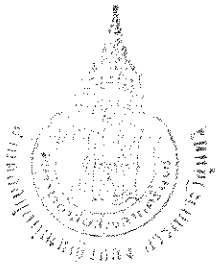
Development of Sample Preparation Techniques for the Analysis of Semivolatile Organic Compounds (SVOCs) in Water



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ชื่อวิทยานิพนธ์	การพัฒนาเทคนิคเตรียมตัวอย่างสำหรับวิเคราะห์สารประกอบอินทรีย์กึ่งระเหยง่ายในตัวอย่างน้ำ
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บทคัดย่อ

วิทยานิพนธ์นี้ศึกษาการพัฒนาเทคนิคการเตรียมตัวอย่างแบบง่าย โดยใช้วัสดุที่มีคุณสมบัติเลือกผ่านสำหรับการแยกสาร ได้แก่ ฮอลโลไฟเบอร์เมมเบรน (hollow fiber membrane) และวัสดุเหลือใช้ในห้องปฏิบัติการ ได้แก่ คาปิลาลีคอลัมน์ที่ใช้กับแก๊สโครมาโทกราฟ พัฒนาวัสดุดังกล่าวเป็นระบบการสกัดแบบอินไลน์ (in-line) โดยต่อพ่วงเข้ากับเครื่องแก๊สโครมาโทกราฟ-แมสสเปกโตรมิเตอร์ (gas chromatograph-mass spectrometer, GC-MS) เพื่อใช้วิเคราะห์สำหรับสารประกอบอินทรีย์กึ่งระเหยง่ายปริมาณน้อยที่ตกค้างในน้ำทั้งในเชิงคุณภาพและเชิงปริมาณ

เทคนิคแรกที่พัฒนาขึ้นเป็นวิธีการเตรียมตัวอย่างโดยอาศัยชุดเมมเบรน อินเลต (membrane inlet, MI) ที่ภายในระบบบรรจุด้วยฮอลโล ไฟเบอร์เมมเบรนต่อพ่วงกับ GC-MS เพื่อวิเคราะห์ตัวอย่างน้ำที่มีการปนเปื้อนด้วยสาร 4,4′-ดีดีที (4,4′- DDT) โดยฮอลโลไฟเบอร์เมมเบรน จะทำหน้าที่เป็นตัวกลางในการแยกสารอย่างจำเพาะเจาะจงโดยสกัดสาร 4,4′-DDT ออกจากตัวอย่างน้ำในขณะที่ตัวอย่างผ่านไปในชุด MI นอกจากนี้ได้ใช้ชุดกล่องทำความร้อนที่ประดิษฐ์ขึ้นเองในห้องปฏิบัติการเพื่อเพิ่มประสิทธิภาพของการแยกสารโดยกระบวนการเพอวาโพเรชัน (pervaporation) ก่อนที่สารจะเข้าสู่ GC-MS เพื่อทำการวิเคราะห์ในโหมดเลือกตรวจวัดจำเพาะไอออนหรือซิมโหมด (selected ion monitoring, SIM) จากการศึกษาพบว่าสภาวะที่เหมาะสมของเทคนิคการเตรียมตัวอย่างที่พัฒนานี้คือ อัตราการไหลของตัวอย่างและแก๊สพาที่ 6 และ 9 มิลลิลิตรต่อนาที ความร้อนที่ใช้ในการคายการดูดซับของสารจากเมมเบรนที่ 60 องศาเซลเซียส ทำให้เทคนิคนี้มีคุณสมบัติของการวิเคราะห์ที่ดีกล่าวคือ สามารถตรวจวัดสาร 4,4′-DDT ได้ที่ระดับความเข้มข้นต่ำถึง 90 ไมโครกรัมต่อลิตร มีช่วงความเป็นเส้นตรงอยู่ในช่วง 0.10 - 1.0 มิลลิกรัมต่อลิตร โดยให้ค่าสัมประสิทธิสหสัมพันธ์ (correlation coefficient, r) มากกว่า 0.99 และประสิทธิภาพของการสกัดตัวอย่างอยู่ในช่วง 83-94% และค่าเปอร์เซ็นต์ความเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า 10

เทคนิคการเตรียมตัวอย่างแบบคาปิลลารี ไมโครแอคเทร็คชัน (capillary microextraction) เป็นระบบการสกัดตัวอย่างอีกระบบหนึ่งที่พัฒนาขึ้น โดยมีวัตถุประสงค์จะนำคอลัมน์ทางโครมาโทกราฟีที่เหลือใช้มาทำเป็นอุปกรณ์สกัดสาร เพื่อสามารถวิเคราะห์สาร SVOCs หกชนิดที่ตกค้างในตัวอย่างน้ำได้พร้อมกัน เทคนิคนี้ใช้คอลัมน์ชนิดเอชพี-5 (HP-5) ยาว 100 เซนติเมตร ขนาด

