Harvey, 2000).

CHAPTER 3

Results and discussion

This research focused on the sample preparation for determination of trihalomethanes *i.e.*, chloroform ($CHCl_3$), dichlorobromomethane ($CHCl_2Br$), dibromochloromethane ($CHClBr_2$), and bromoform ($CHBr_3$) in water. Water samples were extracted with headspace extraction techniques *i.e.*, static headspace (SHS) and headspace single-drop microextraction (HS-SDME) and analyzed by gas chromatography equipped with electron capture detector (GC-ECD) and 2 m × 2.0 mm i.d., glass column, 1% SP-1000 on Carbopack B 60/80 mesh. The optimum conditions of GC-ECD, SHS, and HS-SDME were investigated; the results were then applied to real analyze water samples.

3.1 Optimization of GC-ECD conditions for trihalomethanes analysis

The analytical method for trihalomethanes in water must be sensitive and selective, due to its very low concentration and interference matrices. Gas chromatography (GC) is suitable for trihalomethanes analysis because these compounds are volatile organic compounds whereas have appreciable vapor pressures in the temperature range typical for GC (Nollet, 2006). The gas chromatography was gas-liquid chromatography, GLC which the mobile phase is purify of nitrogen gas and the stationary phase is 1% SP-1000 on Carbopack B 60/80 mesh pack in the 2 m × 2.0 mm i.d., glass column. The mechanism of the separation is based on the partitioning of the trihalomethanes between mobile phase and liquid phase (stationary phase).

The detection system is electron capture detector (ECD) which is a sensitive and selective detector for analysis of trihalomethanes because halomethanes compose of the halogen that is highly electronegative atoms, which provides the response signal for the halomethanes that separated by the chromatographic column (Grob and Barry, 2004). The ECD is schematically presented in Figure 3.1. The main part of the detector is radiation source; the most common of ECD uses Nickel 63.

The basic of the ECD is that the β-ray from Nickel 63 collide with the carrier gas molecules to produce electrons; producing a standing current is measured. The presence of the halogen species which were eluted from the column; functionally present in an organic molecule, could capture an electron to form a negatively charged species (Equation 3.1).

$$CX + e^- \longrightarrow CX^- + \text{Energy} \qquad (3.1)$$

These entities would then cause a reduction in the standing or background current and decreasing of this current is registered (Grob and Barry, 2004).

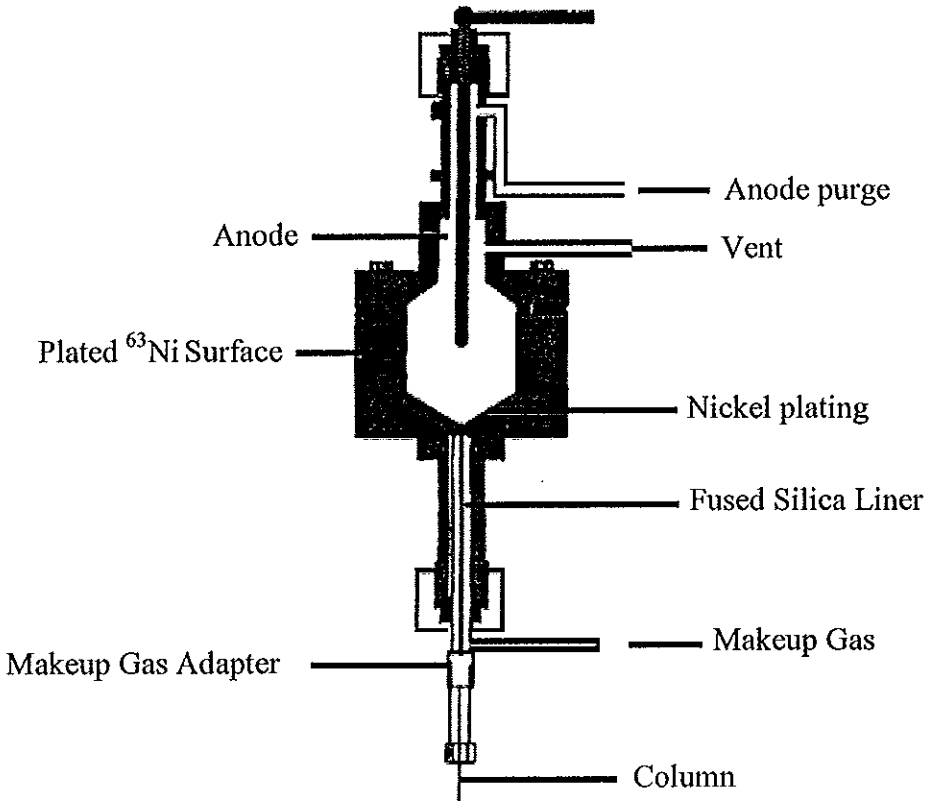


Figure 3.1 Schematic diagram of an electron capture detector (ECD).

In order to achieve specific goals such as higher speed of analysis or improved peak-to-peak resolution, optimization of a number of important variables and their interactions must be done (Grob and Barry, 2004). GC-ECD conditions for analysis of trihalomethanes in water were optimized *i.e.,* carrier gas flow rate, column temperature program, injector temperature and detector temperature. These optimum conditions were obtained by considering the best resolution, highest response and less analysis time.

3.1.1 Carrier gas (N_2) flow rate

Column efficiency and peak resolution is affected by carrier gas flow rate; each column will have an optimum flow rate for the carrier gas used for the analysis (Scott, 1998). The optimum carrier gas flow rate of column was obtained from the van Deemter plot (Figure 3.2) that considered the relationship between the height equivalent to a theoretical plate (HETP) and the carrier gas flow rate. The van Deemter equation that expresses the extent to which a component band spreads as it passes through the column in terms of physical constants and the velocity of the mobile phase as the sum of the eddy diffusion, molecular diffusion and mass transfer effect as show in Equation 3.2 (Grob and Barry, 2004).

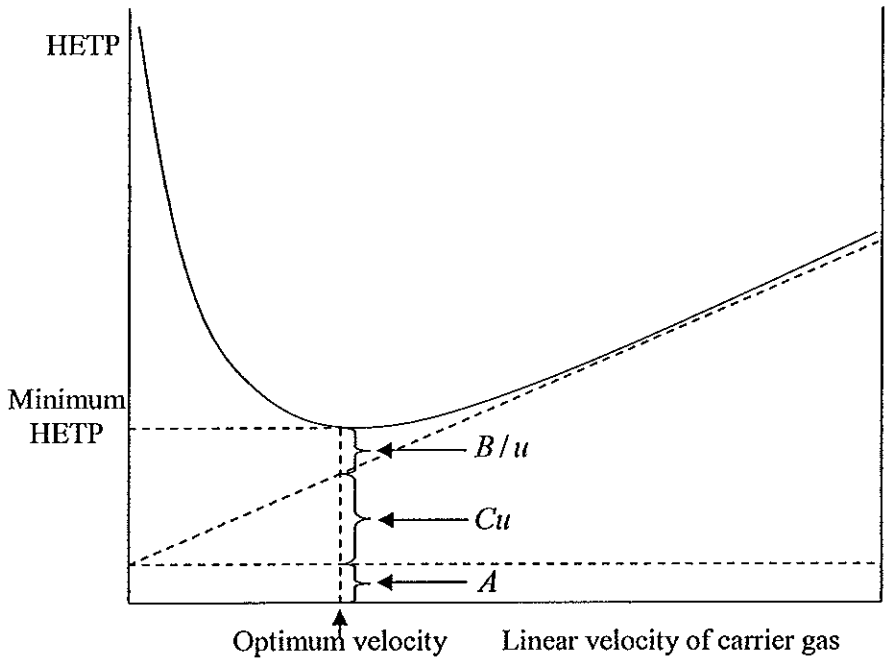


Figure 3.2 The van Deemter plot

$$\text{HETP}\,(H) = A + \frac{B}{u} + Cu \qquad (3.2)$$

Where A is eddy diffusion term: a constant that accounts for the effect of "eddy" diffusion in column.

B is longitudinal or ordinary diffusion term: a constant that accounts for the molecular diffusion of the vapor in the direction of the column axis.

C is resistance to mass transfer term: a constant proportional to the resistance of the column packing to mass transfer of solute through it.

u is the linear velocity of carrier gas (mobile phase).

The van Deemter equation shows the effect of H with changes in linear gas velocity. This equation represents a hyperbola that a minimum velocity, at $u = (\text{B/C})^{1/2}$ and a minimum H value (H_{min}) at $A + 2(BC)^{1/2}$. The constants can be calculated from an experimental plot of H vesus linear gas velocity as shown in Figure 3.2.

In this work, 2 m x 2.0 mm i.d., 1% SP-1000 on Carbopack B 60/80 mesh glass column, was used for the analysis. In this column a liquid phase coated on solid support which has the practical size is 60/80 mesh. Therefore, the A, B and C terms has effect on column efficiency and peak resolution.

In practice, the terms of A, B and C in the equation, are difficult to obtain. However, the plate theory assumes that the column is divided into a number of zones called theoretical plates (N). The zone thickness or height equivalent to a theoretical plate (HETP) is determined by assuming that there is perfect equilibrium between the gas and liquid phases within each plate. The indication of column efficiency in the term of HETP (or H) is determined by Equation 3.3.

$$H = \frac{L}{N} \tag{3.3}$$

Where L is length of column in centimeters and H measures the efficiency of the column per unit length. Small H values (large N values), therefore, mean more efficient. The plate number (N) of a column can be calculating from Equation 3.4.

$$N = 16\left(\frac{t_R}{w}\right)^2 \tag{3.4}$$

Where t_R is the retention time of the peak and w is the base peak width. If a width at height ($w_{\frac{1}{2}}$) was used instead of a width (w) at the base, the plate number could be calculating by Equation 3.5 (Tibor and Esther, 1999) (Figure 3.3).

$$N = 5.54\left(\frac{t_R}{w_{\frac{1}{2}}}\right)^2 \tag{3.5}$$

Where ($w_{\frac{1}{2}}$) is peak width at half height

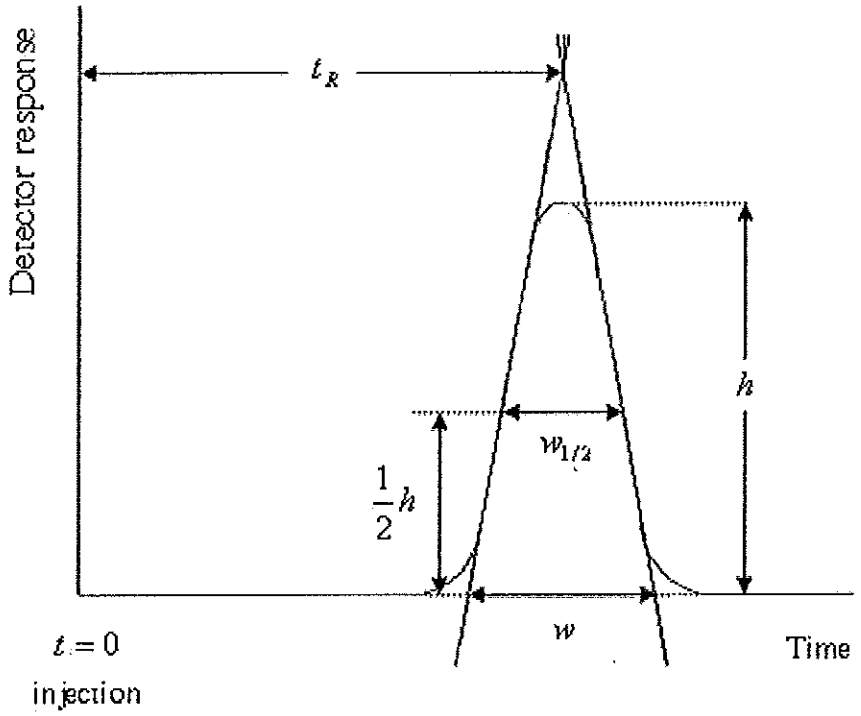


Figure 3.3 Measurement used in calculating total theoretical plates.

N was calculated from Equation 3.5, and substituted in Equation 3.3, with a known L term, column length, to obtain HETP. The results are shown in Table 3.1 and Figure 3.4. From the van Deemter plot, flow rate at 20 mL min^{-1} provided the lowest HETP for all compounds. So, the optimum flow rate at 20 mL min^{-1} was obtained with the maximum column efficiency.

Table 3.1 The height equivalent to a theoretical plate (HETP) of trihalomethanes at various carrier gas (N_2) flow rates.

Flow rate (mL min^{-1})	HETP × 10^{-2} (cm)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
10	1.20±0.09	0.5±0.1	0.36±0.03	0.59±0.09
20	0.89±0.02	0.303±0.003	0.33±0.03	0.41±0.01
30	1.15±0.01	0.663±0.002	0.416±0.001	0.477±0.001
40	1.37±0.01	0.771±0.003	0.741±0.001	0.69±0.08

*5 replications

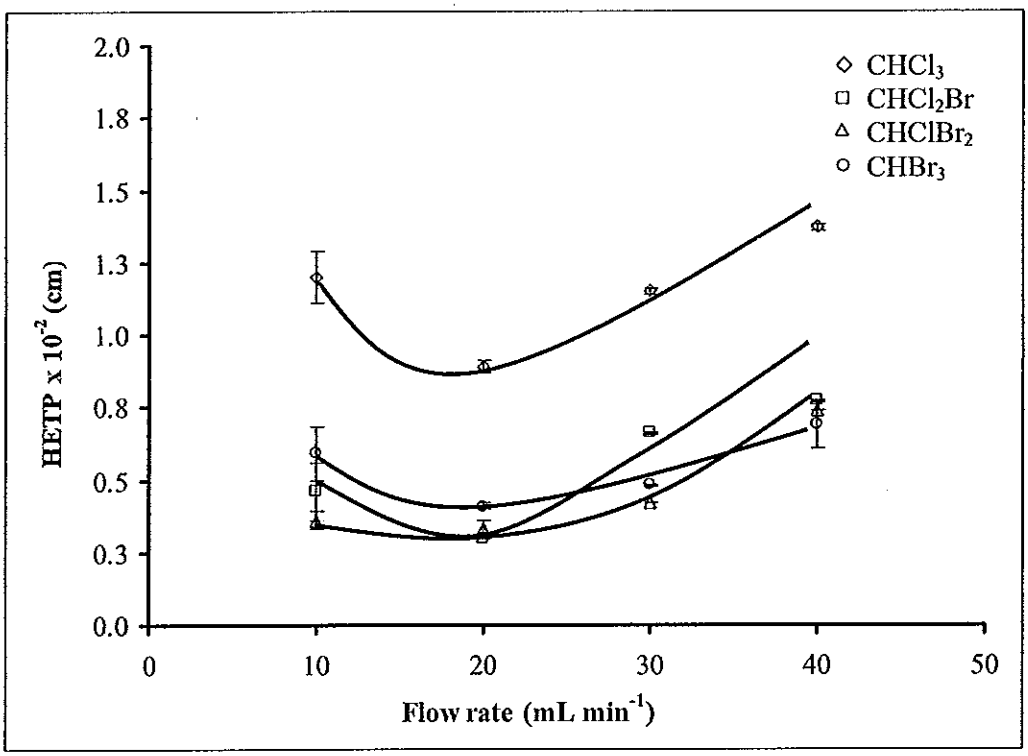


Figure 3.4 The van Deemeter plots of trihalomethanes.

3.1.2 Column temperature

Column temperature is one of the important parameters in gas chromatography technique since it leads to peak resolution and minimizes time of analysis. Temperature programming becomes necessary when a sample contains solutes having polarities and/or molecular weight that extend over a wide range. If separated isothermally, it may well result in the less retained solutes being adequately resolved and eluted in a reasonable time. However, the higher molecular weight solutes will be held on the column for an inordinately long period, and the solute peaks, when they are eluted, likely to be flat which are difficult to evaluate quantitatively (Scott, 1998). The components of interest while narrowing peak widths, increasing integrating detectability and sample throughput and reducing analysis time (Grob and Barry, 2004). Since trihalomethanes have a wide range of boiling points between 60 and 150 °C which can not be separated satisfactorily in an isothermal run column temperature programmings were investigated. The column temperature programming consists of initial temperature, initial holding time, ramp rate, final temperature and final holding time. The optimum column temperature programming was obtained by considering the best resolution, highest response and less analysis time. The result of each step of the column temperature programming as follows.

Step I- Initial temperature: the results are shown in Table 3.2 and Figure 3.5. The analysis time decreased when the temperature increased. Initial temperature at 70 °C and 80 °C provided the high response but initial temperature at 80 °C used less time. Thus, 80 °C was chosen as the optimum initial temperature.

Table 3.2 Effect of initial temperatures on the response and analysis time of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$).

Initial temperatures (°C)	Peak area × 10^6 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
50	1.67±0.02	9.4±0.2	7.0±0.2	4.05±0.07
70	1.99±0.06	11.3±0.3	8.3±0.3	4.9±0.2
80	1.96±0.05	11.3±0.2	8.8±0.3	5.1±0.1
90	1.70±0.01	10.04±0.09	7.6±0.2	4.6±0.1
100	1.75±0.01	9.8±0.1	7.49±0.08	4.5±0.1

*5 replications, RSD < 4%

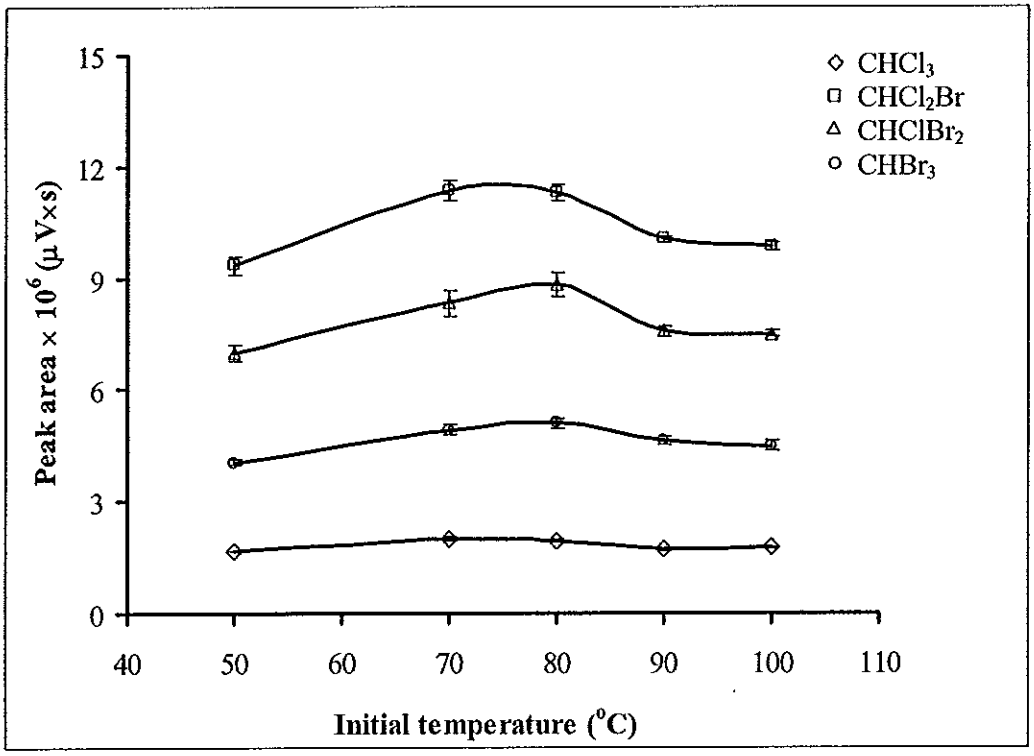


Figure 3.5 Response of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$) at various initial temperatures.

Step II- The responses at various initial holding times are shown in Table 3.3 and Figure 3.6. As can be seen the holding time has not much affect on the response. Thus, the time at 0 minute was chosen as the optimum initial holding time.

Table 3.3 Effect of initial holding time on the response and analysis time of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$).