เส้นผ่าศูนย์กลาง 0.32 มิลลิเมตร และความหนาของฟิลม์ 0.25 ไมโครเมตร มาต่อเข้ากับวาล์วสองตัว ซึ่งทำหน้าที่ในขั้นตอนป้อนตัวอย่าง สกัดตัวอย่าง ชะตัวอย่างและนำตัวอย่างที่ชะเข้าสู่เครื่อง GC-MS เพื่อวิเคราะห์ต่อไป โดยสารประกอบอินทรีย์กึ่งระเหยง่ายหกชนิดที่วิเคราะห์ เป็นสารที่จัดอยู่ในบัญชีรายชื่อของสารที่เป็นพิษได้แก่ เฮกซะคลอโรบิวตะไดอีน (hexachlorobutadiene) เดลดริน (dieldrin) 4,4′-ดีดีที (4,4′-DDT) เบนโซ[บี]ฟลูออแรนทีน (benzo[*b*]fluornathene) เบนโซ[เอ]ไพรีน (benzo[*a*]pyrene) และไดเบนโซ[เอ,เอช]แอนทราซีน (dibenzo[*a,h*]anthracene) จากการศึกษาพบว่าสภาวะที่เหมาะสมสำหรับเทคนิคการเตรียมตัวอย่างนี้ประกอบด้วยอัตราการไหลของตัวอย่าง 1.0 มิลลิลิตรต่อนาที ปริมาตรของตัวอย่าง 7 มิลลิลิตร ใช้ เอทานอล (5% โดยปริมาตร) เป็นสารปรับสภาวะ (organic modifier) และอะซีโตไนไตรล์เป็นตัวทำละลายในการชะ เทคนิคนี้สามารถตรวจวัดสารได้ระดับความเข้มข้นต่ำในช่วง 0.01 ถึง 0.1 นาโนกรัมต่อลิตร โดยมีช่วงความเป็นเส้นตรง 0.01 นาโนกรัมต่อลิตรถึง 1 มิลลิกรัมต่อลิตร โดยให้ค่าสัมประสิทธิสหสัมพันธ์ (correlation coefficient, r) มากกว่า 0.99 นอกจากนี้ยังให้ค่าเปอร์เซ็นต์การได้กลับคืนของสารทั้งหกชนิดมากกว่า 80 ที่ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ต่ำกว่า 10 เปอร์เซ็นต์

เมื่อได้ประยุกต์ใช้เทคนิคการเตรียมตัวอย่างทั้งสองและยืนยันประสิทธิภาพของเทคนิคกับตัวอย่างจริง ซึ่งสุ่มจากน้ำบรรจุขวดและ/หรือน้ำประปาในเขตอำเภอหาดใหญ่ จังหวัดสงขลา รวมทั้งน้ำดิบจากอ่างเก็บน้ำ มหาวิทยาลัยสงขลานครินทร์ จากผลการทดสอบพบว่าทั้งสองเทคนิคสามารถนำไปใช้วิเคราะห์สารประกอบอินทรีย์กึ่งระเหยง่ายได้ทั้งเชิงคุณภาพและปริมาณ ซึ่งข้อมูลที่ได้รับนับว่ามีประโยชน์ต่อการนำไปใช้ในการควบคุมคุณภาพน้ำได้ต่อไป

Thesis Title	Development of Sample Preparation Techniques for the Analysis of Semivolatile Organic Compounds (SVOCs) in Water
Author	Mrs. Pensiri Peeraprasompong
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Abstract

This thesis focuses on the development of simple sample preparation techniques based on materials which are selectively separated the analytes such as hollow fiber membrane and capillary chromatographic column. These materials have been developed to be used as in-line extraction system coupled to gas chromatograph-mass spectrometer (GC-MS) for qualitative and quantitative analysis of trace residual semivolatile organic compounds (SVOCs) in water sample.

The first developed technique was the membrane inlet (MI) unit where a set of hollow fiber membranes was coupled to GC-MS for an in-line analysis of water contaminated with 4,4′-DDT. The hollow fiber membrane acted as a selective barrier to extract 4,4′-DDT from water sample passing through the MI unit. Laboratory-built heating box was used to enhance pervaporation of analyte before being stripped into GC-MS by stripping gas and analyzed with selected ion monitoring (SIM) mode. The optimum conditions of this method consists of sample and stripping gas flow rate at 6 and 9 mL min^{-1}, respectively, and a desorption temperature at 60 degree. These conditions provided excellent analytical performance, *i.e.*, low detection limit; 90 μg L^{-1}, linear range in the range of 0.01-1.0 mg L^{-1} with correlation coefficient (r) more than 0.99 and extraction efficiency in the range of 83-94% with relative standard deviation less than 10%.

The second method, capillary microextraction (CME), was developed with the aim of using the capillary chromatographic column to be an extractor that was able to simultaneously analyze six residual SVOCs in water sample. This method employed the HP-5 capillary column (100 cm × 0.32 mm × 0.25 μm film thickness)

and two switching valves to load, extract, desorb and transport the target analytes into GC-MS for analysis. Six toxic priority list compounds, *i.e.*, hexachlorbutadiene, dieldrin, 4,4'-DDT, benzo[*b*]fluoranthene, benzo[*a*]pyrene and dibenzo[*a,h*]-anthracene were investigated. The optimum conditions were; sample flow rate 1.0 mL min^{-1}, sample volume 7 mL, purging time 50 seconds, ethanol (5% v/v) used as organic modifier and acetonitrile used as eluting solvent. This developed method could detect the target analytes at trace level in the range of 0.01-0.1 ng L^{-1} and provide linearity in the range of 0.01 ng L^{-1} to 1.0 mg L^{-1} with correlation coefficient (r) more than 0.99. In addition, high recovery (more than 80%) was obtained with relative standard deviation less than 10%.

Both developed methods were validated with real samples sampling in Hat Yai City, Songkhla province. The result showed that both methods were successfully applied for trace level analyses of SVOCs in water samples. The qualitative and quantitative information were obtained and useful for water quality control.

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The completion of this thesis would be quite impossible without the help provided by many people, whom I would like to thank.

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Pensiri Peeraprasompong

The Relevance of The Research Work to Thailand

Water is vital for life and presently water quality problem is an important problem in many countries including Thailand. Semivolatile organic compounds (SVOCs) are toxic substances that can contaminate water from many routes, *i.e.*, industrial, agricultural, and human activities, accidental spill, and waste discharges. Moreover, these compounds can affect human health even at trace level, *i.e.*, carcinogenic effect. Therefore, a high efficiency and effective analytical method(s) for trace analysis of target SVOC compounds are required, especially a sample preparation method(s) which is an important step before instrumental analysis. In this work we focused on the development of sample preparation methods that can provide an equivalent or better than current methods in term of accuracy, precision, recovery, less time consuming, more economical and can be considered a "green chemistry" method. The developed techniques can be applied to real samples. The results can help to provide some basic information to the society who can make decisions, initiate new regulation of the limit of contaminant level of SVOCs in water and focus on the evaluation and maintenance of aquatic ecosystem onward.

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Chapter 1

Introduction

1.1 Background and Rationale

The presence of a number of chemical contaminants in water, soil and air are of great concern due to their possible affects on human health and environment. This is especially in water where many toxic or hazardous contaminants are either directly introduced into the aquatic environment or eventually surface turned up in this compartment due to the aquatic runoff of agricultural and industrial sites or transfer from the atmosphere into surface water which is a vital source of drinking water (Barceló, 2003; Cole *et al.*, 1999; U.S. EPA, 2005a). Therefore, water quality has received considerable attention in environmental legislation and strict regulations have been imposed in order to control the quality (U.S. EPA, 2005a). Consequently, the demands for determination of these pollutants in water matrices are rapidly increasing.

Water contaminants, with different chemical characteristics, can roughly be divided into organic, inorganic and metal species pollutants (Bruzzoniti *et al.*, 2000). Organic pollutants are the ones of most concern in water quality, particularly volatile and semivolatile organic compounds (VOCs and SVOCs) (Allen *et al.*, 2001; Allsopp and Johnston, 2000). VOCs are defined as organic chemicals having a low boiling point and high vapor pressure relative to their water solubility (Henry's law constant > 10^{-3} atm m^3 mol^{-1}) (Thomas, 1982) and have often included components of gasoline, fuel oils and lubricants as well as organic solvents, fumigants, some inert ingredients in pesticides, and some by products of chlorine disinfectant (Clark *et al.*, 1998). SVOCs are defined as organic chemicals having a boiling point higher than water and may volatilize slowly with low vapor pressure (3×10^{-7}< Henry's law constant <10^{-5} atm m^3 mol^{-1}) (Thomas, 1982) when exposed to temperatures above room temperature (U.S. EPA, 2006b).

SVOCs are a prominent group of toxic contaminant in water. They are mostly synthetic substances from a range of chemical groups (Barceló, 2003; Ome and Kegley, 2004), some of which are persistent organic chemicals and can be built up (bioaccumulated) in the tissues of animals and humans (Claxton, 1985; Govind *et al.*, 1991; Lekkas *et al.*, 2004; Simcik, 2004; Wolska *et al.*, 1998; Yoshida *et al.*, 2004; Zhao *et al.*, 2005). Larger molecules of SVOCs such as pesticides, chlorinated hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), phenol, polychlorinated biphenyl (PCBs) and dioxins can pose a serious health risk for human even at trace concentration ($\mu g\ L^{-1}$ or $mg\ L^{-1}$) and can have a direct impact on the environment (Allen *et al.*, 2001; Gehrke *et al.*, 2001; Ghaoui, 1993; Pandit *et al.*, 2001; Wolska *et al.*, 1998). Therefore, these compounds are playing important roles in water analysis.

Many SVOCs are still being produced and used, in the industrialized and non-industrialized countries, especially in tropical Southeast Asia (Barceló, 2003; Jones and Voogt, 1999), including Thailand. In many public water systems, pollution exceeds safe levels and these were caused by industrial, agricultural, and human activities, accidental spill, and waste discharges (Haygarth and Jarvis, 2002). This includes most manufacturing facilities that use huge quantities of freshwater to carry their waste discharge to the environment (Liu *et al.*, 2000). This waste water or effluent was discharged into streams, lakes, or oceans, which in turn dispersed SVOCs pollutants and finally entered the food chains (Allsopp and Johnston, 2000). Therefore, SVOCs which are released into the environment not only cause local contamination problems but also contribute to pollution in areas of the world far away from their source (Zhang *et al.*, 2003).

Songkhla is a province in southern Thailand that is expanding rapidly with high economic growth. Industries in these areas are continually growing and could probably be the cause of water pollution. Therefore, some actions must be taken to address, prevent, protect and manage the existing SVOCs problems.