Initial holding time (min)	**Peak area × 10^6 (μV×s)***			
	$CHCl_3$	**$CHCl_2Br$**	**$CHClBr_2$**	**$CHBr_3$**
0	1.86±0.05	10.20±0.02	7.3±0.3	5.4±0.1
1	1.89±0.03	10.3±0.2	7.3±0.3	4.37±0.08
2	1.84±0.05	9.9±0.2	8.0±0.3	4.35±0.08
3	1.85±0.05	10.2±0.2	8.0±0.2	4.3±0.1

*5 replications, RSD < 4%

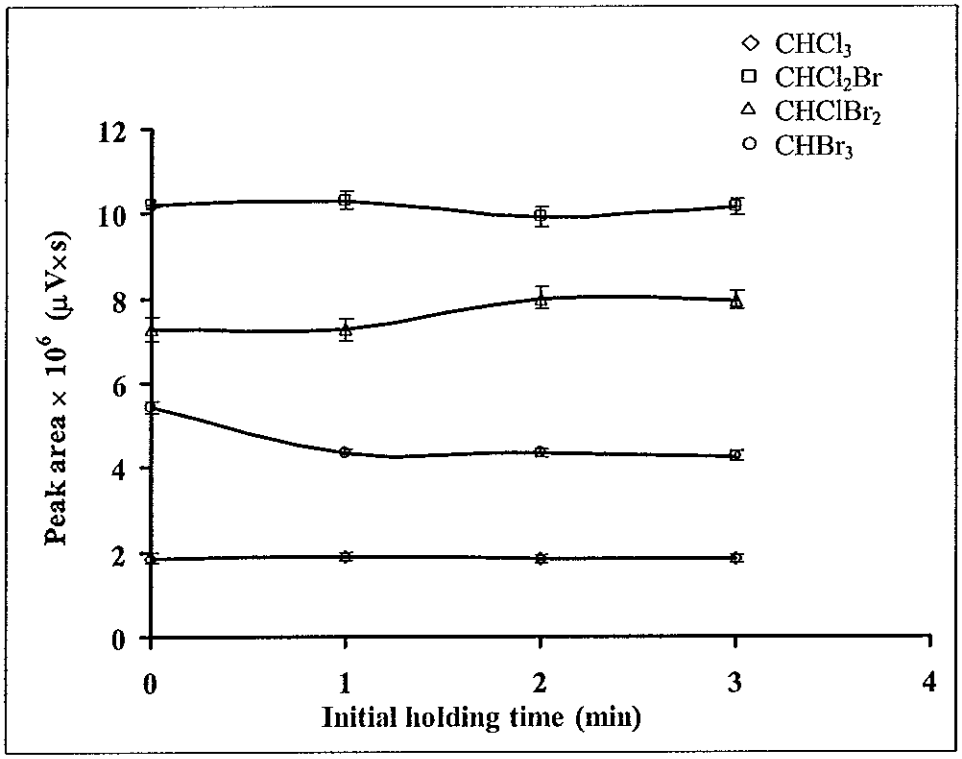


Figure 3.6 Response of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$) at various initial holding times.

Step III- The effect of ramp rate of temperature is shown in Table 3.4 and Figure 3.7. The responses of each analyte are rather constant when increased the ramp rate. Although, increasing ramp rate provided short analysis time, ramp rate higher than 10 °C min^{-1} caused baseline drifte. For this reason, the ramp rate of 10 °C min^{-1} was selected as the optimum ramp rate.

Table 3.4 Effect of ramp rate temperature on the response and analysis time of Standard solution trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$).

Ramp rate (^oC min^{-1})	Peak area × 10^6 (μV×s)*				Analysis time (min)
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$	
5	2.04±0.03	10.7±0.4	6.8±0.2	4.20±0.09	21.87
10	1.77±0.04	10.87±0.08	6.8±0.2	4.62±0.08	14.23
15	1.70±0.07	10.7±0.1	6.6±0.3	4.5±0.2	11.57
20	1.65±0.09	10.6±0.2	6.5±0.4	4.2±0.3	9.84

*5 replications, RSD < 4%

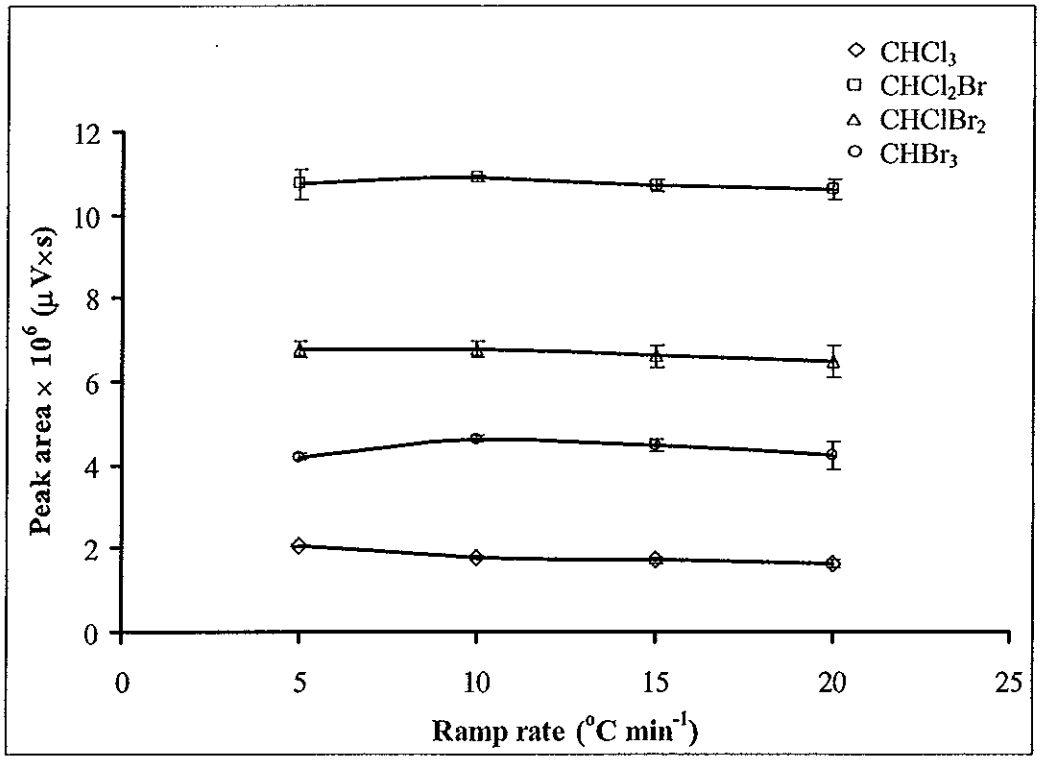


Figure 3.7 Response of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$) at various ramp rates.

Step IV- Final temperature was investigated from 170 to 200 °C with an increment of 10 °C. Higher temperature was not tested because the maximum acceptable temperature of 1% SP-1000 on Carbopack B, 60/80 mesh column is 225 °C and operating above the recommended temperature will cause column deterioration, and probably detector contamination with consequent increase in noise level and loss of sensitivity (Scott, 1998). The results were obtained as in Table 3.5 and Figure 3.8. The response increased as the final temperature increased. The highest response was at 180 °C. Thus, the temperature at 180 °C was selected as the optimum final temperature.

Table 3.5 Effect of final temperatures on the response and analysis time of standard solution trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$).

Final temperature (°C)	Peak area × 10^6 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
170	1.55±0.04	8.4±0.1	4.68±0.06	2.97±0.07
180	1.56±0.05	9.1±0.2	5.3±0.2	2.93±0.06
190	1.43±0.03	8.6±0.1	5.4±0.2	2.90±0.06
200	1.43±0.03	8.16±0.09	4.68±0.09	2.58±0.08

*5 replications, RSD < 4%

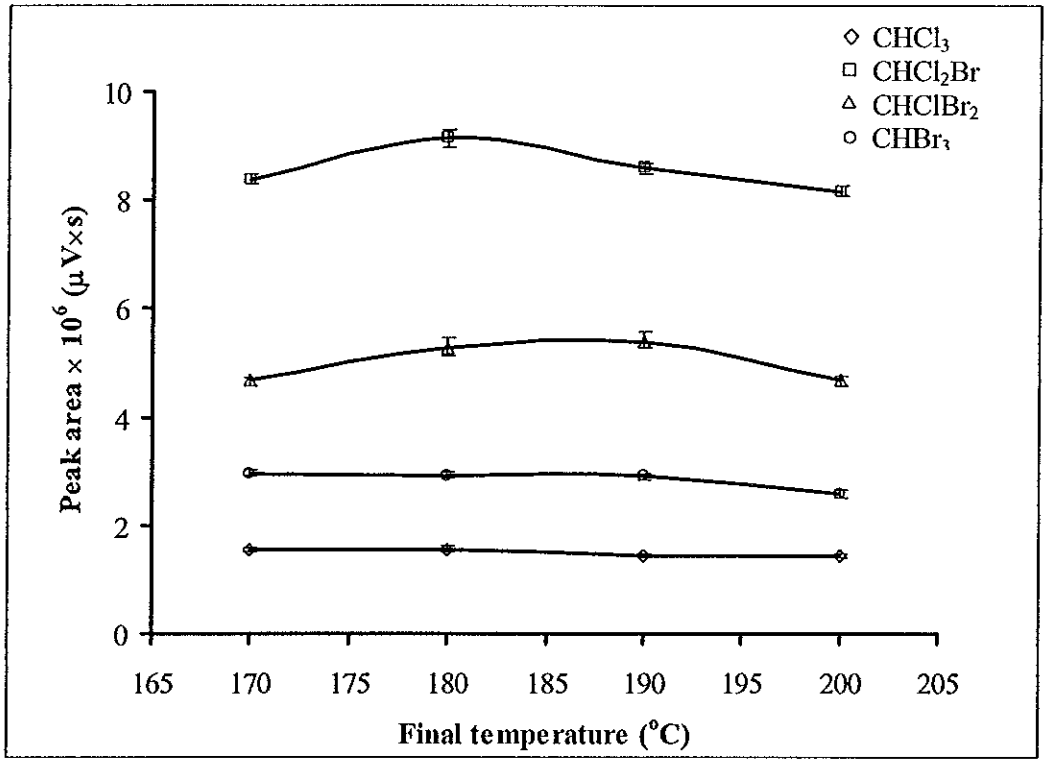


Figure 3.8 Response of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$) at various final temperatures.

Step V- The last step, the final holding time was obtained by considering the time that $CHBr_3$ (the last compound) was eluted completely from the column. The purpose of final holding time was for the signal return to baseline. It had no effect on the response and baseline resolution. Therefore, to give the corrected result and decrease analysis time, the final temperature at 180 °C was held for 7 minutes. This was enough to allow the signal to go back to the baseline.

In summary, the optimum column temperature programming applied in GC-ECD was: initial temperature 80 °C, ramped at 10 °C min^{-1} to 180 °C and held for 7 minutes (Figure 3.9). The total analysis time was 17 minutes which is less than the 20 minutes reported by Kuivinen and Johnsson in 1999. These conditions could minimize the elution time of trihalomethanes with good resolution, increasing detectability and reducing analysis time.

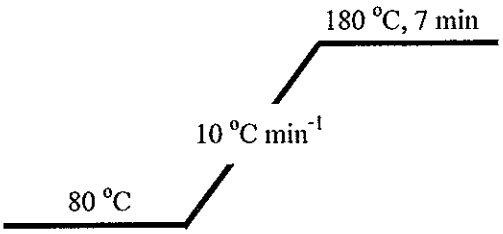


Figure 3.9 Optimum column temperature programming for trihalomethanes.

3.1.3 Injector temperature

Injection port is an important part of the system that performs the physical task of transferring the sample from a syringe into the gas chromatograph. The temperature of the injection port should be high enough to vaporize a liquid sample instantaneously because if the temperature is too low, separation is poor and resulting in broad spectral peaks or no peak develops at all. However, if the injection temperature is too high, the sample components may decompose or change its structure. For trihalomethanes, the results in Table 3.6 and Figure 3.10 show the highest response at 180 °C and this was selected to be an optimum injector temperature.

Table 3.6 Effect of injector temperatures on the response of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$).

Injector temperature (°C)	Peak area × 10^6 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
150	1.79±0.07	8.1±0.3	4.80±0.09	2.65±0.05
160	1.74±0.03	8.2±0.2	4.7±0.1	2.81±0.07
170	1.80±0.03	8.3±0.3	4.95±0.08	2.9±0.1
180	2.06±0.07	9.37±0.07	5.81±0.07	3.23±0.06
190	1.92±0.05	9.34±0.06	5.76±0.07	3.30±0.08
200	2.01±0.02	9.5±0.2	6.0±0.2	3.3±0.1

*5 replications, RSD < 4%

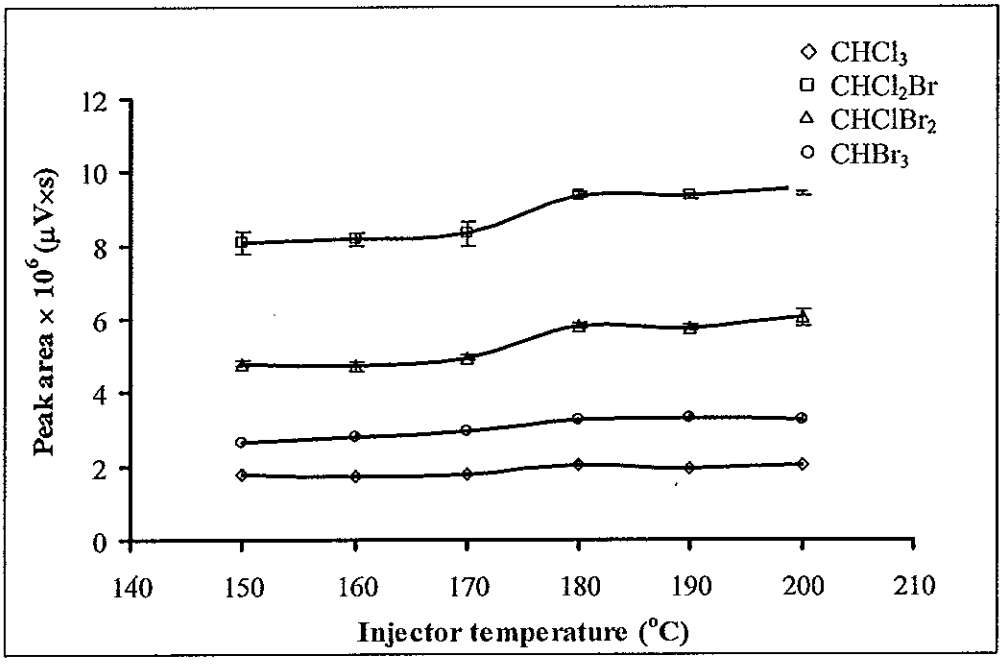


Figure 3.10 Response of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$) at various injector temperatures.

3.2.4 Detector temperature

The sensitivity of the analysis may either increase or decrease with an increase in temperature (Scott, 1998). To prevent water condensation the detector temperature is always set above 100 °C. Table 3.7 and Figure 3.11 showed the effect of the detector temperatures. The highest response was obtained at 240 °C.

Table 3.7 Effect of detector temperatures on the response of standard solution trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$).

Detector temperature (°C)	Peak area × 10^6 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
200	1.30±0.05	8.9±0.2	5.6±0.2	3.35±0.05
225	1.50±0.03	9.8±0.2	6.0±0.2	3.4±0.1
230	1.67±0.02	10.1±0.1	6.12±0.07	3.69±0.06
240	1.87±0.04	10.27±0.07	6.6±0.2	3.9±0.1
250	1.87±0.07	10.2±0.3	6.2±0.2	3.9±0.1
260	1.89±0.06	9.0±0.2	5.6±0.2	3.56±0.08
270	1.94±0.05	8.8±0.2	5.3±0.1	3.3±0.1

*5 replications, RSD < 4%

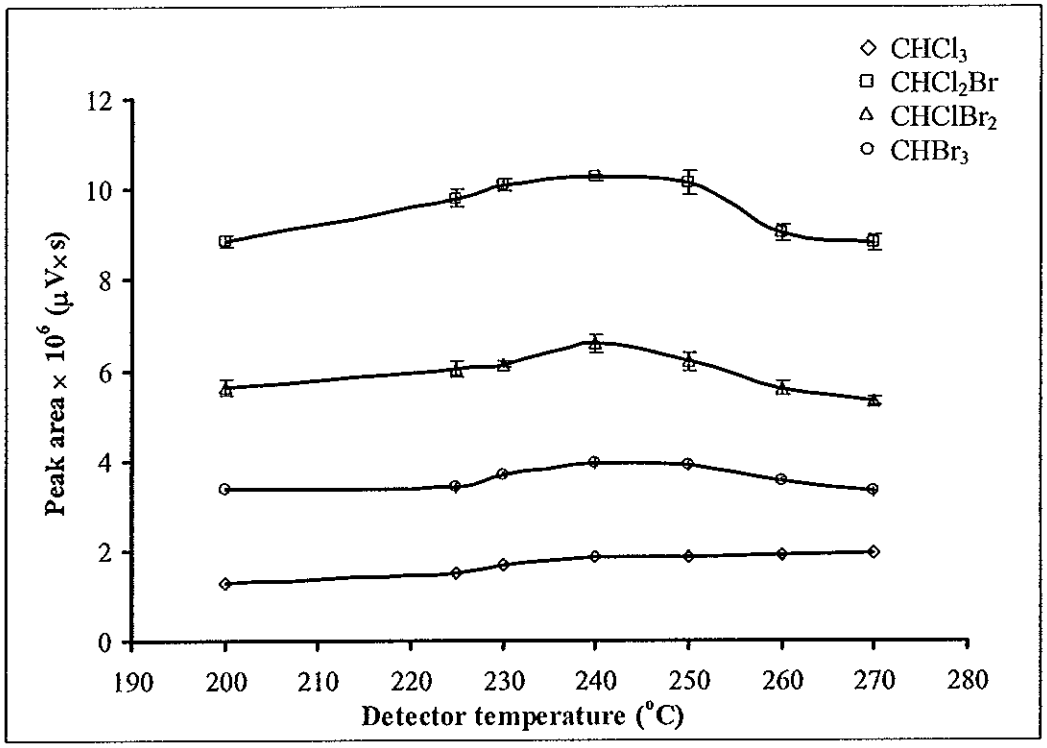


Figure 3.11 Response of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$) at various detector temperatures.

3.1.5 Summary of GC-ECD conditions

The optimum GC-ECD conditions for trihalomethanes analysis on glass column (2 m × 2.0 mm, 1% SP-1000 on Carbopack B, 60/80 mesh) and detected with electron capture detector are summarized in Table 3.8.

Table 3.8 Optimum conditions of GC-ECD for trihalomethanes.

Conditions	Optimum values
Carrier gas flow rate	20 mL min^{-1}
Column temperature program	
- Initial temperature	80 °C
- Initial holding time	0 minutes
- Ramp rate temperature	10 °C min^{-1}
- Final temperature	180 °C
- Final holding time	7 minutes
Injector temperature	180 °C
Detector temperature	240 °C

3.2 Optimization of static headspace (SHS) conditions

In headspace extraction parameters affecting extraction efficiency, sensitivity, quantitation, and reproducibility are including equilibration temperature, phase ratio, and equilibration time.

The analyte partition coefficient (K) and phase ratio (β) are the dominant factors for controlling headspace sensitivity (Grob and Barry, 2004). The effect of K and β on sensitivity of static headspace extraction analysis depends on the solubility of the analyte in the sample matrix. The partition coefficient (K) is the ratio of concentration of the analyte between the two phases in equilibrium, governed by the extraction temperature and β is derived from the relative volume of the two phases. Volatile analytes have a high partition coefficient (highly soluble); temperature will have a greater influence than the phase ratio. For analyte with a low partition coefficient (less soluble), the opposite will be true. The volumes of sample and headspace have a greater influence on sensitivity than the temperature. Headspace sensitivity (the obtained peak area) depends on the combined effect of K and β (Equation 3.6). The sensitivity of the headspace will increase as the distribution coefficient (K) and the phase ratio (β) decrease (Figure 3.12).

$$A \propto C_g = \frac{C_o}{K + \beta} \tag{3.6}$$

Where A is the chromatographic peak area for the analyte.

β is the phase ratio.

C_g is the concentration of the analyte in the headspace.

C_o is the initial concentration of the analyte in the liquid sample.

K is the partition coefficient, $K = \frac{C_S}{C_g}$, C_S is the concentration of the analyte in the liquid sample.

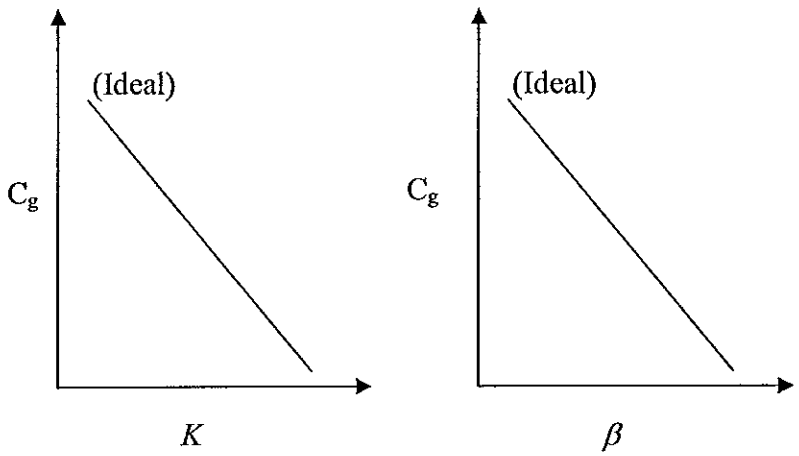


Figure 3.12 Influence of the partition coefficient (K) and phase ratio (β) on extraction efficiency (Wardencki *et al.*, 2007).

3.2.1 Equilibrium temperature

The partition coefficient of a given analyte can be changed by changing the temperature as shown in the relationship of K versus T in Equation 3.7 (Kolb and Ettre, 1997).

$$\log K = \frac{B'}{T} - C' \tag{3.7}$$

Where B' and C' are substance-specific constants

T is the absolute temperature

Thus, an increase in temperature decreases the value of the partition coefficient. When the sample temperature is increased, the vapor pressure of the analyte is increased and consequently the concentration of analyte in the headspace is increased. This can improve the extraction efficiency.

The optimization of equilibration temperature was investigated and the results are shown in Table 3.9 and Figure 3.13. The equilibrium temperature at 90 °C gave the highest response; nonetheless, this temperature has condensation of analyte in the syringe. Thus, equilibration temperature at 80 °C was chosen as the optimum equilibrium temperature.

Table 3.9 Effect of equilibrium temperatures on the response of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$).