In the past, multi-component determinations of SVOCs in waters were neither easy nor simple. As the number of target compounds needed to be monitored increases, there is a great demand for sensitive analytical methods. Different chromatographic procedures suitable for different classes of pollutants have been

developed and are currently available such as liquid chromatography (Belmonte Vega *et al.*, 2005; Berhanu *et al.*, 2006; Cháfer-Pericás *et al.*, 2006; Han *et al.*, 2005; Huang *et al.*, 2004; Jiang *et al.*, 2006; Kayali *et al.*, 2006), capillary electrophoresis (Blanco *et al.*, 2005; García-Ruiz *et al.*, 2005; Hernández-Borges *et al.*, 2005; King *et al.*, 2004; Kronholm *et al.*, 2004; Nozal *et al.*, 2004; Safarpour *et al.*, 2004), capillary electrochromatography (Fintschenko *et al.*, 2001; Messina *et al.*, 2005; Rossi and Desiderio, 2005) and gas chromatography (Basheer *et al.*, 2005; Brown and Emmert, 2006; Chia and Huang, 2006; El-Beqqali *et al.*, 2006; Kawaguchi *et al.*, 2006a; León *et al.*, 2003; Llorca-Porcel *et al.*, 2006; Olivella, 2006; Tahboub *et al.*, 2005). Although these analytical techniques are available, it is not possible to extract all the information from complex samples (Braune *et al.*, 2005) or introduce the sample directly into the analytical instrument due to the presence of particles or too low a concentration (Baltussen, 2000). Therefore, these methods still need sample preparation steps, *i.e.*, preconcentration or enrichment and matrix removal.

Sample preparation of trace SVOCs contaminants in water samples are often accomplished by extraction techniques, *i.e.*, liquid-liquid extraction (Brito *et al.*, 2002; Cai *et al.*, 2006; Fujiwara *et al.*, 2000; Jiang *et al.*, 2005; Kataoka, 2003; Vorkamp *et al.*, 2004; Xiao *et al.*, 2001), solid phase extraction (Blanco *et al.*, 2005; Brossa *et al.*, 2003; Columé *et al.*, 2001; Huck and Bonn, 2000; Kharat *et al.*, 2002; Liang *et al.*, 2006; Pereiro *et al.*, 2004), solid phase microextraction (Chang *et al.*, 2003; Fang *et al.*, 2006; González-Toledo *et al.*, 2001; Huang *et al.*, 2004; Lord and Pawliszyn, 2000; Mohammadi *et al.*, 2005; Peñalver *et al.*, 2002), stir bar sorptive extraction (Benijts *et al.*, 2001; Blasco *et al.*, 2002; García-Falcón *et al.*, 2004a; Itoh *et al.*, 2005; Kawaguchi *et al.*, 2006b; Peñalver *et al.*, 2003), membrane extraction (Barri *et al.*, 2006; Basheer *et al.*, 2004; Berhanu *et al.*, 2006; Chao *et al.*, 2002; Liu *et al.*, 2003b; Tudorache and Emnéus, 2005), in-tube solid phase microextraction (Globig and Weickhardt, 2005; Gou and Pawliszyn, 2000; Mitani and Kataoka, 2006; Nardi, 2003c; Wang *et al.*, 2004; Wu *et al.*, 2002) and capillary extraction (Nardi, 2003b; Nardi, 2003c). Each of these extraction techniques has its own advantages, *i.e.*, liquid liquid extraction (LLE), solid phase extraction (SPE) and solid phase microextraction (SPME) are selective, SPME and stir bar sorptive extraction (SBSE) use less or no solvent whilst SPE, SPME and SBSE are cost effective and simple to

use. However, some problems still exist, these include channeling in the adsorbent within the cartridge in solid phase extraction (Poole *et al.*, 2000), limited lifetime and fragility of polymeric fiber in solid phase microextraction (Kabir *et al.*, 2004), long extraction time and high bleed when use thick film coating of sorptive phase, and poor recovery when extracting both polar and non-polar compounds in stir bar sorptive extraction (Baltussen *et al.*, 2002; Montero *et al.*, 2004). Therefore, alternative techniques for extraction and concentration are needed in order to provide sensitive and selective analyses of trace SVOCs contaminated in water, and these were developed and discussed in this thesis.

1.2 Objectives of the research

This study focused on the development of alternative small scale sample preparation systems coupled with chromatographic analysis to achieve faster, high throughput, reproducible and cost effective sample preparation procedures. Two systems were developed to extract organic compounds, especially SVOCs contaminated in water. The first system was a membrane inlet unit coupled with gas chromatography-mass spectrometry (GC-MS) for in-line analysis of 4,4′-DDT in water. This was a solvent free method using the hollow fiber membrane as an extraction unit in order to increase surface area for separation and reducing the sample preparation time. The second technique, capillary microextraction, was developed for in-line analysis of multi residues of SVOCs in water using a short capillary column as a microextractor in order to isolate and extract SVOCs from water. A broken capillary column that does not have enough length for ordinary gas chromatography was employed as a simple microextraction column using very minimal organic solvent. Both systems were implemented and validated with real samples.

1.3 Benefits

It is expected that these new in-line sample preparation techniques can be implemented for trace analyses of SVOCs contaminated in water. The proposed methods should be equivalent or better than current methods in term of accuracy, precision, recovery, less time consuming and more economical. Furthermore, the analytical results can provide the basic information that will help our society to make

decisions, initiate new regulation of the limit of contaminant level of SVOCs in water and focus on the evaluation and maintenance of aquatic ecosystem onward.

Chapter 2

Semivolatile organic compounds (SVOCs)

2.1 Introduction and classification

SVOCs are an environmentally significant group of contaminants which have a boiling point higher than water and which may vaporize when exposed to temperatures above room temperature (Lauritsen and Ketola, 1997; U.S. EPA, 2006b). These include, for example, polycyclic aromatic hydrocarbons and pesticides which may be found in burn pits, chemical manufacturing plants and disposal areas, contaminated marine sediments, disposal wells and leach fields, electroplating/metal finishing shops, firefighting training areas, hangars/aircraft maintenance areas, landfills and burial pits, leaking collection and system sanitary lines, leaking storage tanks, radiologic/mixed waste disposal areas, oxidation ponds/lagoons, pesticide /herbicide mixing areas, solvent degreasing areas, surface impoundments, and vehicle maintenance areas and wood preserving sites (FRTR, 2005). With their hydrophobic property, SVOCs preferentially distribute into hydrophobic organic phases, such as sediment organic carbon and lipids in animal tissue (Claxton, 1985) and then create adverse effects, *i.e.*, mortality, reduced fecundity, and inhibited or abnormal growth to animal and human health (U.S. EPA, 1995; U.S. EPA, 2005a; U.S. EPA, 2006b). Therefore, scientists have become aware of these compounds which can disrupt the endocrine system and reproduction of fish, reptiles, and mammals (Colborn *et al.*, 1993).

For classification, Federal Remediation Technologies Roundtable (FRTR) has classified SVOCs in to two simple guideline categories, *i.e.*, non-halogenated and halogenated (FRTR, 2005) while ALS Environmental Australia has classified SVOCs based on the United State Environmental Protection Agency (U.S. EPA) method 8270 (ALS, 2006) as follows.

- Phenols
- Polycyclic aromatic hydrocarbons (PAHs)
- Phthalate esters
- Nitrosamines
- Nitroaromatics and ketones
- Haloethers
- Chlorinated hydrocarbons
- Anilines and benzidines
- Organochlorine pesticides
- Organophosphorous pesticides
- Micellaneous compounds, *i.e.*, methanesulfonate methyl, methanesulfonate ethyl, cis-isosafrole, trans-isosafrole, safrole, 1,3,5-trichlorobenzene, 1,2,4,5-tetrachlorobenzene, tetrachlorophenol, hexachlorophene, isodrin, diallate

In addition, polychlorinated biphenyls (PCBs) which were established in U.S.EPA Method 8082, together with dioxins and dibenzofurans in U.S.EPA Method 8280 and 8290 are also included in the SVOCs class (ATSDR, 2006; U.S. EPA, 2003a).

To consider the risk of SVOCs to the environment, the Agency for Toxic Substances and Disease Registry (ATSDR) and U.S. EPA set up a list of priority substances that are most commonly found at facilities on the National Priorities List (NPL). The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) will then use this list, which will be revised periodically, to reflect additional information on hazardous substances. Each substance on the CERCLA Priority List of Hazardous Substances will be a candidate to become the subject of a toxicological profile prepared by ATSDR (ATSDR, 2006). The present top 20 hazardous substances on the CERCLA Priority List of Hazardous Substances are listed in Table 2.1.

Table 2.1 CERCLA Priority List of Hazardous Substances for 2005 (ATSDR, 2006)

1.Arsenic	11. Chloroform
2. Lead	12. 4,4′-DDT
3. Mercury	13. Aroclor 1254
4. Vinyl Chloride	14. Aroclor 1260
5. Polychlorinated Biphenyls	15. Dibenzo[*a,h*]anthracence
6. Benzene	16. Trichloroethylene
7. Polycyclic aromatic hydrocarbons	17. Dieldrin
8. Cadmium	18. Chromium,Hexavalent
9. Benzo[*a*]pyrene	19. Phosphorus, white
10. Benzo[*b*]fluoranthene	20. Chlordane

2.2 Target analytes

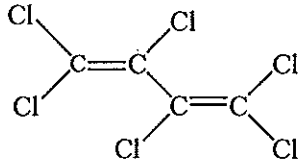
In this work six SVOC target analytes which are potential water contaminants and have different molecular structures have been selected from the CERCLA Priority List. They consisted of one chlorinated hydrocarbon, *i.e.*, hexachlorobutadiene, two pesticides, *i.e.*, 4,4′-DDT and dieldrin, and three PAHs, *i.e.*, benzo[*a*]pyrene, benzo[*b*]fluoranthene and dibenzo[*a,h*]anthracence.

2.2.1 Hexachlorobutadiene (ATSDR, 1994)

CAS No.: 87-68-3

Chemical formula: C_4Cl_6

Structure formula:



Synonyms: *Hexachloro-1,3-butadiene, 1,3-Hexachlorobutadiene, 1,1,2,3,4,4-Hexachloro-1,3-butadiene, Perchlorobutadiene, HCBD, Dolen-Pur*