Equilibrium temperature (°C)	Peak area × 10^6 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
50	1.32±0.03	7.4±0.2	4.2±0.1	2.7±0.1
60	1.64±0.06	1.0±0.5	5.8±0.3	3.77±0.03
70	2.47±0.08	15±0.5	9.2±0.3	6.2±0.1
80	3.5±0.1	20.4±0.5	12.3±0.4	8.2±0.1
90	4.52±0.09	26.8±0.5	17.4±0.6	12.0±0.2

*5 replications, RSD < 4%

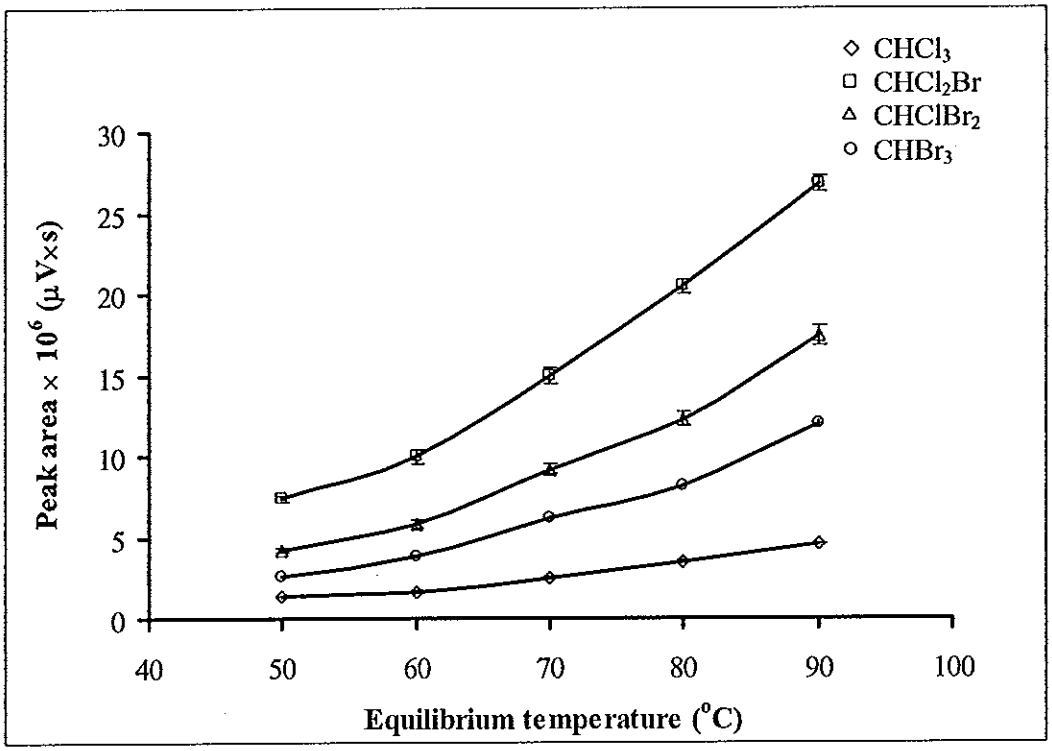


Figure 3.13 Response of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$) at various equilibrium temperatures.

3.2.2 Phase ratio

The phase ratio (β) is the ratio of the volume of gas phase in the vial to the volume of sample phase and is expressed as

$$\beta = \frac{V_g}{V_S} = \frac{V_V - V_S}{V_s} = \frac{V_g}{V_V - V_g} \tag{3.8}$$

Where V_g is the volume of the gas phase

V_s is the volume of the condensed phase (sample)

V_V is the total volume

The optimization of phase ratio was investigated.The results (Table 3.10, Figure 3.14) indicated that when phase ratio decreased, the signals increased and the highest responses were obtained at phase ratio equal to 0.5.

Table 3.10 Effect of phase ratio on the response of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$).

Phase ratio	Peak area × 10^6 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
0.2	3.5±0.1	22.3±0.6	13.3±0.4	7.7±0.1
0.5	3.9±0.1	22.4±0.9	13.1±0.2	8.0±0.3
1	2.99±0.08	19.1±0.7	11.9±0.4	7.6±0.2
2	2.45±0.07	15.1±0.4	10.1±0.3	6.8±0.2
5	1.28±0.04	8.7±0.3	6.2±0.2	4.9±0.1

*5 replications, RSD < 4%

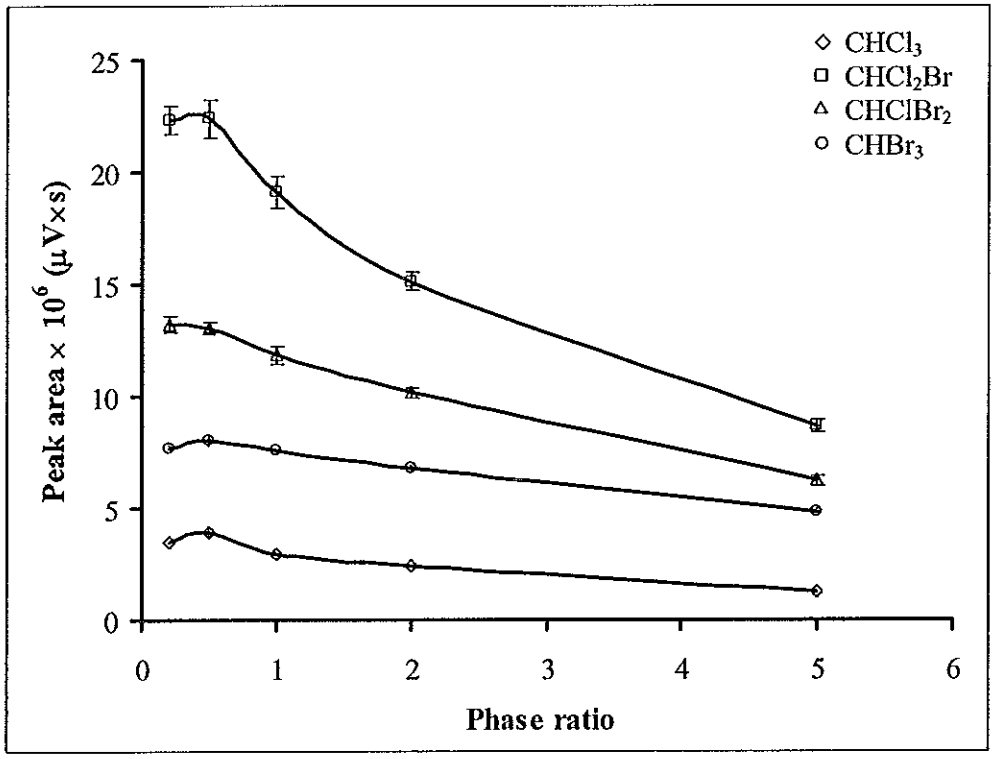


Figure 3.14 Response of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$) at various phase ratios.

3.2.3 Equilibrium time

Equilibrium time is the time that the headspace system provide the constant peak area and this depended on the distribution of the volatile compounds from liquid phase into gas phase (Kolb and Ettre, 1997). The equilibration time was investigated and the results are shown in Table 3.11 and Figure 3.15. The response increased from 15 to 20 minutes and became constant. Thus, equilibration time at 20 minutes was chosen.

Table 3.11 Effect of equilibrium time on the response of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$).

Equilibrium time (°C)	Peak area × 10^6 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
15	2.8±0.1	18.1±0.9	9.5±0.1	6.74±0.09
20	3.4±0.1	21.8±0.5	13.0±0.5	7.8±0.1
25	3.32±0.07	21.45±0.08	11.5±0.2	6.9±0.1
30	3.2±0.1	21.9±0.6	11.5±0.2	7.6±0.1

*5 replications, RSD < 4%

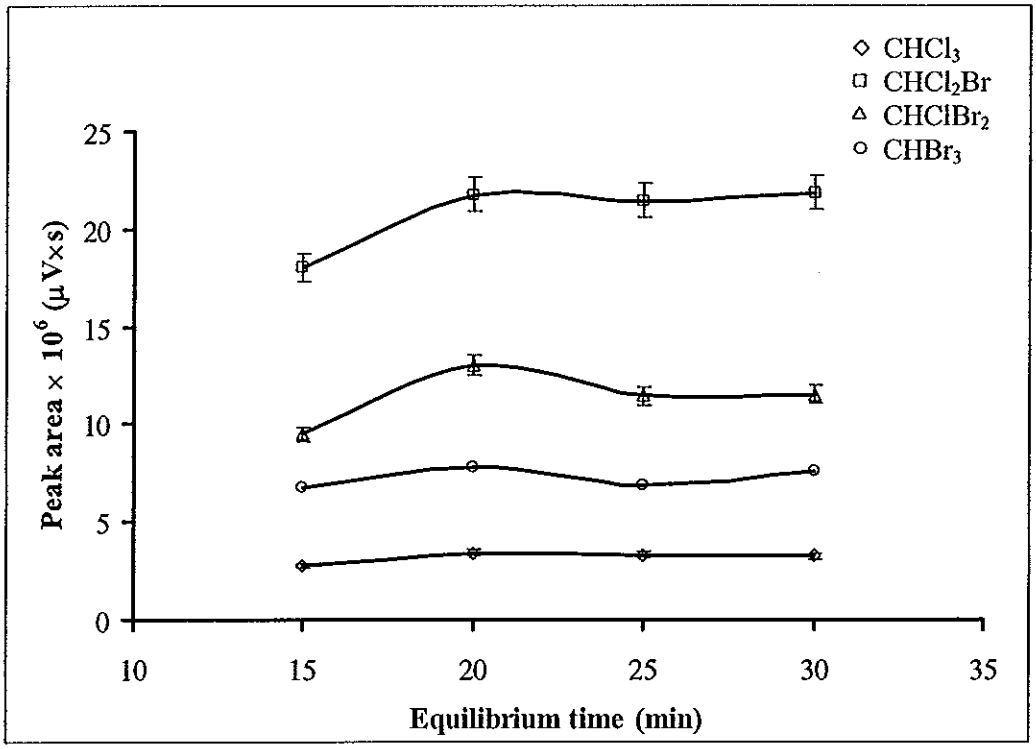


Figure 3.15 Response of standard solution of trihalomethanes of standard solution of trihalomethanes (40 μg L^{-1} $CHCl_3$, $CHCl_2Br$, and $CHClBr_2$, 120 μg L^{-1} $CHBr_3$) at various equilibrium times.

3.2.4 Summary of static headspase (SHS) conditions

The optimum SHS conditions for trihalomethanes analysis are summarized in Table 3.12. The chromatograms obtained using these sample preparation conditions is shown in Figure 3.16.

Table 3.12 Optimum conditions of SHS for the trihalomethanes analysis.

Conditions	Value
Equilibrium temperature	80 °C
Phase ratio	0.5
Equilibrium time	20 minutes

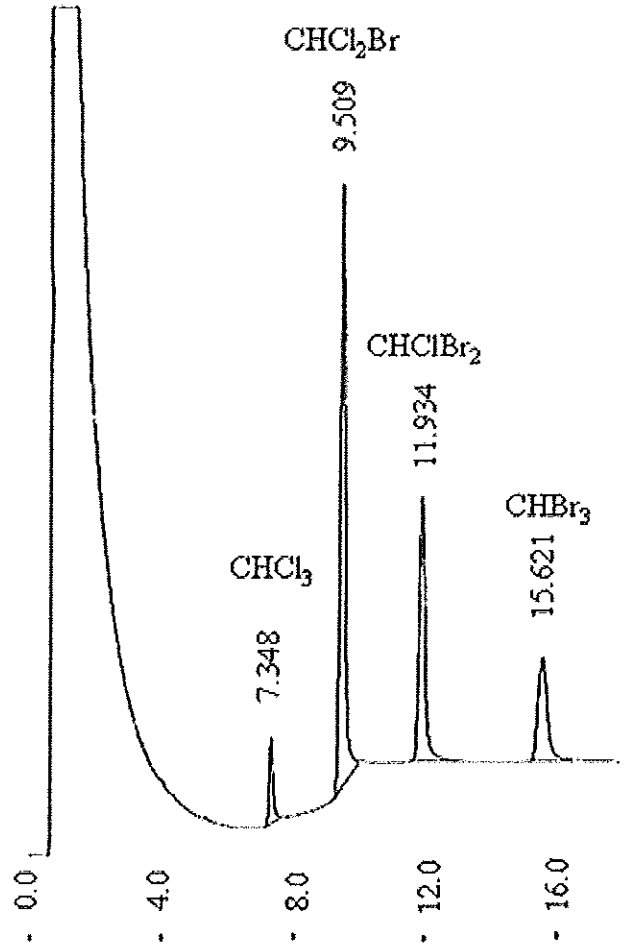


Figure 3.16 The chromatogram of trihalomethanes (2 μg L^{-1}) at optimum SHS-GC-ECD conditions.

3.3 Linear dynamic range (LDR)

Linear dynamic range or linearity of an analytical method is the ability to obtain the test results of variable data which are directly proportional to the concentration (amount of analyte) in the sample. The dynamic range was investigated by serial dilutions of a stock standard solution. Linearity is achieved when the coefficient of determination (R^2) is equal or greater than 0.99 (FDA, 2000). The slope of the regression line will provide the sensitivity of the regression. The y-intercept provides the analyst with an estimate of the variability of the method (Chung Chow,

2004). Table 3.13 and Figure 3.17 show the response of trihalomethanes at various concentrations. The system provided a wide linear dynamic range from 0.5 to 75 μg L^{-1} for $CHCl_3$, 0.1 to 60 μg L^{-1} for $CHCl_2Br$, 0.1 to 70 μg L^{-1} for $CHClBr_2$, and 0.1 to 180 μg L^{-1} for $CHBr_3$, with $R^2 > 0.99$ and the relative standard deviations (RSD) lower than 4%.

Table 3.13 Response of trihalomethanes at various concentrations.

Concentration (μg L^{-1})	Peak area × 10^6 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
0.05	1.04±0.08	1.05±0.24	0.54±0.03	0.032±0.009
0.1	1.1±0.1	1.16±0.06	0.56±0.05	0.030±0.009
0.5	1.5±0.5	3.7±0.1	1.9±0.2	0.36±0.02
1	1.69±0.06	6.1±0.3	3.1±0.2	0.61±0.02
2	2.5±0.2	11.5±0.5	6.3±0.2	1.32±0.04
10	11±0.9	71±4	37±2	7.09±0.04
30	33±1	218±9	140±5	25±1
50	55±2	345±7	234±9	40.2±0.9
60	68±2	410±15	277±4	48±1
70	86±2	421±12	319±9	55±1
75	89±1	429±12	324±3	58±2
80	90±2	-	334±2	61±2
100	92±2	-	-	79±2
150	-	-	-	109±6
180	-	-	-	130±4
190	-	-	-	132±8
200	-	-	-	131±6

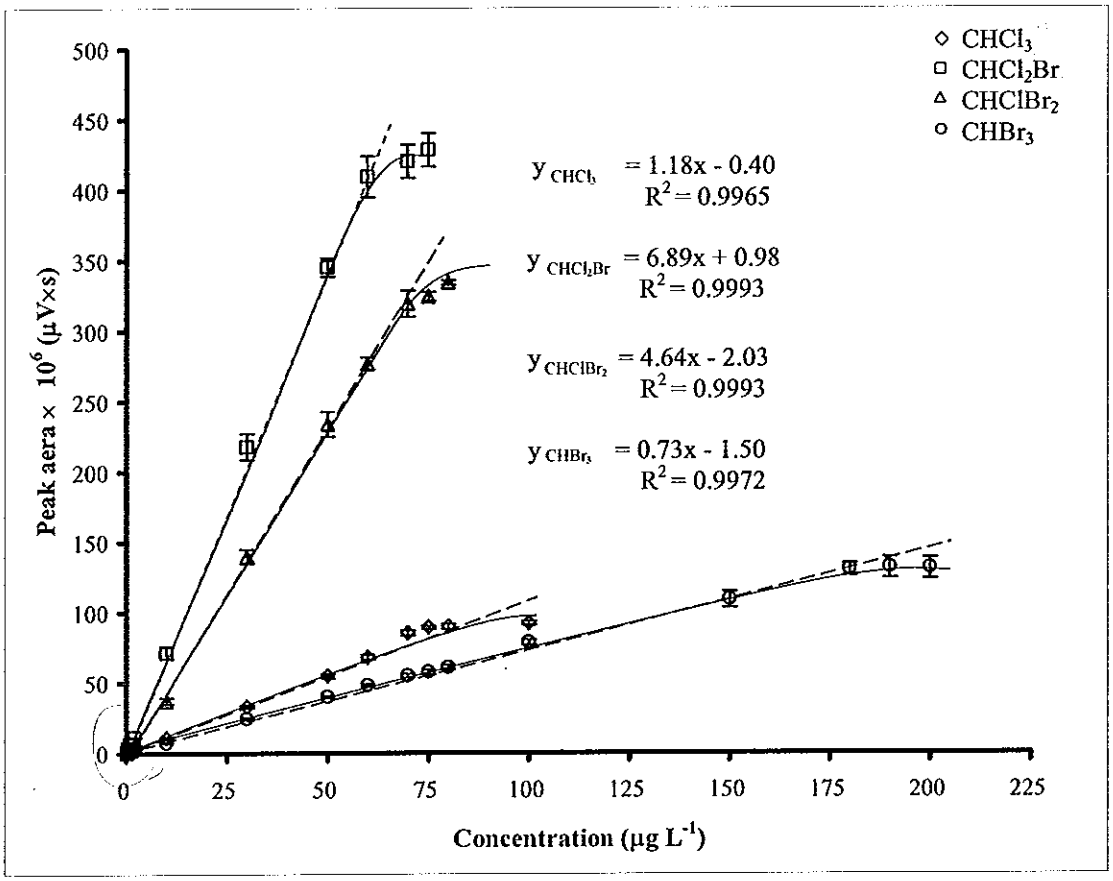


Figure 3.17 Linear dynamic ranges of trihalomethanes by SHS-GC-ECD technique.

3.4 Limits of detection (LOD)

The limits of detection is the lowest concentration of the analyte that an analytical process can be reliable detects. Average peak area from 20 blank injections was used to calculate the limit of detection using the IUPAC method (Long and Winefordner, 1983). Mean value of blank response, $\overline{X}_B$ and standard deviation (S_B) were calculated using these following equations;

$$\overline{X}_B = \frac{\sum_{j=1}^{n_B} X_{Bj}}{n_B} \tag{3.9}$$

$$S_B = \sqrt{\frac{\sum_{j=1}^{n_B}\left(X_{Bj} - \bar{X}_B\right)^2}{n_B - 1}} \tag{3.10}$$

In defining the smallest detectable signal (X_L), IUPAC states that

$$X_L = \bar{X}_B + kS_B \tag{3.11}$$

Where k is a numerical factor chosen in accordance with the confidence level desired and the accepted value is 3 at a confidence level of 99.7%, S_B is the standard deviation for 20 times of injections. C_L was then obtained as a function of X_L.

$$C_L = \frac{\left(X_L - \bar{X}_B\right)}{m} \tag{3.12}$$

Where m is the analytical sensitivity (slope of the calibration curves), and C_L is the smallest concentration that can be detected with reasonable certainty for a given analytical procedure. Because the mean blank reading, $\bar{X}_B$ is not always 0 the signal must be background corrected. Equation 3.13 was obtained after substitution Equation 3.11 into 3.12.

$$C_L = \frac{kS_B}{m} \tag{3.13}$$

Where C_L is the limit of detection.

S_B is the standard deviation of the blank response: 20 times.

m is the analytical sensitivity (slope of the analytical curve).

k is the numerical factor chosen according to the confidence level where k=3 or 3σ limits of detection, corresponds to a confidence level of about 99.7%.

From Equation 3.13 and the data in Table 3.14, limit of detection for $CHCl_3$, $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$ were obtained, 0.22, 0.04, 0.06, and 0.35 μg L^{-1} (Table 3.15), respectively which indicating that this technique is sensitive to detect trace amount of trihalomethanes in water.

Table 3.14 The maximum response of 20 blank injections.

Retention time (t_R, min)	**Maximum response × 10^4 (μV×s)**
7.35	7.25
7.36	8.93
7.32	6.10
7.29	6.48
7.29	5.87
7.32	6.18
7.30	6.36
7.35	7.25
7.36	7.85
7.37	8.24
7.37	8.12
7.37	7.74
7.33	6.23
7.31	8.02
7.31	7.77
7.31	7.72
7.31	7.53
7.30	8.08
7.35	7.84
7.31	6.95
$\bar{x}$	**7.33**
$\mathbf{S^2}$	**0.86**

Table 3.15 Limit of detection (LOD) of trihalomethanes

Compounds	Limit of detection (LOD) ($\mu g\ L^{-1}$)
$CHCl_3$	0.22
$CHCl_2Br$	0.04
$CHClBr_2$	0.06
$CHBr_3$	0.35

3.5 Limit of quantitation (LOQ)

The limit of quantitation (LOQ) is regarded as the lower limit for precise quantitative measurements (Corley, 2002). The LOQ was calculated as the analyte concentration response giving a 10 times of standard deviation of the response of blank. The LOQs of trihalomethanes were investigated in 2.13.2 and as shown in Table 3.16. The LOQ ranged from 0.12 to 1.17 $\mu g\ L^{-1}$.

Table 3.16 Limit of quantitation (LOQ) of trihalomethanes.