Molecular weight: 260.76

Boiling point: 215°C

Melting point: -19 to -22°C

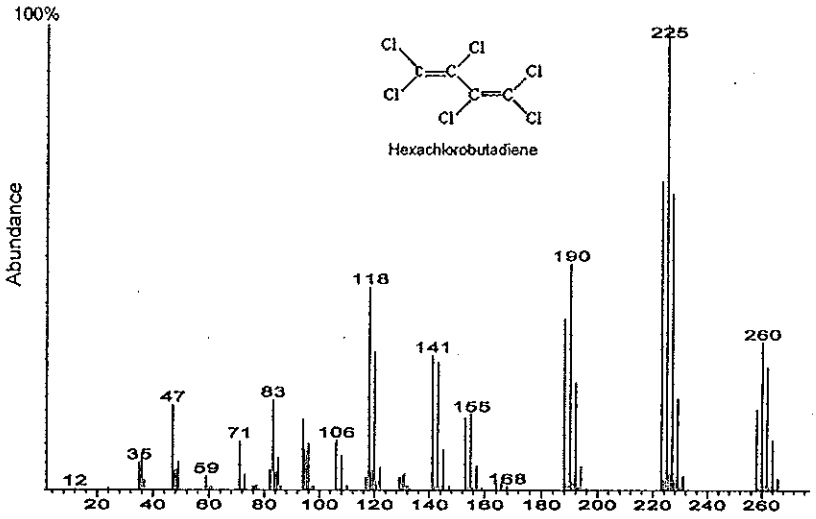
Log K_{ow}: 4.78 mg L^{-1}

Vapor pressure: 0.02 kPa , 0.15 mm Hg at 20°C

Solubility in water: 3.2 mg L^{-1} at 20°C

Appearance: Heavy, clear, colorless liquid

Mass spectrum (Hewlett Packard, 1998b):



Hexachlorobutadiene is a non flammable, incombustible, clear and colorless liquid which has a turpentine-like odor (HSDB, 2000). It does not occur naturally in the environment but is formed during the processing of other chemicals such as tetrachloroethylene, trichloroethylene and carbon tetrachloride (IPCS, 1993). Generally, this chemical is used as an intermediate product in rubber manufacturing, during chlorofluorocarbon and lubricant production, in transformer and hydraulic fluids, as a fluid for gyroscopes, as a heat transfer liquid, solvent, laboratory reagent, and as wash liquor for removing tetracarbon (C_4) and higher hydrocarbons (ATSDR, 1995; Howard, 1989). Hexachlorobutadiene is released into water via industrial effluents, by leaching from landfill or soil, or the urban runoff (ATSDR, 1994). After being released into water during disposal of waste, it could have bioaccumulation potential (WHO, 1994). Therefore, human may be exposed to this compound indirectly via drinking contaminated water, contaminated food or direct skin contact (ATSDR, 1994). In addition, hexachlorobutadiene is poorly soluble in water but small

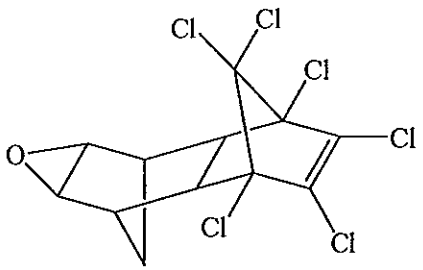
amount could be found in some public drinking water (≤ 1 μg L^{-1}) (U.S. EPA, 2003b). From a health perspective, there is no data for humans exposed to hexachlorobutadiene but the effect of this compound was studied in rats and this indicated that it could increase the risk of kidney cancer (NTP, 1991). Therefore, U.S. EPA has defined that hexachloro-butadiene is a possible human carcinogen (Group C) and the guideline for exposure to this compound in drinking water recommends that it should not exceed 0.05 μg L^{-1} for long period (7 years) (ATSDR, 1994; U.S. EPA, 1991).

2.2.2 Dieldrin (ATSDR, 2002a)

CAS No.: 60-57-1

Chemical formula: $C_{12}H_8Cl_6O$

Structure formula:



Synonyms: *4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a7,7a-octahydro-2,7:3,6-Dimethanonaphth[2,3-b]oxirene; 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene; 3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphth(2,3-b)oxirene, alvit, compound 497, dieldrix, diledrite, HEOD, illoxol, quintox, octalox and numerous further trade names, especially for mixtures containing dieldrin.*

Molecular weight: 380.91

Boiling point: 385°C

Melting point: 176-177°C

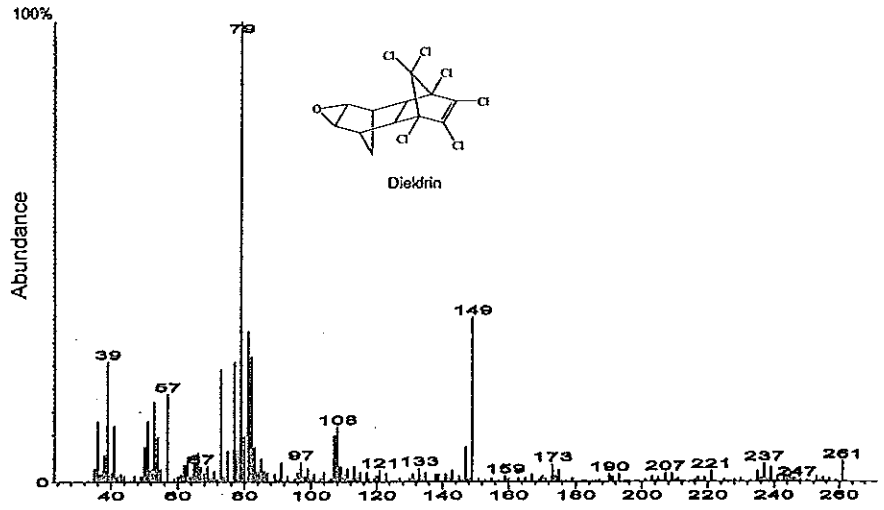
Log K_{ow}: 6.2 mg L^{-1}

Vapor pressure: 400 μPa, 3 ×10^{-6} mm Hg at 20°C

Solubility in water: 0.186 mg mL^{-1} at 20°C

Appearance: White crystals to light tan flakes

Mass spectrum (Hewlett Packard, 1998b):