Compounds	Limit of quantitation (LOQ) ($\mu g\ L^{-1}$)
$CHCl_3$	0.73
$CHCl_2Br$	0.12
$CHClBr_2$	0.19
$CHBr_3$	1.17

3.6 Matrix interference

Analysis of trihalomethanes in water, natural organic matter, inorganic salts (Ndabigengesere and Narasiah, 1998) and the other disinfection by-products such as haloacetic acids, haloacetonitriles, haloketones, chloral hydrate, and chloropicrin (Nikolaou *et al.*, 2005), could interfered with the interest analytes (trihalomethanes). It can lead to either a suppression or enhancement of the sample signal compared to the calibrate signal for the same analytes. Interference would usually affect the slope of the calibration curve, so that it will be different from the slope of the analyte of interest, so the calibration curve slope in the method of additions may affect the linearity of the curve. This effect has the potential to indicate the possible present of a hidden interference (Eurachem, 1998).

Matrix effect of this method was evaluated in tap water and well water. The matrix-matched calibration curve was used for this study by spiking various amounts of known trihalomethanes standard solution into the sample as described in 2.12. The presence of interference can be evaluated by observing the slope of standard curve and the spiked sample calibration (matrix-matched calibration curve) and determined whether or not it is parallel to the standard curve because of the slope of the calibration curve will affect the linearity of the curve. If the matrix effect is not present, the instrument responses consider from both the slope of the standard curve and the spiked sample calibration should be the same (Eurachem, 1998; Roper *et al.*, 2001). The results are shown in Tables 3.17-3.20 and Figures 3.18-3.21. When the standard and the spiked sample calibration curves were compared, the two sets of data do have parallel regression lines, which indicated that these should be not interference from components of water sample on trihalomethanes analysis.

Table 3.17 Effect of matrix on the responses of $CHCl_3$ in tap water and well water samples.

Concentration of $CHCl_3$ ($\mu g\ L^{-1}$)	Peak area $\times 10^5$ ($\mu V \times s$)*± SD		
	Standard $CHCl_3$	Well water	Tap water
0.5	0.62±0.05	0.32±0.02	0.28±0.06
2	1.89±0.07	2.5±0.2	1.38±0.05
10	7.9±0.6	7.6±0.4	7.3±0.1
30	19.2±0.9	17.9±0.4	18.9±0.9
50	28±1	27.9±0.6	28±1

*5 replications, RSD ≤ 10%

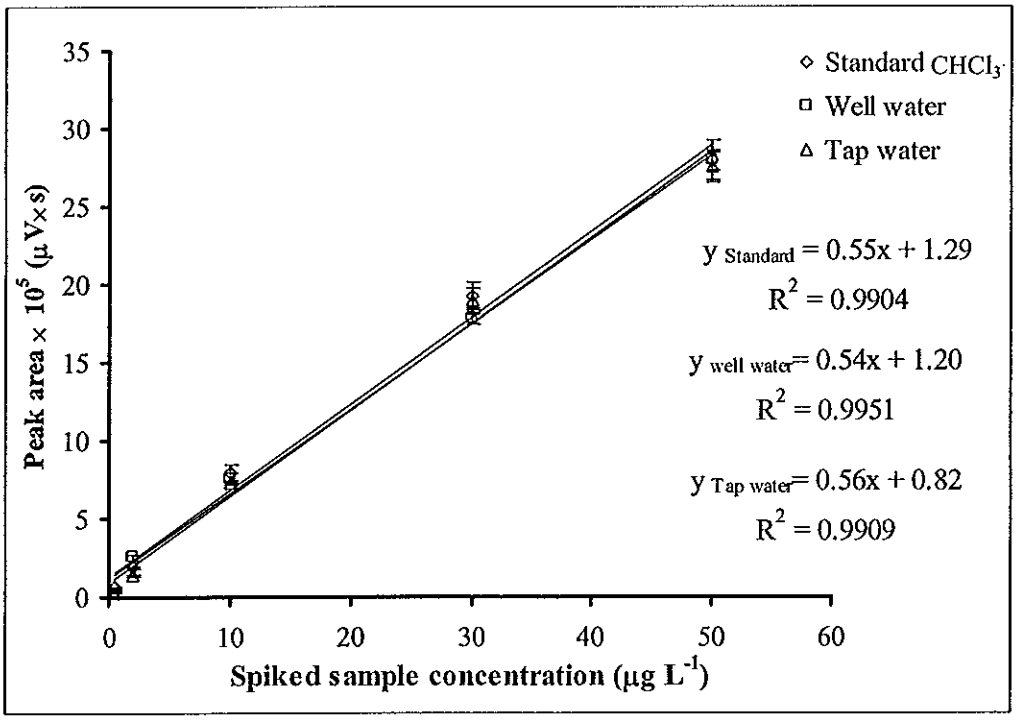


Figure 3.18 Matrix-matched calibration curve of $CHCl_3$ of tap water and well water samples.

Table 3.18 Effect of matrix on the responses of $CHCl_2Br$ in tap water and well water samples.

Concentration of $CHCl_2Br$ ($\mu g\ L^{-1}$)	Peak area $\times 10^5$ ($\mu V \times s$)*± SD		
	Standard $CHCl_2Br$	Well water	Tap water
0.5	2.3±0.2	2.5±0.3	2.3±0.3
2	8.3±0.4	10.7±0.8	8.3±0.4
10	66±5	67.4±1.5	66±5
30	227±4	230±4	230±11
50	330±5	328±9	333±8

*5 replications, RSD ≤ 14%

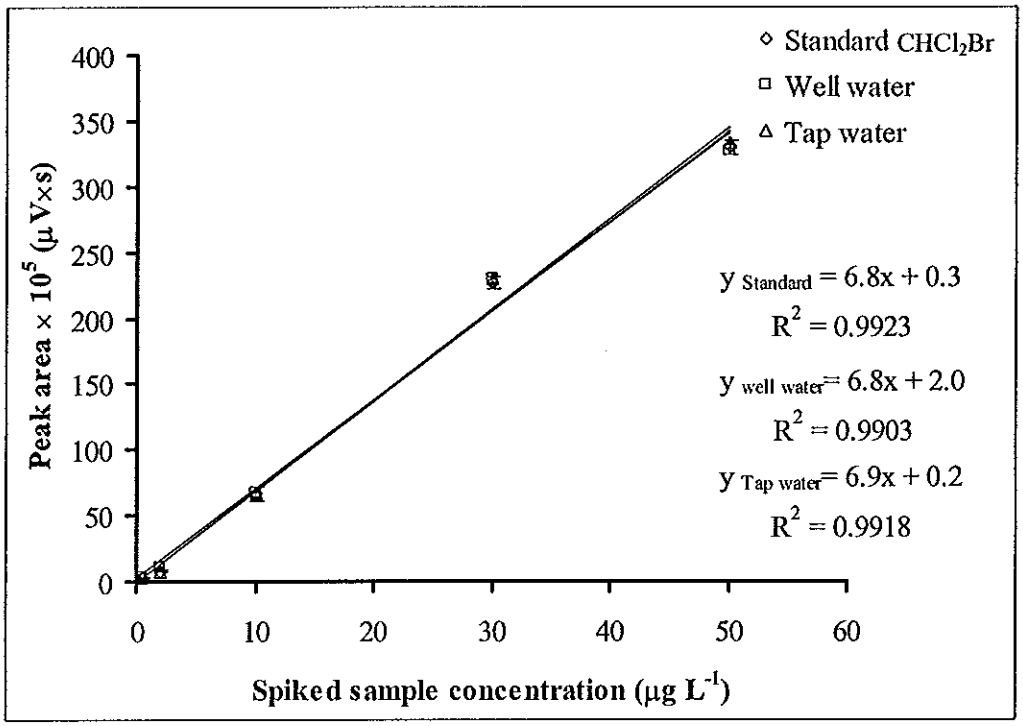


Figure 3.19 Matrix-matched calibration curve of $CHCl_2Br$ of well water and tap water samples.

Table 3.19 Effect of matrix on the responses of $CHClBr_2$ in well water and tap water samples.

Concentration of $CHClBr_2$ ($\mu g\ L^{-1}$)	Peak area $\times 10^5$ ($\mu V \times s$)*± SD		
	Standard $CHClBr_2$	Well water	Tap water
0.5	1.5±0.2	2.1±0.2	1.6±0.2
2	4.88±0.09	6.5±0.4	5.0±0.1
10	39±4	44±2	39±3
30	114±3	111±5	119±3
50	212±7	213±8	215±14

*5 replications, RSD ≤ 12%

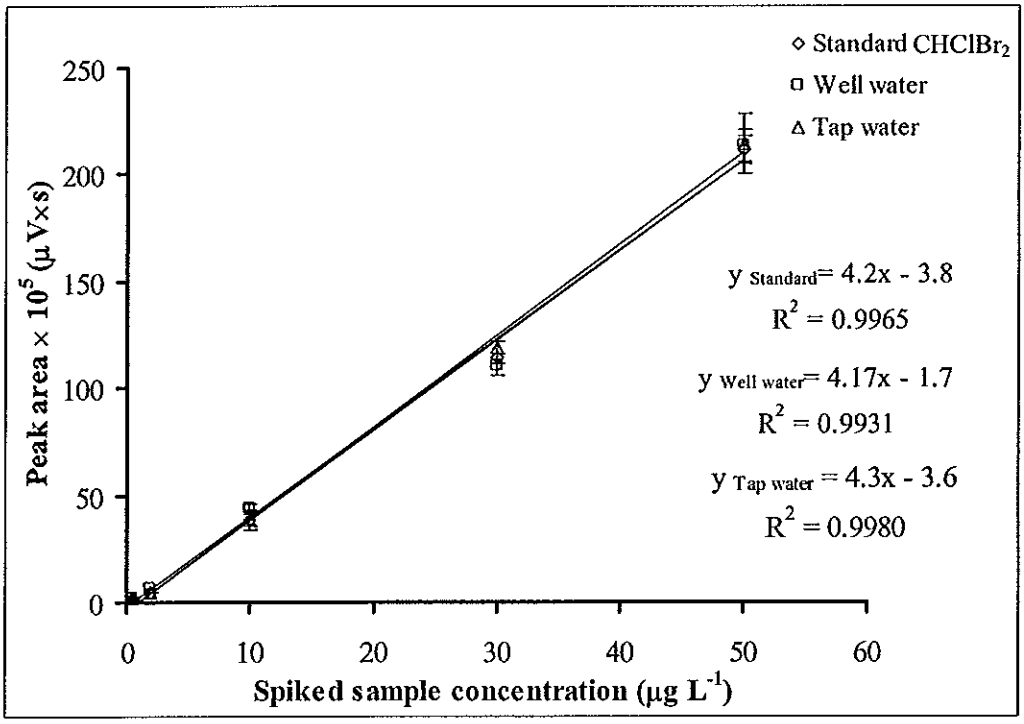


Figure 3.20 Matrix-matched calibration curve of $CHClBr_2$ of well water and tap water samples.

Table 3.20 Effect of matrix on the responses of $CHBr_3$ in well water and tap water samples.

Concentration of $CHBr_3$ ($\mu g\ L^{-1}$)	Peak area $\times 10^5$ ($\mu V \times s$)*± SD		
	Standard $CHBr_3$	Well water	Tap water
0.5	0.30±0.02	0.22±0.02	0.23±0.06
2	1.00±0.03	0.95±0.04	1.01±0.02
10	6.6±0.4	7.1±0.3	6.6±0.4
30	19.9±0.7	19.2±0.4	20.8±0.5
50	34±1	33±1	34±4

*5 replications, RSD ≤ 10%

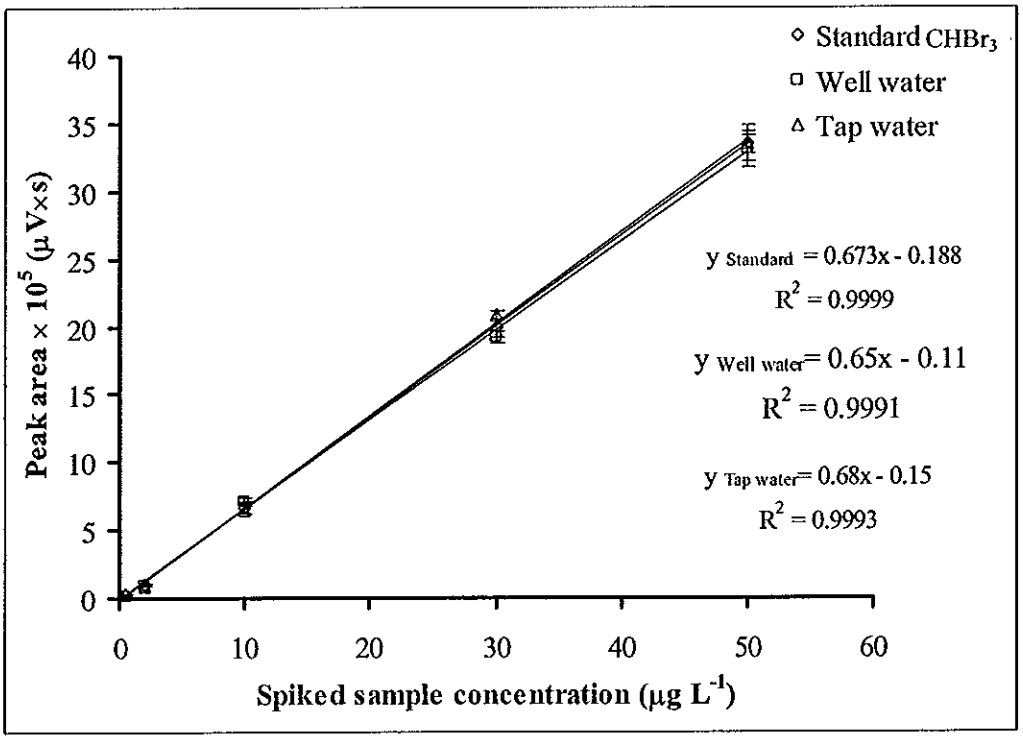


Figure 3.21 Matrix-matched calibration curve of $CHBr_3$ of well water and tap water samples.

Table 3.21 Statistical values for the comparison between the slopes of $CHCl_3$ standard curve and matrix-matched calibration curve of well water and tap water samples using two-way ANOVA by R software.

Matrix	*Df*	Sum Sq	Mean Sq	*F*	*P*
Well water	4	9.2×10^{10}	2.3×10^{10}	2.0568	0.1047
Tap water	4	5.2×10^{10}	1.3×10^{10}	1.3632	0.2639

Table 3.22 Statistical values for the comparison between the slopes of $CHCl_2Br$ standard curve and matrix-matched calibration curve of well water and tap water samples using two-way ANOVA by R software.

Matrix	*Df*	Sum Sq	Mean Sq	*F*	*P*
Well water	4	1.4×10^{12}	3.5×10^{11}	1.2455	0.3074
Tap water	4	2.0×10^{12}	5.0×10^{11}	1.4549	0.2339

Table 3.23 Statistical values for the comparison between the slopes of $CHClBr_2$ standard curve and matrix-matched calibration curve of well water and tap water samples using two-way ANOVA by R software.

Matrix	*Df*	Sum Sq	Mean Sq	*F*	*P*
Well water	4	9.2×10^{11}	2.3×10^{11}	1.4119	0.2475
Tap water	4	2.1×10^{12}	5.3×10^{11}	1.7124	0.1662

Table 3.24 Statistical values for the comparison between the slopes of $CHBr_3$ standard curve and matrix-matched calibration curve of well water and tap water samples using two-way ANOVA by R software.

Matrix	*Df*	Sum Sq	Mean Sq	*F*	*P*
Well water	4	1.8×10^{11}	4.6×10^{10}	1.1857	0.3318
Tap water	4	1.2×10^{11}	2.9×10^{10}	1.1439	0.3499

Significant codes: '*' (α = 0.01), '**' (α = 0.05), '***' (α = 0.001)

From the results of the interaction between responses and concentrations in standard and matrix groups, it can be concluded that these is no significant difference between the slopes of regression line in each group of standard curve and matrix-matched calibration curve at all levels. Thus, the standard calibration curve could be used as an accurate determination of the unknown concentration.

3.8 Method validation

Method validation is performed to create confidence in the analysis procedures. It provides an assurance of reliability during normal use (Swartz, 1997). To ensure the reliability of the results, method validation was evaluated by using standards, spiked samples, reagent and method blanks.

Accuracy, in terms of mean recovery, and precision, in term of %RSD, were assessed by replicated analyses of samples, spiked at 2 different concentrations within the calibration range. Recoveries were obtained ranged from 95 to 108% and 89 to 105% for trihalomethanes at spiked concentration 0.5 and 10.0 $\mu g\ L^{-1}$, respectively and RSDs ranged from 4 to 11 % (Table 3.25-3.26). The accuracy and precision data demonstrated that the method meets EPA method 8061 and 8000 regulatory method requirement of 70-120% recovery and a RSD less than 20%. The

results indicated that the static headspace method followed by gas chromatography can be used to extract trihalomethanes from water sample with high recoveries and excellent precision.

Table 3.25 Recovery of trihalomethanes of water samples at spiked concentration of 0.5 μg L^{-1}.

Samples	Recovery* (%) ± SD			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
Well water	102±11	104±7	103±9	98±6
Tap water	108±6	105±4	104±4	95±7

*5 replications, RSD < 11%

Table 3.26 Recovery of trihalomethanes of water samples at spiked concentration of 10.0 μg L^{-1}.

Samples	Recovery* (%) ± SD			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
Well water	101±7	102±6	104±5	97±6
Tap water	105±9	103±7	104±6	89±7

*5 replications, RSD < 10%

3.9 Qualitative and quantitative analysis of trihalomethanes in water samples

3.9.1 Qualitative analysis

The optimum conditions of SHS-GC-ECD were used to analyse trihalomethanes in water samples. For qualitative analysis, the most frequently used technique for the tentative identification of an eluted component is based on the retention times (t_R) of the eluted component and those of known standard solution (Gudzinowicz, 1967). The average t_R of $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$ were 7.37±0.05, 9.55±0.06, 11.97±0.08 and 15.65±0.13 minutes, respectively.

3.9.2 Quantitative Analysis

Quantitative analysis of trihalomethanes was done by considering the response, *i.e.,* peak area, which was related to the concentration of the analytes. Three types of water samples *i.e.,* drinking water, well water, and tap water were sampling and analysed by SHS-GC-ECD at optimum conditions and the representative chromatogram of the three types of water sample are shown in Figures 3.22-3.24.

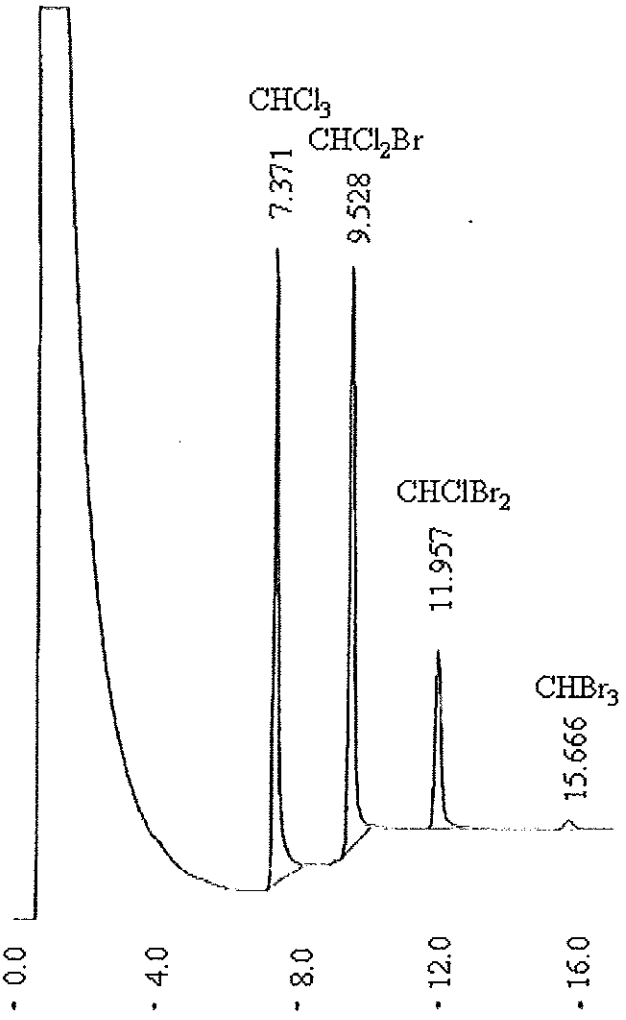


Figure 3.22 SHS-GC-ECD chromatogram of trihalomethanes from well water sample.

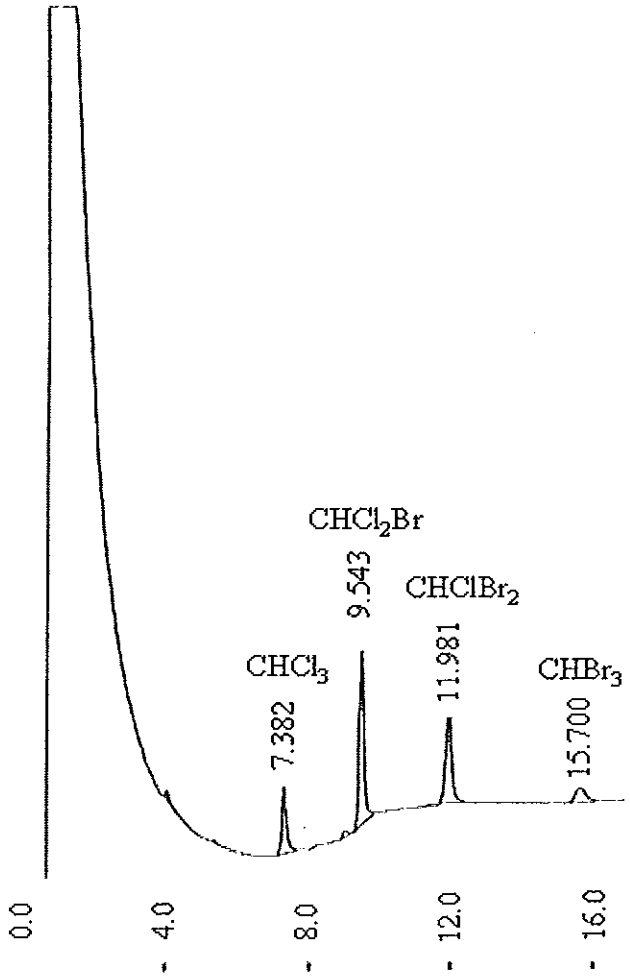


Figure 3.23 SHS-GC-ECD chromatogram of trihalomethanes from tap water sample.

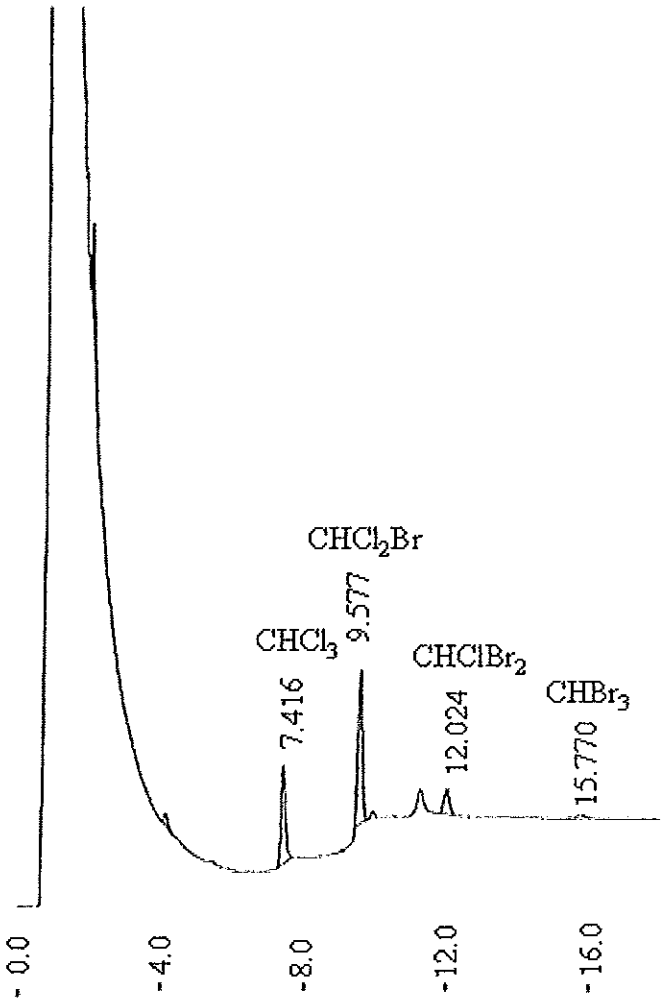


Figure 3.24 SHS-GC-ECD chromatogram of trihalomethanes from drinking water sample.

Trihalomethanes concentrations in water samples were evaluated by using the calibration curve to quantify trihalomethanes in water. The results are shown in Tables 3.27-3.29, the concentrations of total trihalomethanes in well, tap, and drinking water samples were in the range from 4.2±1 to 11±1 μg L^{-1}, 5.8±0.8 to 25±1 μg L^{-1}, and ND to 5±1 μg L^{-1}. These do not exceed the WHO guideline values of trihalomethanes in drinking water where the maximum contaminant levels (MCLs) for $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$ are limited at 200, 60, 100, and 100 μg L^{-1}, respectively and the sum of ratio of concentration of each to its respective

guideline value do not exceed 1 (WHO, 2004), and also not exceed the US EPA and EU guideline values of 80 (US EPA, 2001) and 150 μg L^{-1} (EU, 1998), respectively.

Table 3.27 Trihalomethanes concentrations of well water samples determined by calibration curves.

Sampling date	Concentration of trihalomethanes (μg L^{-1})*					THMs ratio**
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$	Total THMs	
14/11/2548	2.5±0.6	0.28±0.09	1.0±0.1	0.37±0.09	4.2±0.1	0.03
22/11/2548	8±1	0.6±0.1	1.0±0.1	ND	10±1	0.06
22/12/2548	5.3±0.7	2.7±0.3	2.2±0.2	0.6±0.1	11±1	0.1

* 5 replications, RSD ≤ 11%, ND= Not detectable

** The sum of ratio of concentration of each to its respective guideline value.

Table 3.28 Trihalomethanes concentrations of tap water samples determined by calibration curves.

Samples	Concentration of trihalomethanes (μg L^{-1})*					THMs ratio**
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$	Total THMs	
Tap water 1	5.3±0.9	0.4±0.1	0.22±0.09	ND	5.9±1.1	0.04
Tap water 2	5.2±0.6	0.38±0.09	0.20±0.09	ND	5.8±0.8	0.03
Tap water 3	6±1	0.4±0.1	0.3±0.1	ND	7±1	0.04
Tap water 4	13±1	7.1±0.2	4.0±0.2	0.460±0.006	25±1	0.23

* 5 replications, RSD ≤ 15%, ND= Not detectable

** The sum of ratio of concentration of each to its respective guideline value.

Table 3.29 Trihalomethanes concentrations of drinking water samples determined by calibration curves.

Samples	Concentration of trihalomethanes (μg L^{-1})*					THMs ratio**
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$	Total THMs	
Drinking water 1	5±1	ND	ND	ND	5±1	0.02
Drinking water 2	ND	ND	ND	ND	ND	-
Drinking water 3	1.0±0.6	ND	ND	ND	1.0±0.6	0.005
Drinking water 4	2.3±0.7	0.4±0.1	0.3±0.1	0.4±0.1	3±1	0.02

*5 replications, RSD ≤ 17%, ND= Not detectable

** The sum of ratio of concentration of each to its respective guideline value.

3.10 Optimization of headspace single-drop microextraction (HS-SDME) conditions

The HS-SDME was applied to enhance the extraction efficiency of trihalomethanes in water. The theory indicates that the analytes in headspace are transferred into the organic solvent extruded from the tip of a GC microsyringe needle; a dynamic equilibrium was finally established between the concentration of the analytes in headspace and the analytes in the organic solvent drop (Besharati-Seidani *et al.*, 2005). During the extraction processes, there are three phase involved: the condensed phase, its headspace, and the extracting phase. These should be two interfaces, the condensed phase/headspace interface and the headspace/extracting solvent interface.

The distribution of analyte between the three phases may be described by the following equilibrium constants (Theis *et al.*, 2001),

$$K_{hw} = \frac{C_h}{C_w} \tag{3.14}$$

$$K_{oh} = \frac{C_o}{C_h} \tag{3.15}$$

$$K_{ow} = \frac{C_o}{C_w} = K_{hw}K_{oh} \tag{3.16}$$

Where K_{hw} is the headspace-water distribution constant, K_{oh} is the organic drop-headspace distribution constants, and K_{ow} is the (overall) organic drop-water distribution constant.

Mass transfer in the headspace is considered as a fast process because diffusion coefficients of the analytes in the gas phase are usually 10^4-10^5 times larger than diffusion coefficients in the liquid phase. During a HS-SDME, chemicals in the headspace over a condensed phase are directly extracted and concentrated in the

extracting solvent, which makes this technique advantageous over conventional techniques for headspace analysis (Shariati-Feizabadi *et al.*, 2003).

The amount of the analyte n extracted by the microdrop at equilibrium is described by the following Equation 3.17 (Przyjazny and Kokosa, 2002):

$$n = \frac{K_{ow} V_d C_o V_s}{K_{ow} V_o + K_{hs} V_h + V_s} \tag{3.17}$$

Where C_0 is the initial concentration of the analyte in the matrix; and V_o, V_s and V_h the volumes of the drop, the sample, and the headspace, respectively. Equation 3.17 after some manipulation becomes

$$\frac{1}{n} = \frac{1}{C_o}\left[\frac{1}{V_s} + \frac{1}{K_{ow} V_o}\left(\frac{K_{hs} V_h}{V_s} + 1\right)\right] \tag{3.18}$$

In Equation 3.18 (Zhao *et al.*, 2004), relationships between n and other parameters such as K_{ow}, V_o, V_s and V_h are clearer. These parameters and their relationships are most important for the optimum of extraction conditions.

The HS-SDME conditions for the analysis of trihalomethanes in water were optimized to enhance the extraction efficiency. These included type of organic solvent, phase ratio, stirring rate, salting-out effect, organic drop volume, extraction time, and extraction temperature.

3.10.1 Type of organic solvent

The selection of an appropriate extraction solvent is the major importance for the optimization of the SDME process, which is dependent on the polarity of the target analytes. As in liquid-liquid extraction, the selection of extraction solvent is based on the principle of "like dissolves like" (Cao *et al.*, 2006). The choice of extracting solvent is based on the following consideration. First, it should provide high solubility for target analyte. Second, it should have low volatility in order to be stable at the extraction period and third, it should separate from the analyte peaks in the chromatogram. However, other parameters must also be

considered for the selection of the extracting solvent, *e.g.*, selectivity, extraction efficiency, and level of toxicity (Psillakis and Kalogerakis, 2002; Zhao *et al.*, 2004).

In order to determine which solvent would be optimal for the extraction of trihalomethanes, four solvents according to the polarity *i.e.*, *n*-hexane, 1-octanol, *p*-xylene, and ethylene glycol (Table 3.30, solvent's properties) were investigated as in 2.8.1.

Table 3.30 Properties of each organic solvent. (Mackay *et al*, 2006).

Type of organic solvent	Boiling point (°C)	Vapor pressure (Pa, 25°C)	Water solubility
n-hexane	68.73	20,180	insoluble
p-xylene	138.37	1,165	insoluble
1-octanol	195.15	8.0	slightly soluble
ethylene glycol	197.3	12.26	soluble

Among these solvents, 1-octanol gave the best extraction efficiency and its peak was easily separated from the analyte peak, while *n*-hexane and *p*-xylene provided an unstable organic drop and ethylene glycol peak can not be separated from the analyte peak. Therefore, 1-octanol was chosen as extracting organic solvent.

3.10.2 Phase ratio

Phase ratio plays an important role in headspace microextraction analysis. According to the Equation 3.8 an increase in sample volume and consequently a decrease in headspace volume enhance the extracted amount of analyte, which improves the sensitivity (Tadeusz and Janusz B., 1997). The effect of phase ratio was investigated and the results are shown in Table 3.31 and Figure 3.25. When phase ratio decreased the response of analytes increased until phase ratio at 0.4, which gave the highest response. After that, responses decreased because it did not

reach the equilibrium of temperature and time. Thus, the phase ratio at 0.4 was chosen for further analysis.

Table 3.31 Effect of phase ratio on the extraction efficiency of 10.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

Phase ratio	Peak area × 10^5 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
0.1	1.33±0.05	29±2	38±2	44±2
0.4	1.30±0.02	29.1±0.6	38.7±0.6	45.1±0.8
1.0	1.13±0.02	24.7±0.9	32±2	35±2
2.3	0.85±0.03	18.8±0.7	25±1	27±1

*5 replications, RSD < 6%

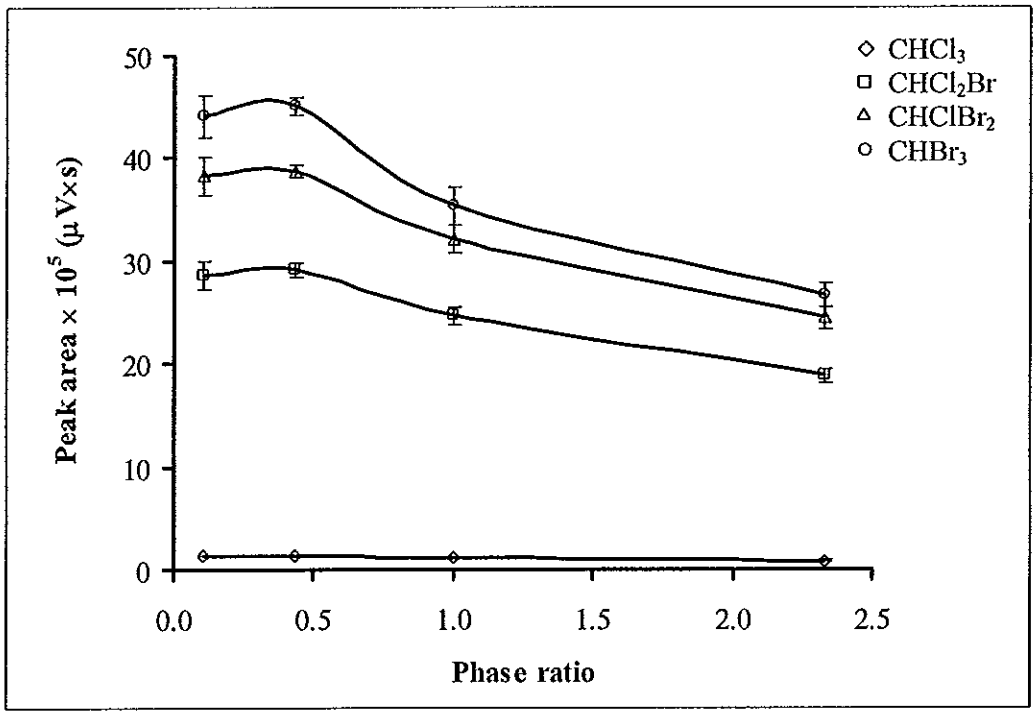


Figure 3.25 Effect of phase ratio on the extraction efficiency of 10.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

3.10.3 Stirring rate

Headspace single-drop microextraction is base on an equilibrium distribution process; the maximum amount of analytes will be extracted at the equilibrium conditions between three phases. Stirring of the solution can improve mass transfer in the aqueous phase and induces the convection in the headspace where equilibrium between the aqueous and vapor phases can be achieved more rapidly. So, sample stirring reduces the time required to reach the equilibrium and extraction time by enhancing the diffusion of the analytes towards the microdrop, especially for higher molecular mass analytes (Shariati-Feizabadi *et al.*, 2003).

Base on the penetration theory of mass transfer of solute, the aqueous phase mass-transfer coefficient (β_{aq}) is given by

$$\beta_{aq} = 2\left(\frac{D_{aq}}{\pi t_e}\right)^{\frac{1}{2}} \tag{3.19}$$

Where D_{aq} is the diffusion coefficient in the aqueous phase, and t_e the exposure time of a small fluid volume element of one phase momentarily in contact with the other phase. According to the theory, β_{aq} increase with increasing stirring rate because at a faster stirring rate value of t_e is smaller (Zhao *et al.*, 2004).

The effect of stirring rate on the extraction efficiency was studied in the range 500 to 1,250 rpm in 2.8.3. As shown in Table 3.32 and Figure 3.26, the highest extraction efficiency was obtained at the stirring rate of 1,000 rpm, after that the response of analytes decreased because the drop was unstable at higher stirring rate. Therefore, the optimum stirring rate was 1,000 rpm.

Table 3.32 Effect of stirring rate on the extraction efficiency of 10.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

Stirring rate (rpm)	Peak area × 10^5 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
500	2.64±0.08	46.3±0.5	45.3±0.7	41.1±0.8
700	2.57±0.06	46±1	45.4±0.5	44±2
1,000	2.85±0.04	52±1	52±2	51±1
1,250	2.7±0.2	49±4	48±4	43±3

*5 replications, RSD < 9%

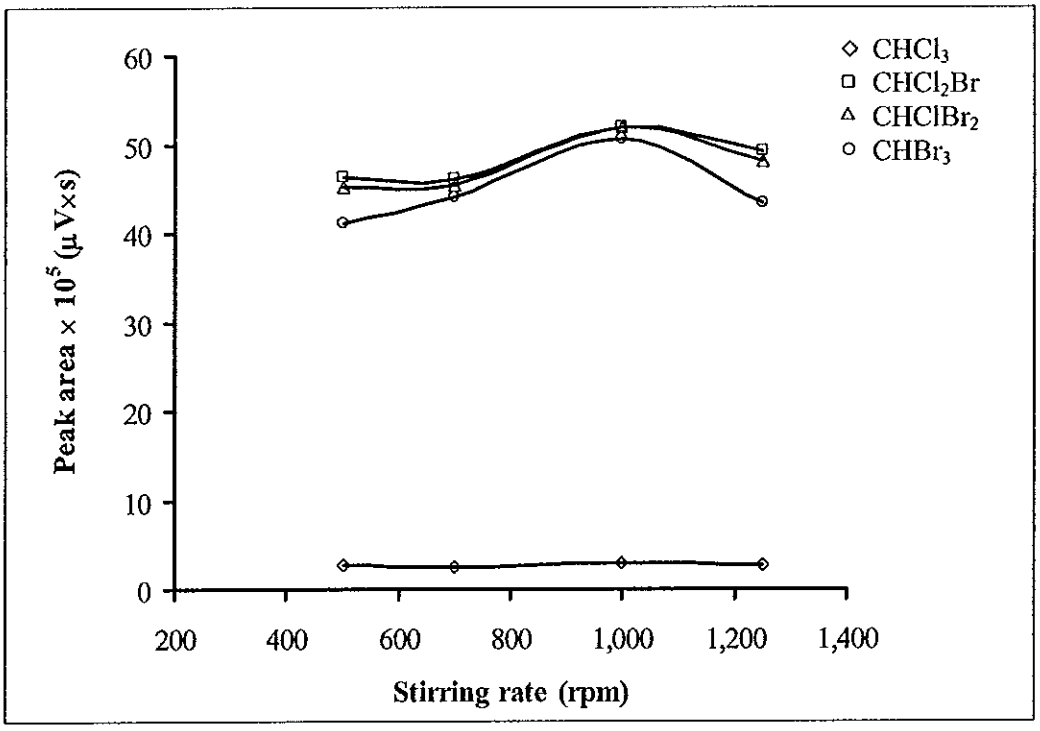


Figure 3.26 Effect of stirring rate on the extraction efficiency of 10.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

3.10.4 Salting-out effect

The addition of salt into the sample can increase ionic strength of the solution resulting in the decreasing of the solubility of target analytes in aqueous sample and enhancing their partition into an organic drop (salting-out effect). Ionic strength of a solution is the concentration of ions in the molar concentrations (C_i) and charges of each ion (Z_i), and the sum is over all ions in the solution (Maron and Prutton, 1969).

$$I = 0.5\sum\left(C_i Z_i^2\right) \tag{3.20}$$

According to equation 3.20, the ionic strength depends on the molar concentrations and charges of each ion. In this work, type of salt and concentrations of salt was evaluated for their extraction efficiency.

3.10.4.1 Type of salt

Four types of salt *i.e.* sodium chloride, potassium chloride, sodium sulphate, and sodium carbonate were investigated. The results show that sodium carbonate with the highest ionic strength gave the highest response (Table 3.33 and Figure 3.27). Thus, the sodium carbonate was selected as the optimum salt.

Table 3.33 Effect of type of salt on the extraction efficiency of 5.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

Type of salt	Peak area × 10^5 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
NaCl	4.7±0.3	89±7	74±5	55±4
KCl	3.10±0.04	60±1	554±2	48±3
Na_2SO_4	5.6±0.7	136±14	108±13	83±9
Na_2CO_3	8.2±0.2	234±7	240±10	147±13

*5 replications

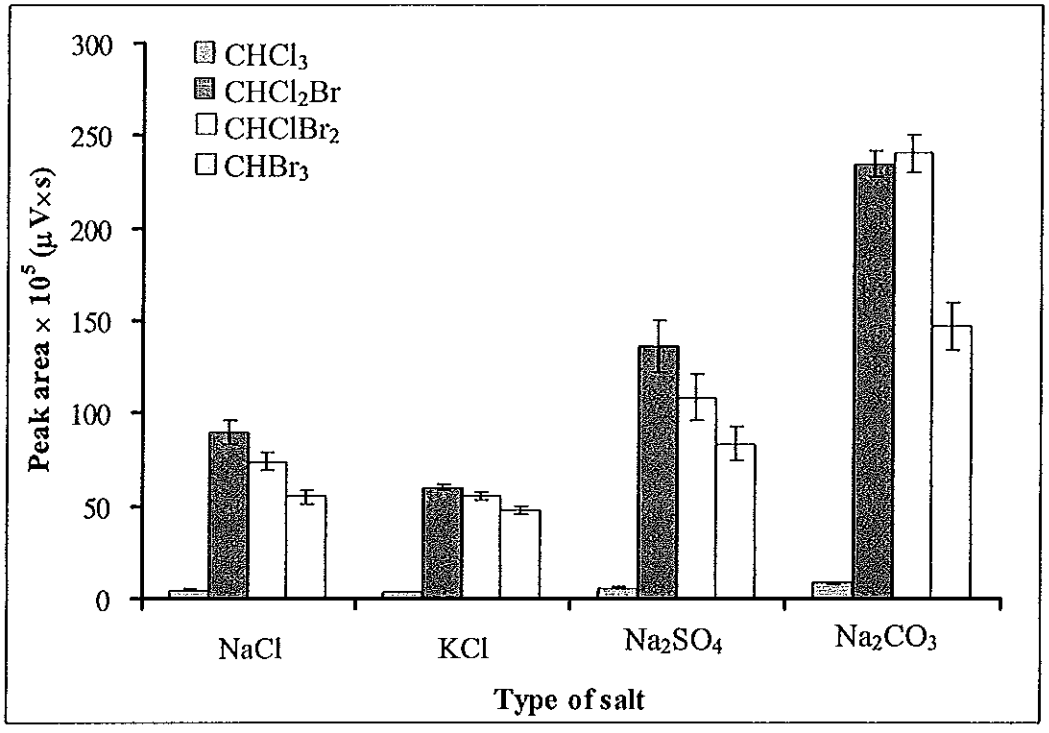


Figure 3.27 Effect of type of salts on the extraction efficiency of 5.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

3.10.4.2 Concentrations of salt

Concentrations of sodium carbonate (Na_2CO_3) were evaluated in 2.8.4.2. Results shown in Table 3.34 and Figure 3.28 indicated that the response signal increase when amount of salt increased until 0.3 g mL^{-1}, the responses tend to be constant. Hence, the amount of sodium carbonate at 0.3 g mL^{-1} was selected for salting out.