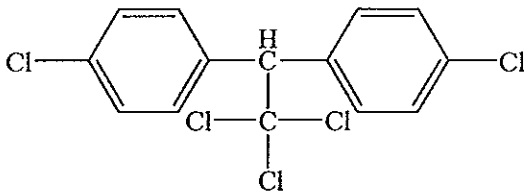
Dieldrin is a polychlorinated Diels–Alder adduct that was first manufactured in the 1950s for use as an insecticide instead of DDT (IPCS, 1989) since dieldrin proved to be a highly effective insecticide (ATSDR, 2002a; WWF, 2005). It was later banned in several countries during the early 1970s because its acute toxic, mutagenic and teratogenic effects on mammals (ATSDR, 2002a; Kanthasamy *et al.*, 2005). This compound is generally white with a mild chemical odor and a tan color where a less pure commercial powder (HSDB, 2000). It is made in the laboratory and does not occur naturally in the environment (IPCS, 1989). It is an extremely persistent organic pollutant that does not easily break down, therefore, dieldrin is still being found in the environment but at very low levels (Skaates *et al.*, 2005). In addition, it tends to accumulate in adipose tissue as it is passed along the food chain (ATSDR, 2002a). Therefore, U.S. EPA has classified dieldrin in group B2 (probable human carcinogen) (Chiu *et al.*, 2005; Kanthasamy *et al.*, 2005; WWF, 2005). The guideline for exposure to this compound in drinking water is that it should not exceed 0.03 μg L^{-1} in order to limit the lifetime risk of developing cancer from exposure (ATSDR, 2002a; FWEC, 1996).

2.2.3 4,4'-DDT (ATSDR, 2002b)

CAS No.: 50-29-3

Chemical formula: $C_{14}H_9Cl_5$

Structure formula:



Synonyms:*Dichlorodiphenyltrichloroethane, 1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane, 2,2-bis(p-Chlorophenyl)-1,1,1-trichloroethane, 1,1'-(2,2,2-Trichloroethylidene)bis(4-chlorobenzene), Anofex, Cesarex, Chlorophenothane, Dedelo, p,p'-DDT, Dichlorodiphenyltrichloroethane, Dinocide, Didimac, Digmar, ENT 1506, Genitox, Guesapon, Guesarol, Gexarex, Gyron, Hildit, Ixodex, Kopsol, Neocid, OMS 16, Micro DDT 75, Pentachlorin, Rukseam, R50 and Zerdane*

Molecular weight: 354.5

Boiling point: 260°C

Melting point: 108.5-109°C

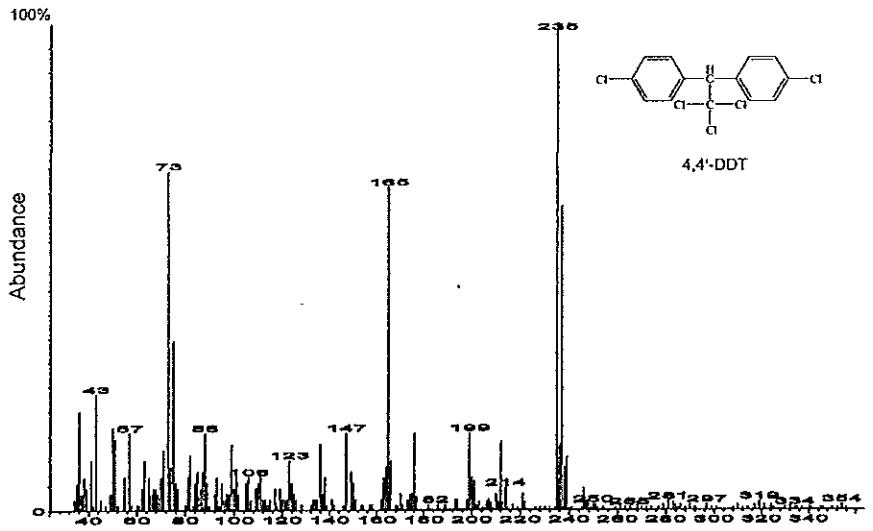
Log K_{ow}: 6.36 mg L^{-1}

Vapor pressure: 1.87×10^{-7} mm Hg at 25°C

Solubility in water: < 1 mg mL^{-1} at 20°C

Appearance: Colorless crystals or white powder, waxy solid (technical product)

Mass spectrum (Hewlett Packard, 1998b):