Table 3.34 Effect of concentration of sodium carbonate on the extraction efficiency of 5.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

Concentration of Na_2CO_3 (g mL^{-1})	**Peak area × 10^5 (μV×s)***			
	$CHCl_3$	**$CHCl_2Br$**	**$CHClBr_2$**	**$CHBr_3$**
0	1.47±0.02	13.0±0.6	14.3±0.5	8.2±0.3
0.1	3.3±0.1	30±2	34±1	19.8±0.9
0.2	5.2±0.3	43±3	46±5	25±1
0.3	6.8±0.4	62±2	59±5	28±2
0.4	5.9±0.7	61±5	56±5	30±3

*5 replications

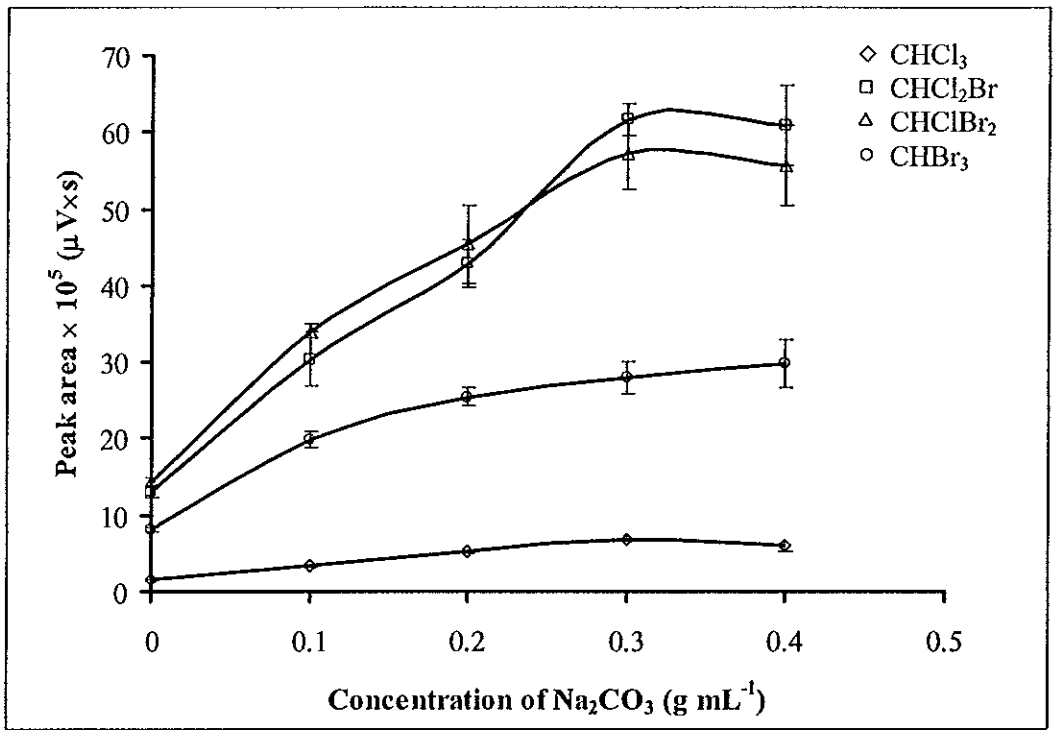


Figure 3.28 Effect of concentration of sodium carbonate on the extraction efficiency of 5.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

3.10.5 Organic drop volume

The amount of extracted analyte depends on the organic drop volume and the response signals are shown in Table 3.35 and Figure 3.29. The use of a large organic drop resulted in an increase analytical response. However, larger drops are difficult to manipulate and not reliable for the drop hanging. Thus, a microdrop volume of 2.0 μL was used as it ensured the formation of a stable and reproducible microdrop.

Table 3.35 Effect of organic drop volume on the extraction efficiency of 5.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

Organic drop volume (μL)	Peak area × 10^5 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
1.0	5.3±0.1	95±4	78±2	63±2
2.0	10.2±0.6	191±8	134±8	101±7
3.0	11±2	190±23	129±16	86±11
4.0	2.1±1.5	27±22	20±16	16±13

*5 replications

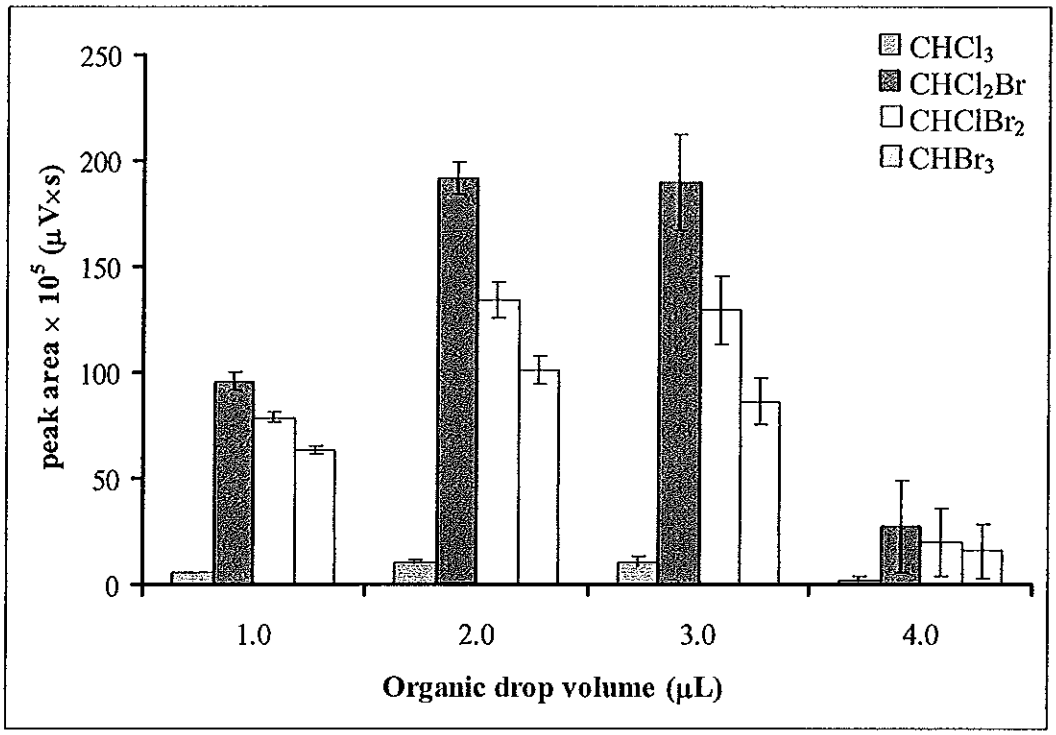


Figure 3.29 Effect of organic drop volume on the extraction efficiency of 5.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

3.10.6 Extraction time

The amount of the analyte transferred into the microdrop is expected to increase with increasing exposure time to the headspace of the sample. However, the HS-SDME is not an exhaustive extraction method and the analyte partitions among the sample phase, the headspace and the microdrop. Thus, the amount of the analyte transferred into the microdrop reaches its maximum when the equilibrium is established. The extraction time was investigated by varying the exposure time of the microdrop in the headspace of a sample from 1 to 10 minutes, and monitoring the analytical signal and the result are shown in Table 3.36 and Figure 3.30. There is an increase in response with sampling time between 1 and 5 minutes. The increase became much slower after that because it reached to the equilibrium of the analytes. Therefore, the extraction time of 5 minutes was selected as the optimal extraction time.

Table 3.36 Effect of extraction time on the extraction efficiency of 5.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

Extraction time (min)	Peak area $\times 10^5$ (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
1.0	7.3±0.1	97±6	43±3	18±1
3.0	10.5±0.6	182±13	90±6	30±2
5.0	11±1	195±15	114±9	40±3
7.5	11±0.6	197±14	105±7	39±2
10.0	11.2±0.4	200±13	109±14	40±3

*5 replications, RSD < 11%

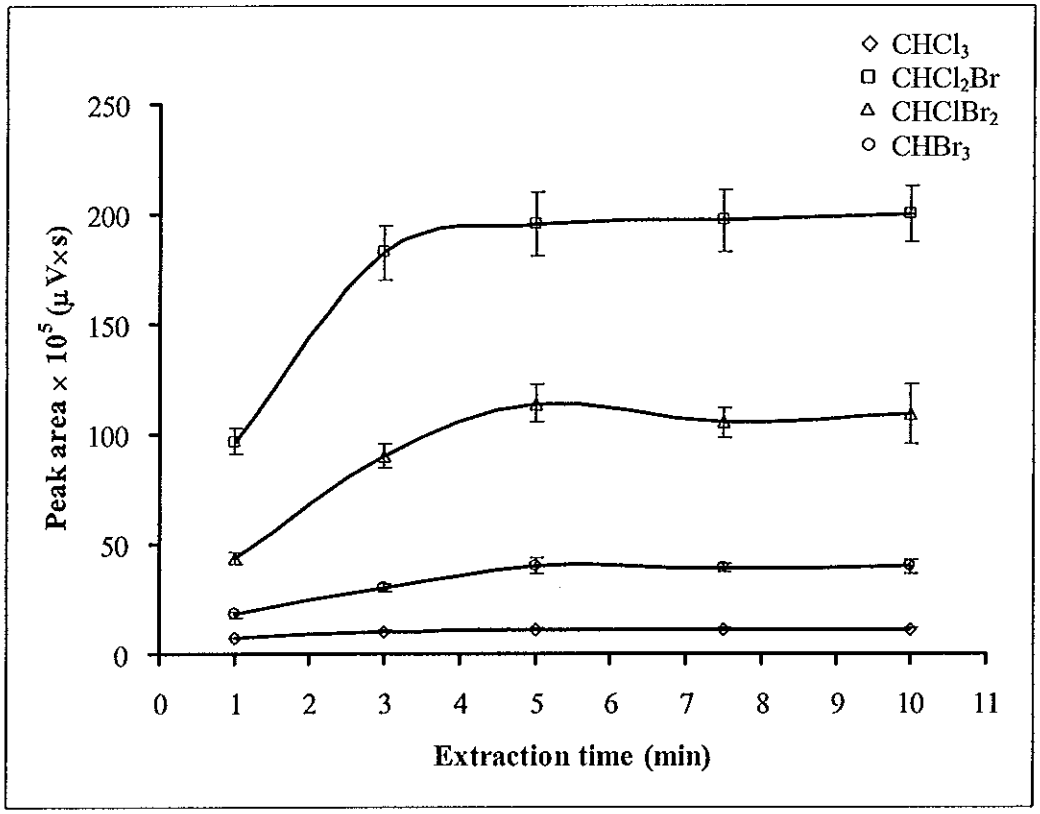


Figure 3.30 Effect of extraction time on the extraction efficiency of 5.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

3.10.7 Extraction temperature

To find a reasonable extraction temperature, the temperature of a heating block was calibrated using thermometer to obtain the correct temperature. The results are shown in Table 3.37 and Figure 3.31.

Table 3.37 Relationship between the set temperature and the actual temperature of the heating block. (*in most cases all measurements provided standard deviation less than 1, in this case we record the least count).

Set temperature (°C)	Correct temperature ± least count*,**
16	26±1
20	30±1
26	35±1
31	40±1
36	45±1
41	50±1
52	60±1
62	70±1

**10 replications, RSD < 4%

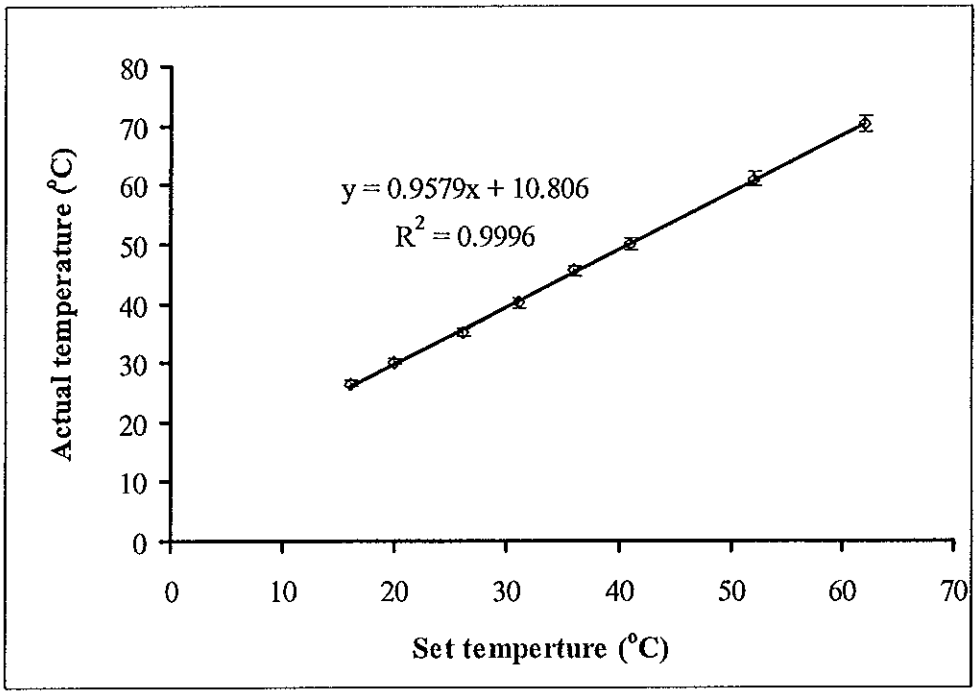


Figure 3.31 Set temperature *versus* actual temperature of the heating block.

Extraction temperature was investigated using the heating block as describe in 2.8.7. The results shown in Table 3.38 and Figure 3.32 indicated that when temperatures increased the response of the analytes decreased. This can be explained that when the extraction temperature increased, the diffusion coefficients of the analytes also increased. This can improve the extraction efficiency. However, by increasing the extraction temperature, the partition coefficients of the analytes between the microdrop and the headspace (K_{oh}) decreased. In addition, a high extraction temperature can speed the evaporation of the solvent phase (Fang *et al.*, 2006). This decreased the efficiency of extraction. Therefore, a temperature of 26 °C (room temperature) was selected for the extraction.

Table 3.38 Effect of extraction temperature on the extraction efficiency of 5.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

Extraction temperature (^{0}C)	Peak area × 10^5 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
26	9.4±0.3	143±5	85±4	31±2
30	9.6±0.4	127±7	69±3	26±1
40	9.5±0.6	127±11	79±5	33±3
50	6.9±0.7	81±10	57±5	28±4

*5 replications

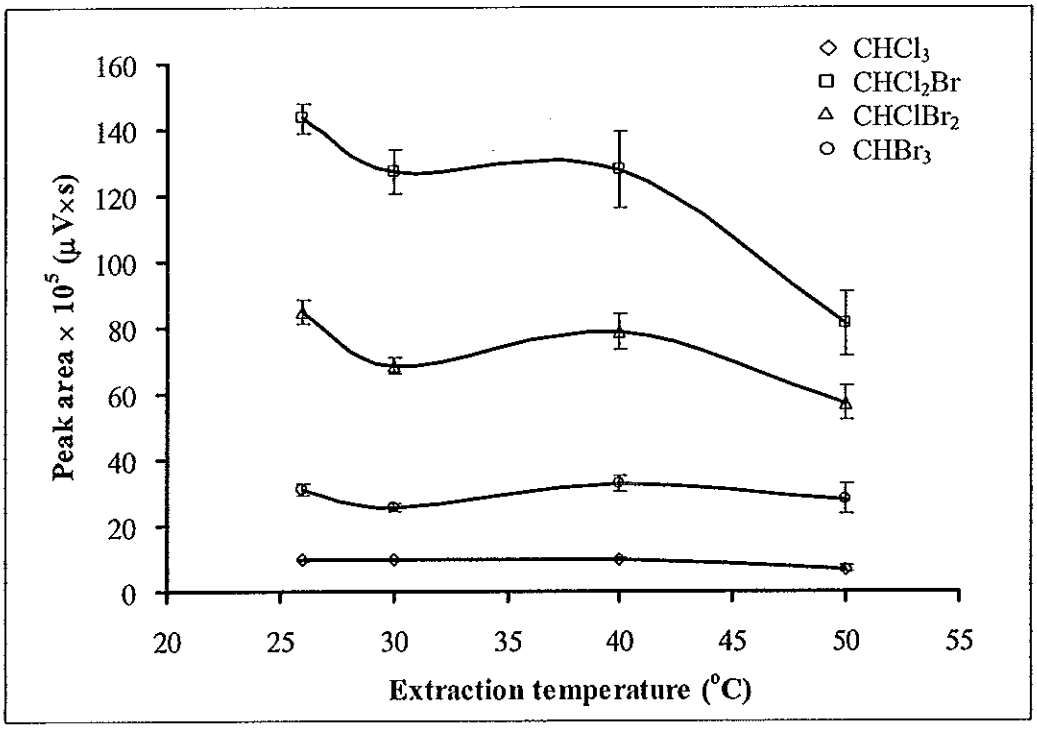


Figure 3.32 Effect of extraction temperature on the extraction efficiency of 5.0 μg L^{-1} trihalomethanes obtained from HS-SDME.

3.10.8 Summary of headspace single-drop microextraction (HS-SDME) conditions

The optimum conditions of headspace single-drop microextraction (HS-SDME) for the analysis of trihalomethanes in water are summarized in Table 3.39. The chromatograms obtained by these sample preparation conditions (Figure 3.33).

Table 3.39 Optimum conditions of HS-SDME for the trihalomethanes analysis.

Parameters	Optimum values
Organic solvent	1-octanol
Phase ratio	0.4
Stirring rate	1,000 rpm
Salting-out effect	
- Type of salt	Na_2CO_3
- Concentration of salt	0.3 g mL^{-1}
Organic drop volume	2 μL
Extraction time	5 minutes
Extraction temperature	26 °C (room temperature)

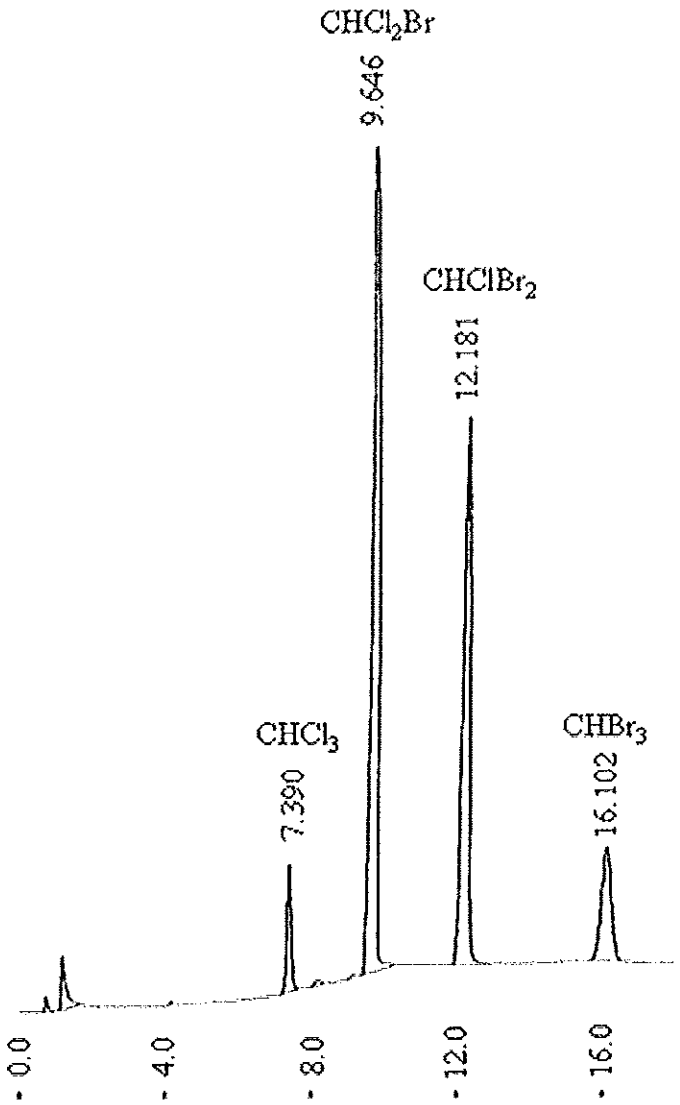


Figure 3.33 Chromatogram of trihalomethanes from 1 μg L^{-1} standard solution at optimum conditions of HS-SDME-GC-ECD technique.

3.11 Linear dynamic range (LDR)

The dynamic range of HS-SDME technique was investigated by serial dilutions of a stock standard solution as describe in the experiment 2.9. Linearity is achieved when the coefficient of determination (R^2) is equal or greater than 0.99 (FDA, 2000). Table 3.40 and Figure 3.34 showed the response of trihalomethanes at various concentrations.

Table 3.40 Response of trihalomethanes at various concentrations

Concentration (μg L^{-1})	Peak area × 10^5 (μV×s)*			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
0.005**	0.16 ±0.01	0.10±0.08	0.10 ±0.06	0.1±0.1
0.01	0.17±0.09	0.11±0.06	0.11±0.06	0.14±0.01
0.05	0.20±0.05	0.48±0.06	0.5±0.2	0.2±0.1
0.1	0.3 ±0.1	1.5±0.2	1.0±0.1	0.6±0.2
0.5	1.09±0.08	18 ±1	7.9±0.1	2.8±0.7
1	2.5±0.3	39±3	17±2	5.6±0.8
5	14.5±0.2	220±8	124±6	47 ±4
10	30±3	328±10	292±15	98 ±10
15	45±4	352±4	339 ±19	139±10
20	65±4	-	352±5	188±8
25	91±5	-	-	211±13
30	138 ±9	-	-	225 ±12

* 5 replications, RSD < 15%,

**5 replications, RSD < 20%

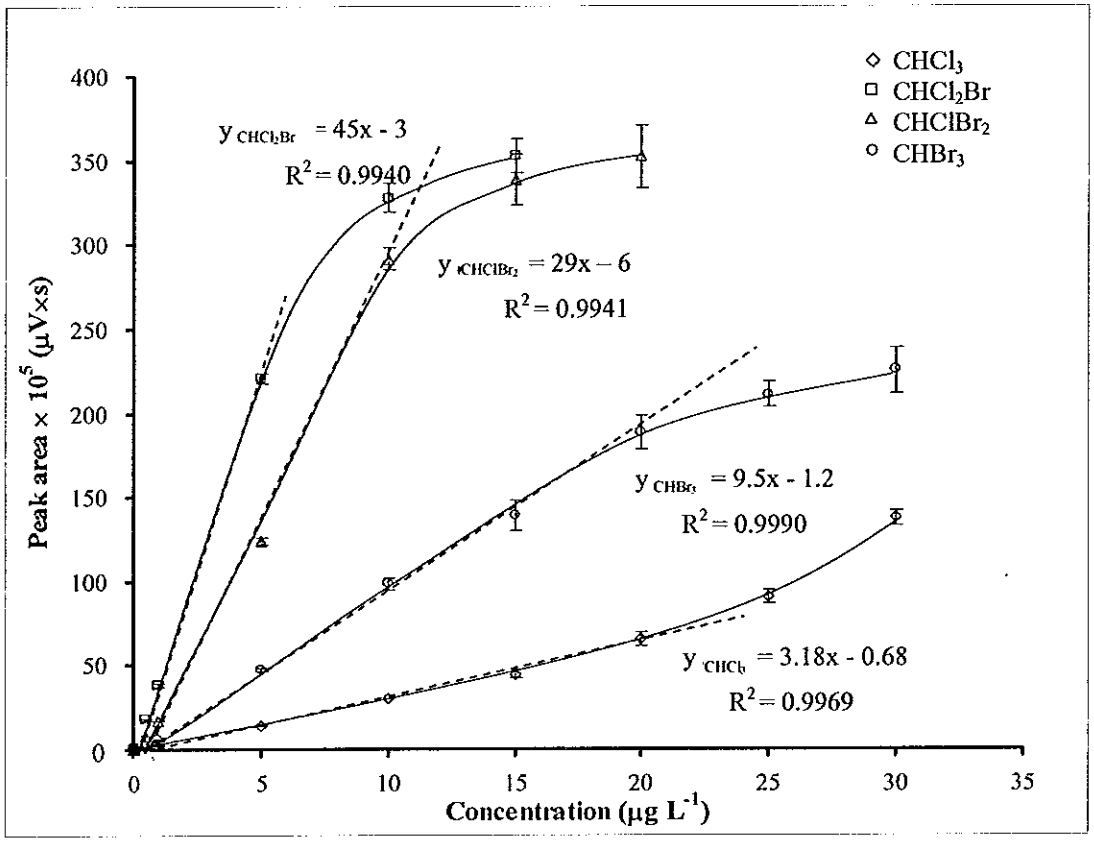


Figure 3.34 Linear dynamic ranges of trihalomethanes by HS-SDME-GC-ECD technique.

The system provided a linear dynamic range from 0.05 to 20 μg L^{-1} for $CHCl_3$, 0.05 to 5 μg L^{-1} for $CHCl_2Br$, 0.01 to 10 μg L^{-1} for $CHClBr_2$, and 0.01 to 20 μg L^{-1} for $CHBr_3$, with $R^2 > 0.99$ and the relative standard deviations (RSD) lower than 15%. When comparing between SHS-GC-ECD and HS-SDME, the SHS-GC-ECD provided better linear range than HS-SDME-GC-ECD for all analytes (Table 3.41).

Table 3.41 Comparison between linear dynamic range of SHS-GC-ECD and HS-SDME-GC-ECD.

Compounds	Linear dynamic rang ($\mu g\ L^{-1}$)	
	SHS-GC-ECD	**HS-SDME-GC-ECD**
$CHCl_3$	0.5-75	0.05-20
$CHCl_2Br$	0.1-60	0.05-5
$CHClBr_2$	0.1-70	0.01-10
$CHBr_3$	0.1-180	0.01-20

3.12 Limit of detection (LOD)

The limit of detection of HS-SDME technique was investigated by using the IUPAC method (Long and Winefordner, 1983). From equation 3.13 and the data in Table 3.42, limit of detections for $CHCl_3$, $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$ were calculated and are shown in Table 43. When comparing between SHS-GC-ECD and HS-SDME-GC-ECD, the HS-SDME-GC-ECD provided better LODs than SHS-GC-ECD for all analytes (Table 3.43).

Table 3.42 The maximum response of 20 blank injections.

Retention time (t_R, min)	Maximum response × 10^4 (μV×s)
7.36	3.69
7.37	3.96
7.38	4.40
7.37	3.99
7.38	4.34
7.36	4.02
7.37	4.06
7.36	3.46
7.35	2.83
7.36	4.01
7.36	3.37
7.36	3.82
7.36	4.01
7.35	3.36
7.36	4.13
7.36	4.34
7.36	3.37
7.36	4.34
7.36	3.37
7.35	3.82
$\bar{x}$	**3.83**
S^2	**0.42**

Table 3.43 Comparison between limit of detection of SHS-GC-ECD and HS-SDME-GC-ECD.

Compounds	Limit of detection (LOD, μg L^{-1})		$\frac{LOD_{(SHS\text{-}GC\text{-}ECD)}}{LOD_{(HS\text{-}SDME\text{-}GC\text{-}ECD)}}$
	SHS-GC-ECD	HS-SDME-GC-ECD	
$CHCl_3$	0.22	4.00×10^{-2}	5.50
$CHCl_2Br$	0.04	2.85×10^{-3}	14.04
$CHClBr_2$	0.06	4.39×10^{-3}	13.67
$CHBr_3$	0.35	1.34×10^{-2}	26.12

3.13 Limit of quantitation (LOQ)

The limit of quantitation (LOQ) is regarded as the lower limit for precise quantitative measurements (Corley, 2002). The LOQ was calculated as the analyte concentration giving a 10 times of standard deviation of the response. The LOQs of trihalomethanes are shown in Table 3.44. The LOQ ranged from 9.50×10^{-3} to 1.33×10^{-1} μg L^{-1}.

Table 3.44 Limit of quantitation (LOQ) of trihalomethanes

Compounds	Limit of quantitation (LOQ) (μg L^{-1})
$CHCl_3$	1.33×10^{-1}
$CHCl_2Br$	9.50×10^{-3}
$CHClBr_2$	1.46×10^{-2}
$CHBr_3$	4.46×10^{-2}

3.14 Matrix interference

Matrix effect of this method was evaluated in tap water and drinking water samples. The results are shown in Tables 3.45-3.48 and Figures 3.35-3.38. When the slope of standard curve and the spiked sample calibration curves were compared, standard curve and drinking water matrix-matched calibration curve have parallel regression lines but tap water matrix-matched calibration curve do not have parallel regression lines, which indicated that the matrix effect are not present in drinking water but it are present in tap water samples on trihalomethanes analysis.

Table 3.45 Effect of matrix on the responses of $CHCl_3$ in drinking water and tap water samples.

Concentration of $CHCl_3$ ($\mu g\ L^{-1}$)	**Peak area $\times 10^5$ ($\mu V \times s$)*±SD**		
	Standard $CHCl_3$	**Drinking water**	**Tap water**
0.1	0.26±0.04	0.4±0.1	0.11± 0.05
0.5	1.1±0.1	1.0±0.3	1.0±0.2
1	2.5±0.2	2.4±0.4	2.1±0.3
3	8.6±0.6	8.6±0.5	7.5±0.7
4	11.7±0.5	11.4±0.6	9.5±0.8

*5 replications, RSD ≤ 10%

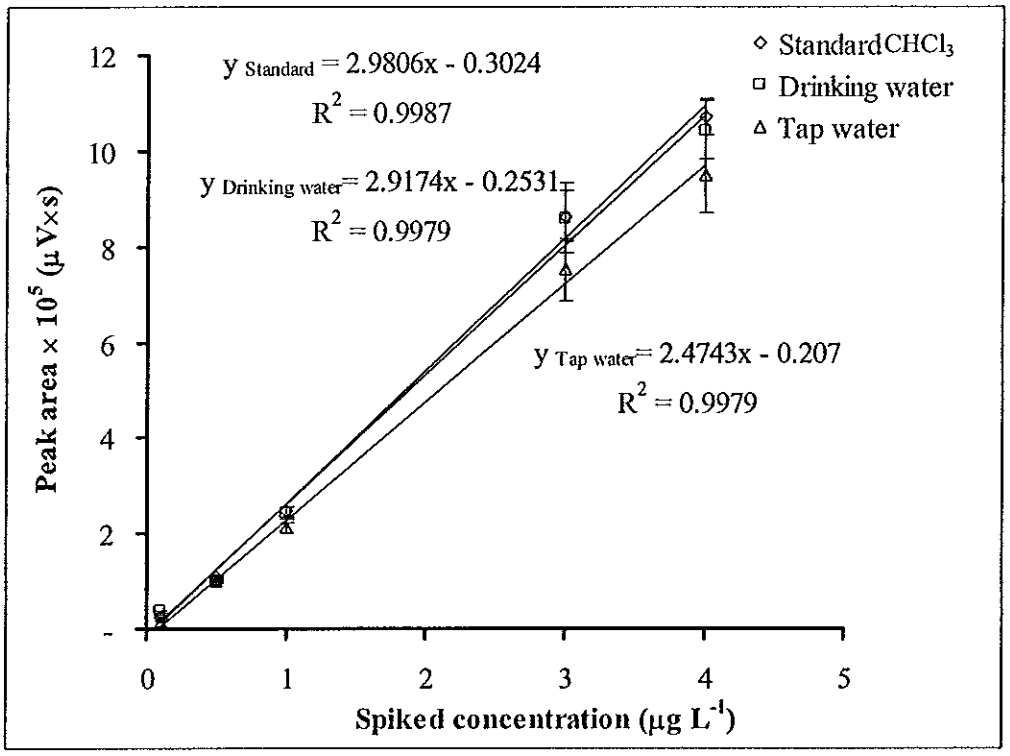


Figure 3.35 Matrix-matched calibration curve of $CHCl_3$ of drinking water and tap water samples.

Table 3.46 Effect of matrix on the responses of $CHCl_2Br$ in drinking water and tap water samples.

Concentration of $CHCl_2Br$ ($\mu g\ L^{-1}$)	Peak area × 10^5 (μV×s)*±SD		
	Standard $CHCl_2Br$	Drinking water	Tap water
0.1	3.3±0.2	2.9±0.1	3.2± 0.2
0.5	16.6±0.6	16.8±0.9	14±2
1	39±2	39±2	37±2
3	130±9	136±5	111±7
4	170±5	169±4	148±6

*5 replications, RSD ≤ 14%.

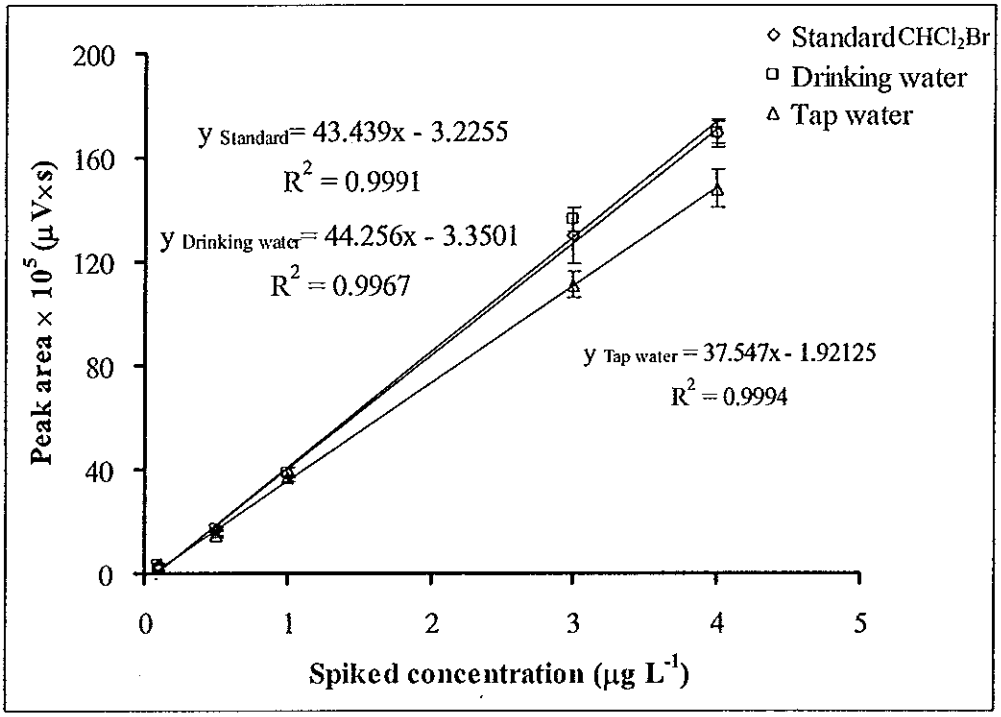


Figure 3.36 Matrix-matched calibration curve of $CHCl_2Br$ of drinking water and tap water samples.

Table 3.47 Effect of matrix on the responses of $CHClBr_2$ in drinking water and tap water samples.

Concentration of $CHClBr_2$ (μg L^{-1})	Response × 10^5 (μV*s)*±SD		
	Standard $CHClBr_2$	Drinking water	Tap water
0.1	1.4±0.2	1.33±0.02	1.14±0.03
0.5	7.2±0.6	7.1±0.3	6.9±0.6
1	16±2	16± 0.8	14±1
3	74±6	73±0.8	65±4
4	95±5	94±4	85± 4

*5 replications, RSD ≤ 12%

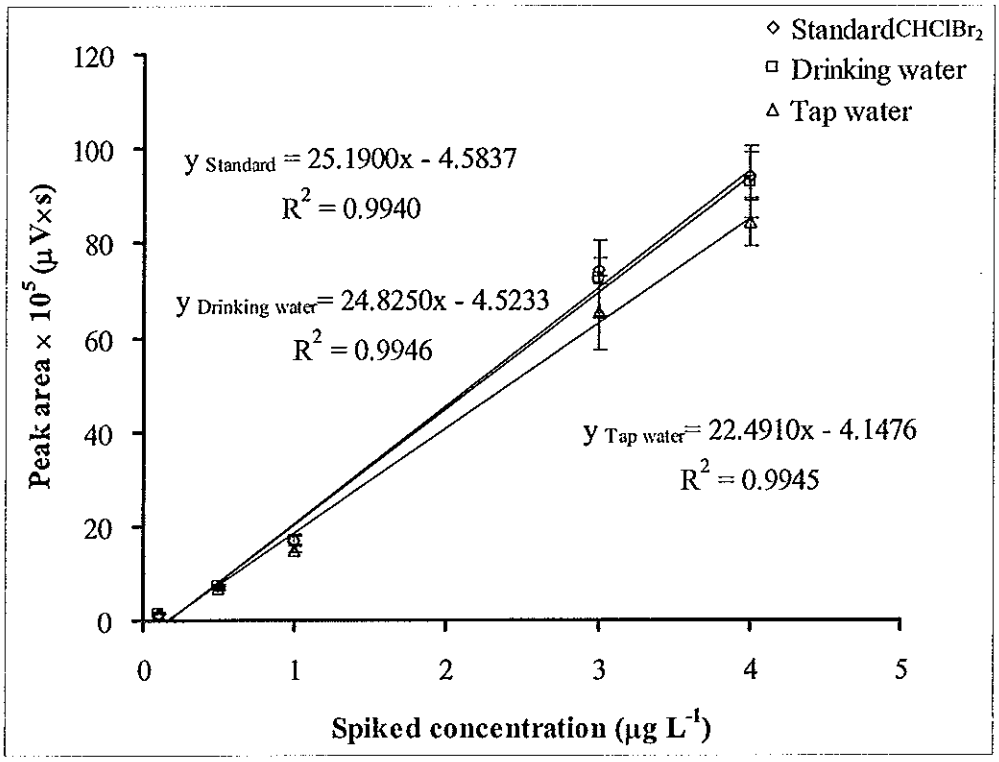


Figure 3.37 Matrix-matched calibration curve of $CHClBr_2$ of drinking water and tap water samples.

Table 3.48 Effect of matrix on the responses of $CHBr_3$ in drinking water and tap water samples.

Concentration of $CHBr_3$ (μg L^{-1})	Peak area × 10^5 (μV×s)*±SD		
	Standard $CHBr_3$	Drinking water	Tap water
0.1	0.22±0.06	0.21±0.02	0.23±0.06
0.5	2.7±0.2	2.7±0.1	2.6±0.2
1	5.5±0.2	5.3±0.2	5.2±0.2
3	23±2	22±2	19.5±0.8
4	29±2	28±0.5	26±1

*5 replications, RSD ≤ 10%

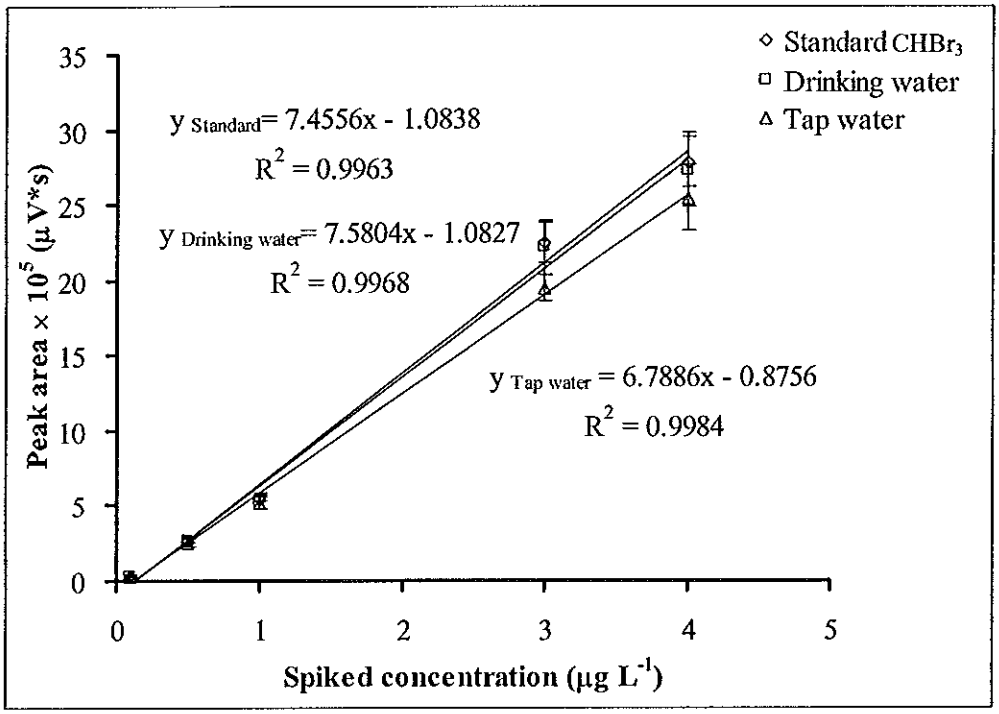


Figure 3.38 Matrix-matched calibration curve of $CHBr_3$ of drinking water and tap water samples.

3.15 Comparison between the slopes of standard and matrix-matched calibration curve.

The results from significant tests of the comparison of the calibration curves are shown in Tables 3.49-3.52.

Where

D_f is degree of freedom, it refers to the number of independent deviation, $D_f = n$-1 (n is the number of concentration = 5-1 = 4)

Sum Sq is sum square; it refers to an interim quantity used in the calculation of an estimate of the population variance

Mean Sq is mean square, it refers to a sum of squared terms divided by the number of degrees of freedom

F is ratio of two variances

P is probability

Table 3.49 Statistical values for the comparison between the slopes of $CHCl_3$ Standard curve and matrix-matched calibration curve of drinking water and tap water samples using two-way ANOVA by R software.

Matrix	*Df*	Sum Sq	Mean Sq	*F*	*P*
drinking water	4	1.59×10^{10}	3.98×10^{9}	1.5653	0.2022
Tap water	4	2.38×10^{11}	5.94×10^{10}	4.7732	0.0030 **

Table 3.50 Statistical values for the comparison between the slopes of $CHCl_2Br$ standard curve and matrix-matched calibration curve of drinking water and tap water samples using two-way ANOVA by R software.

Matrix	*Df*	Sum Sq	Mean Sq	*F*	*P*
Well water	4	4.70×10^{13}	1.57×10^{13}	2.1087	0.1135
Tap water	4	1.03×10^{13}	2.59×10^{12}	12.0410	1.6280×10^{-6} ***

Table 3.51 Statistical values for the comparison between the slopes of $CHClBr_2$ standard curve and matrix-matched calibration curve of drinking water and tap water samples using two-way ANOVA by R software.

Matrix	*Df*	Sum Sq	Mean Sq	*F*	*P*
Drinking water	4	6.5995×10^{11}	1.6499×10^{11}	1.1361	0.3534
Tap water	4	2.1708×10^{12}	5.4269×10^{11}	27.4560	5.300×10^{-11} ***

Table 3.52 Statistical values for the comparison between the slopes of $CHBr_3$ standard curve and matrix-matched calibration curve of drinking water and tap water samples using two-way ANOVA by R software.

Matrix	*Df*	Sum Sq	Mean Sq	*F*	*P*
Drinking water	4	5.44×10^{10}	1.36×10^{10}	1.2952	0.2882
Tap water	4	2.04×10^{11}	5.11×10^{10}	4.1808	0.0064 **

Significant codes: '*' ($\alpha = 0.01$), '**' ($\alpha = 0.05$), '***' ($\alpha = 0.001$)

From the results of the interaction between responses and concentrations in standard and matrix groups, it can be concluded that there is no significant difference between the slopes of regression line between drinking water group of standard curve and drinking water matrix-matched calibration curve at all level. Thus, the standard calibration curve could be used as an accurate determination of the unknown concentration. In case of tap water, the slope of regression line of standard curve and matrix-matched calibration curve were significantly different at various levels of significance as shown in Table 3.53. Thus, the matrix-matched calibration curve is required to an accuratly determine the unknown concentration (Eurachem, 1998; Roper *et al.*, 2001).

Table 3.53 Level of significance (*P* value) from ANOVA for the comparison between the slopes of standard curve and matrix-matched calibration curve of tap water samples.

Trihalomethanes	*P*
$CHCl_3$	0.05
$CHCl_2Br$	0.001
$CHCl_2Br$	0.001
$CHBr_3$	0.05

3.16 Method validation

Accuracy, in terms of mean recovery, and precision, in term of %RSD, were assessed by replicated analyses of samples, spiked at 3 different concentrations within the calibration range. Recoveries were obtained, ranged from 91 to 115%, 85 to 108% and 89 to 110% for trihalomethanes at spiked concentration 0.1, 0.5, and 3 μg L^{-1} respectively. The RSDs were ranged from 5 to 11 % (Tables 3.54-3.56). The accuracy and precision data demonstrated that the method meets EPA method 8061 and 8000 regulatory method requirement of 70-120 recovery and a RSD less than 20%. The results indicated that the headspace single-drop microextraction method followed by gas chromatography can be used for the extraction of trihalomethanes from water sample with high recoveries and excellent precision.

Table 3.54 Recovery of trihalomethanes of water samples at spiked concentration of 0.1 μg L^{-1}.

Samples	Recovery* (%) ± SD			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
Drinking water	106±8	101±8	99±6	99±5
Tap water	115±13	104±9	98±5	91±5

*5 replications, RSD < 11%

Table 3.55 Recovery of trihalomethanes of water samples at spiked concentration of 0.5 μg L^{-1}.

Samples	Recovery* (%) ± SD			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
Drinking water	103±10	102±8	99±8	91±6
Tap water	108±9	97±7	98±9	85±5

*5 replications, RSD < 10%

Table 3.56 Recovery of trihalomethanes of water samples at spiked concentration of 3.0 μg L^{-1}.

Samples	Recovery* (%) ± SD			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
Drinking water	104±5	94±5	97±8	97±5
Tap water	110±12	91±5	89±7	89±8

*5 replications, RSD < 11%

3.17 Qualitative and quantitative analysis of trihalomethanes in water samples

3.17.1 Qualitative analysis

The optimum conditions of HS-SDME-GC-ECD were used to analyse trihalomethanes in water samples. The average t_R of $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$ were 7.37±0.06, 9.66±0.08, 12.13±0.11 and 15.96±0.16 minutes, respectively.

3.17.2 Quantitative analysis

Quantitative analyses of trihalomethanes were done by considering the response, *i.e.,* peak area, which was related to the concentration of the analytes. Two types of water samples *i.e.,* drinking water, and tap water were sampling and analysed by HS-SDME-GC-ECD at optimum conditions and the representative chromatogram of water sample are shown in Figures 3.39.

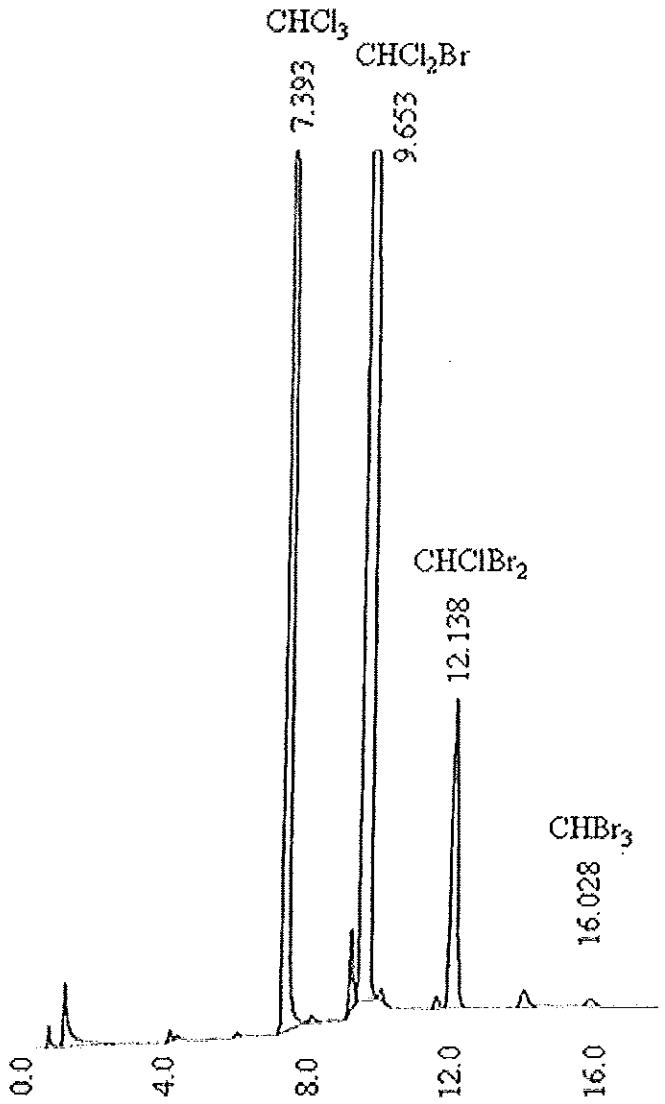


Figure 3.39 HS-SDME-GC-ECD chromatogram of trihalomethanes from tap water sample.

Trihalomethanes concentrations in drinking water were evaluated by using the calibration curve and tap water were evaluated by using the matrix-matched calibration curve. The results in Table 3.57 show that the concentrations of total trihalomethanes in drinking water samples were in the range from ND to 12.9±0.9 μg L^{-1} and tap water samples were in the range from 15±2 to 25±2 μg L^{-1}. These do not exceed the World Health Organization (WHO) guideline values of

trihalomethanes in drinking water where the maximum contaminant levels (MCLs) for $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$ are limited at 200, 60, 100, and 100 μg L^{-1}, respectively and the sum of ratio of concentration of each to its respective guideline value do not exceed 1 (WHO, 2004), and also not exceed the US EPA and EU guideline values of 80 (US EPA, 2001) and 150 μg L^{-1} (EU, 1998), respectively.

Table 3.57 Trihalomethanes concentrations of drinking water sample determined by calibration curves and tap water sample determined by matrix-matched calibration curve.

Samples	Concentration of trihalomethanes (μg L^{-1})*					THMs ratio**
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$	Total THMs	
Drinking water 1	ND	ND	ND	ND	ND	-
Drinking water 2	ND	ND	ND	ND	ND	-
Drinking water 3	1.4±0.2	ND	ND	ND	1.4±0.2	0.01
Drinking water 4	1.6±0.4	ND	ND	ND	1.6±0.4	0.01
Drinking water 5	11.4±0.8	1.12±0.06	0.33±0.03	0.05±0.01	12.9±0.9	0.08
Tap water 1	13±2	1.2±0.1	0.95±0.01	0.17±0.01	15±2	0.10
Tap water 2	16±2	7.1±0.2	1.5±0.1	0.52±0.07	25±2	0.22

*5 replications, RSD ≤ 15%, ND= Not detectable

** The sum of ratio of concentration of each to its respective guideline value.

3.18 Comparison between static headspaces (SHS) and headspace single-drop microextraction (HS-SDME).

Static headspaces (SHS) and headspace single-drop microextraction (HS-SDME) techniques provided linear dynamic range (LDR), limit of detection (LOD), limit of quantitation (LOQ) and relative standard deviation (%RSD) as shown in Table 3.58.

Table 3.58 Analytical performance data of static headspaces (SHS) and headspace single-drop microextraction (HS-SDME) techniques.

Compound	LDR ($\mu g\ L^{-1}$)		LOD ($\mu g\ L^{-1}$)		LOQ ($\mu g\ L^{-1}$)		%RSD	
	SHS	HS-SDME	SHS	HS-SDME	SHS	HS-SDME	SHS	HS-SDME
$CHCl_3$	0.5-75	0.05-20	0.22	4.00×10^{-2}	0.73	1.33×10^{-1}	< 4	< 15
$CHCl_2Br$	0.1-60	0.05-5	0.04	2.85×10^{-3}	0.12	9.50×10^{-3}		
$CHClBr_2$	0.1-70	0.01-10	0.06	4.39×10^{-3}	0.19	1.46×10^{-2}		
$CHBr_3$	0.1-180	0.01-20	0.35	1.34×10^{-2}	1.17	4.46×10^{-2}		

Both static headspaces (SHS) and headspace single-drop microextraction (HS-SDME) are very simple, rapid, less toxic solvent consumtion (only 2 μL), high sensitivity and selectivity, and cost effective method. The HS-SDME gave higher sensitivity than SHS, which provided LODs were better than SHS in all analytes (5.50, 14.04, 13.67, and 26.12 times for $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$, respectively). However, the linear dynamic range and precision of SHS was better than HS-SDME and it require less manual.

CHAPTER 4

Conclusions

Trihalomethanes are the major categories of chlorinated disinfectant by-products detected in chlorinated drinking waters. There is a concern that the presence of trihalomethanes in water would affect humans health from the possible carcinogenic effects. Human are expose to these compound everyday though ingestion, inhalation and dermal absorption routes (Uyak, 2006). Thus, for water safety, these compounds must not present in water in unacceptable quantities and their detection becomes an important issue in water safety analysis.

This work investigated the analytical method for fast, high sensitive and selective of trace determination of trihalomethanes in water. The first part was the optimization of gas chromatograph couple with electron capture detector (GC-ECD) for the analysis of trihalomethanes in water using. Four major trihalomethanes were studied simultaneously, chloroform ($CHCl_3$), dichlorobromomethane ($CHCl_2Br$), dibromochloromethane ($CHClBr_2$), and bromoform ($CHBr_3$). Chromatographic separation was carried out by a glass column: 2 m x 2.0 mm i.d., 1% SP-1000 on Carbopack B, 60/80 mesh. The optimum conditions of GC-ECD technique were obtained as shown.

Conditions	Optimum values
Flow rate: N_2, carrier gas	20 mL min^{-1}
Column temperature program:	
-Initial temperature	80 °C
-Initial holding time	0 minutes
-Ramp rate temperature	10 °C min^{-1}
-Final temperature	180 °C
-Final holding time	7 minutes
Injector temperature	180 °C
Detector temperature	240 °C

These optimum conditions are appropriate for the simultaneous analysis of the trihalomethanes. It provided very good resolution (R ≥1) and selectivity within a short analysis time (≤17 minutes) which is better than earlier report by Kuivinen and Johnsson reported in 1999 where 20 minutes is required for the trihalomethanes analysis.

The second part was the sample preparation of sample preparation for trihalomethanes in water. Headspace (HS) techniques, both static headspaces (SHS) and headspace single-drop microextraction (HS-SDME) were used as sample preparation techniques. The static headspace (SHS) condition were optimized and the optimum values were obtained as shown.

Conditions	value
Equilibrium temperature	80 °C
Phase ratio	0.5
Equilibrium time	20 minutes

Combining static headspace with GC-ECD, this SHS-GC-ECD system provided a wide linear dynamics range from 0.5 to 75 μg L^{-1} for $CHCl_3$, 0.1 to 60 μg L^{-1} for $CHCl_2Br$, 0.1 to 70 μg L^{-1} for $CHClBr_2$, and 0.1 to 180 μg L^{-1} for $CHBr_3$, with $R^2 > 0.99$ and high precision *i.e.,* the relative standard deviations (RSD) lower than 4%. The limit of detections for $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$ were 0.22, 0.04, 0.06, and 0.35 μg L^{-1}, respectively, which indicated that this technique is sensitive enough to detect trace amount of trihalomethanes in water. When comparing the LOD of this method with those reported (Table 4.1), it can be seen that this method gave lower LOD than others.

Table 4.1 LOD reported for the determination of trihalomethanes.

Analytical method	LOD (μg L^{-1})	Reference
SHS-GC-ECD	0.04-0.35	This work
DAI-GC-ECD	3.0-5.0	Golfinopoulos *et al.*, 2001
CMS-GC-ECD	0.3-0.5	Emmert *et al.*, 2004
DI-SDME-GC-ECD	0.23-0.45	Tor and Aydin, 2006
HS-SPME-GC-MS	0.078-0.52	San Juan *et al.*, 2007

The matrix interference was also studied and the statistically test indicated that the matrices of water samples (tap water and well water) do not affect the responses. Thus, the external calibration curve was used for determination of trihalomethanes in water samples. This method was validated to ensure the reliability of the results by using standards, spiked samples, reagent and method blanks. Validation parameters *i.e.,* precision, and recovery were studied. High recoveries were obtained in acceptable level (EPA method 8061, 1996), ranged from 95 to 108% and 89 to 105% for trihalomethanes at spiked concentration 0.5 and 10.0 μg L^{-1} with relative standard deviations (RSD) ranged from 4 and 11%. The limits of quantitation are ranged from 0.12 to 1.17 μg L^{-1}. The qualitative and quantitative analysis of trihalomethanes were performed with real water samples, well water, tap water, and drinking water were sampled from a local soft drink factory, the outlet in our laboratories and nearby sources, and local stores, respectively. The concentrations of total trihalomethanes in well water, tap water, and drinking water samples were in the range from 4.2±1 to 11±1 μg L^{-1}, 5.8±0.8 to 25±1 μg L^{-1}, and ND to 5±1 μg L^{-1}, respectively.

Subsequently, the headspace single-drop microextraction (HS-SDME) technique was investigated and the results were obtained as follows.

Conditions	Optimum values
Organic solvent	1-octanol
Phase ratio	0.4
Stirring rate	1,000 rpm
Salting-out effect:	
-Type of salt	Na_2CO_3
-Concentration of salt	0.3 g mL^{-1}
Organic drop volume	2 µL
Extraction time	5 minutes
Extraction temperature	26 °C (room temperature)

These optimum conditions are appropriate for analysis of trihalomethanes in water. They provide a wide linear dynamic range from 0.05 to 20 µg L^{-1} for $CHCl_3$, 0.05 to 5 µg L^{-1} for $CHCl_2Br$, 0.01 to 10 µg L^{-1} for $CHClBr_2$, and 0.01 to 20 µg L^{-1} for $CHBr_3$, with $R^2 > 0.99$ and the relative standard deviations (RSD) lower than 15%. The limit of detections for $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$ were 4.00×10^{-2}, 2.85×10^{-3}, 4.39×10^{-3} and 1.34×10^{-2} µg L^{-1}, respectively, which indicated that this technique is high sensitive to detect trace amount of trihalomethanes in water. When comparing the LOD of this method with those reported (Table 4.2), it can be seen that this method gave lower LOD.

Table 4.2 LOD reported for the determination of trihalomethanes.

Analytical method	**LOD (µg L^{-1})**	**Reference**
HS-SDME-GC-ECD	2.85×10^{-3}- 0.04	This work
SHS-GC-ECD	0.03-0.06	Kuivinen and Johnsson, 1999
HS-SPME-GC-MS	1.0-2.8	Stack *et al.*, 2000
Purge and trap-GC-ECD	0.025-0.05	Nikolaou *et al.*, 2002
HS-SDME-GC-ECD	0.15-0.4	Zhao *et al.*, 2004

The matrix interference was also studied and the statistically test in drinking water and tap water, indicated that the matrixes of water samples were not presented in drinking water. Thus, the external calibration curve was used for determination of trihalomethanes in this water sample. However, the matrices were presented in tap water, so the matrix-matched calibration curve was used in this case. The method was validated to ensure the reliability of the results by using standards, spiked samples, reagent and method blanks. Validation parameters *i.e.,* precision, and recovery were studied. High recoveries were obtained in acceptable level (EPA method 8061, 1996) ranged from 91 to 115%, 85 to 108% and 89 to 110% for trihalomethanes at spiked concentration 0.1, 0.5, and 3 $\mu g\ L^{-1}$ respectively. The RSDs ranged from 5 to 11 %. The limits of quantitation are ranged from 9.50×10^{-3} to 1.33×10^{-1} $\mu g\ L^{-1}$. The qualitative and quantitative analysis of trihalomethanes in water samples were studied in tap water and drinking water samples which was sampled from laboratories and nearby sources, and local stores, respectively. All water samples were analyzed and evaluated by using the calibration curve and the matrix-matched calibration curve. The concentrations of total trihalomethanes in tap water, and drinking water samples were in the range of 15±2 to 25±2 $\mu g\ L^{-1}$, and ND to 12.9±0.9 $\mu g\ L^{-1}$, respectively.

In summary both static headspace (SHS) and headspace single-drop microextraction (HS-SDME) techniques are very simple rapid, solvent free, high sensitivity and selectivity, and inexpensive. The HS-SDME gave higher sensitivity than SHS. However, HS-SDME required more manual operation. The precision of SHS was better than HS-SDME. The analytical performance data of static headspace (SHS) and headspace single-drop microextraction (HS-SDME) techniques are shown.

Compounds	LDR (μg L^{-1})		LOD (μg L^{-1})		LOQ (μg L^{-1})		%RSD	
	SHS	HS-SDME	SHS	HS-SDME	SHS	HS-SDME	SHS	HS-SDME
$CHCl_3$	0.5-75	0.05-20	0.22	4.00×10^{-2}	0.73	1.33×10^{-1}	< 4	< 15
$CHCl_2Br$	0.1-60	0.05-5	0.04	2.85×10^{-3}	0.12	9.50×10^{-3}		
$CHClBr_2$	0.1-70	0.01-10	0.06	4.39×10^{-3}	0.19	1.46×10^{-2}		
$CHBr_3$	0.1-180	0.01-20	0.35	1.34×10^{-2}	1.17	4.46×10^{-2}		

The determination of trihalomethanes in water by both techniques indicated that the concentrations of total trihalomethanes are ranged between ND to 25±2 μg L^{-1}. These do not exceed the World Health Organization (WHO) guideline values of trihalomethanes in drinking water where the maximum contaminant levels (MCLs) for $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$ are limited at 200, 60, 100, and 100 μg L^{-1}, respectively and the sum of ratio of concentration of each to its respective guideline value do not exceed 1 (WHO, 2004), and also not exceed the US EPA and EU guideline values of 80 (US EPA, 2001) and 150 μg L^{-1} (EU, 1998), respectively. The order of trihalomethanes contaminated in the water samples are $CHCl_3$ > $CHCl_2Br$ > $CHClBr_2$ > $CHBr_3$. The results showed that chloroform is a dominated species of trihalomethanes in water.

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List of Publications and Proceedings

Poster presentations

1. Rattanarungsi, W, Kanatharana, P. and Thavarungkul, P. "Speciation of Trihalomethanes (THMs) in Water by Headspace/Gas Chromatographic Technique" The 32nd Congress on Science and Technology of Thailand (STT2006), Queen Sirikit National Convention Center, Thailand. October 8-12, 2006.

2. Rattanarungsi, W, Kanatharana, P. and Thavarungkul, P. "Headspace-single drop microextraction (HS-SDME) for Trihalomethanes (THMs) Speciation in water" The 5th PERCH-CIC Annual Scientific Congress (PERCH-CIC Congress V), Pattaya, Thailand, May 6-9, 2007.