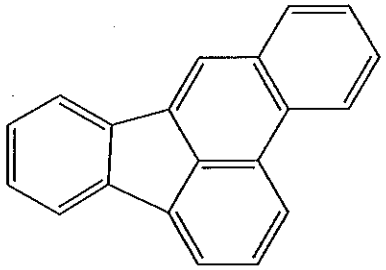
4,4′-DDT is an organochlorine insecticide widely used to control insects in agriculture and those which carry diseases such as malaria (Beard, 2006; Kapp, 2000). Generally, it is a white crystalline solid with no odor or taste and is available in several forms, *i.e.*, aerosols, dust powders, emulsifier concentrates, granules and wet powders (Fifield and Haines, 1996). Although it has been banned in the USA since 1972, some countries still use it to control mosquito-borne malaria (Beard, 2006; Besbelli, 1992; Ome and Kegley, 2004). In addition, because of its extremely low solubility in water, 4,4′-DDT is highly persistent in the environment, with a half life between 2-15 years, and its degradation process occurs very slow (Besbelli, 1992). Therefore, it is possible to leach into water primarily by runoff, atmospheric transport, drift or by direct application (e.g. to control mosquito-borne malaria) (ATSDR, 2000b). These could be the sources of 4,4′-DDT contamination in drinking water, especially near waste sites and landfills that may contain higher levels of 4,4′-DDT (Barra *et al.*, 2005; Binelli *et al.*, 2004; Venkatesan *et al.*, 1996). Considering the health effect, 4,4′-DDT has caused chronic effects on the nervous system, liver, kidneys and immune systems in animals (ATSDR, 2002b; Beard, 2006). Therefore, the U.S. EPA has also classified 4,4′-DDT in the group of B2 (probable human carcinogen) and the guideline for exposure to this chemical in drinking water that it should not exceed 0.002 mg L^{-1} (U.S. EPA, 2005a).

2.2.4 Benzo[*b*]fluoranthene (ATSDR, 1993)

CAS No.: 205-99-2

Chemical formula: $C_{20}H_{12}$

Structure formula:



Synonyms: *3,4-benzofluoranthene, benz[e]acenaphthanthrylene, 3,4-benz[e]acenaphthanthrylane, 2,3-benzofluoranthene, benzofluoranthrene, benzo[e]fluoranthene*

Molecular weight: 252.3

Boiling point: 481°C

Melting point: 163-165°C

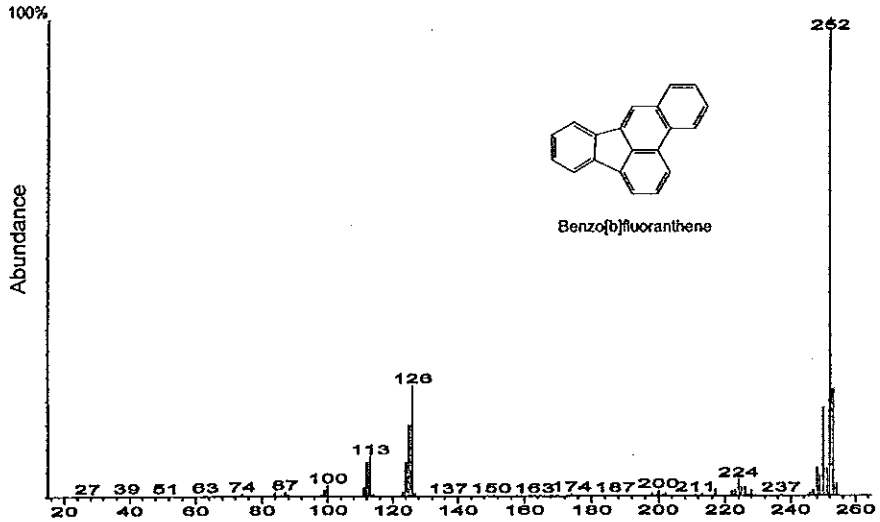
Log K_{ow}: 6.04 mg L^{-1}

Vapor pressure: 5×10^{-7} mm Hg at 20°C

Solubility in water: 0.0012 mg mL^{-1} at 20°C

Appearance: off-white to tan powder

Mass spectrum (Hewlett Packard, 1998b):



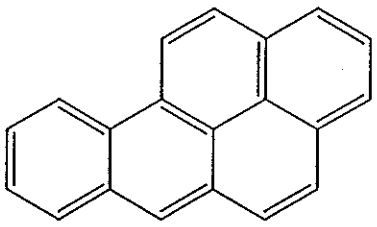
Benzo[*b*]fluoranthene, a crystalline solid, is a polycyclic aromatic hydrocarbon (PAH) with one five member ring and four six member rings (IARC, 1973b). There is no commercial production or known use of this compound (IARC, 1983). It has been found in fossil fuels and occurs ubiquitously in products of incomplete combustion, including in mainstream cigarette smoke, urban air, gasoline engine exhaust, emissions from burning coal and from oil-fired heating, broiled and smoked food, and oils and margarine (IARC, 1983). In the environment, benzo[*b*]fluoranthene is found in soils, groundwater, and surface waters at hazardous waste sites (Irwin, 1997). No report has addressed its carcinogenic effect in humans although because there is sufficient evidence for carcinogenicity in animals, therefore, the U.S. EPA has assigned this compound in a classification of B2 (probable human carcinogen) (IPCS, 1998). To protect human health, the water quality standards under the Clean Water Act (CWA) gives the guideline for the exposure to benzo[*b*]-fluoranthene in drinking water at 0.01 μg L^{-1} (U.S. EPA, 2005a).

2.2.5 Benzo[*a*]pyrene (ATSDR, 1993)

CAS No.: 205-99-2

Chemical formula: $C_{20}H_{12}$

Structure formula:



Synonyms: *1,2-benzopyrene, 6,7-benzopyrene, benzo[a]pyrene, B(a)P, BP, 3,4-benzopyrene, benzo[d,e,f]chrysene, 3,4-benzpyrene, benzpyrene, 3,4-benzylpyrene, 3,4-benz[a]pyrene, 3,4-BP, 3,4-benzopyrene*

Molecular weight: 252.31

Boiling point: 495°C

Melting point: 176.5-177.5°C

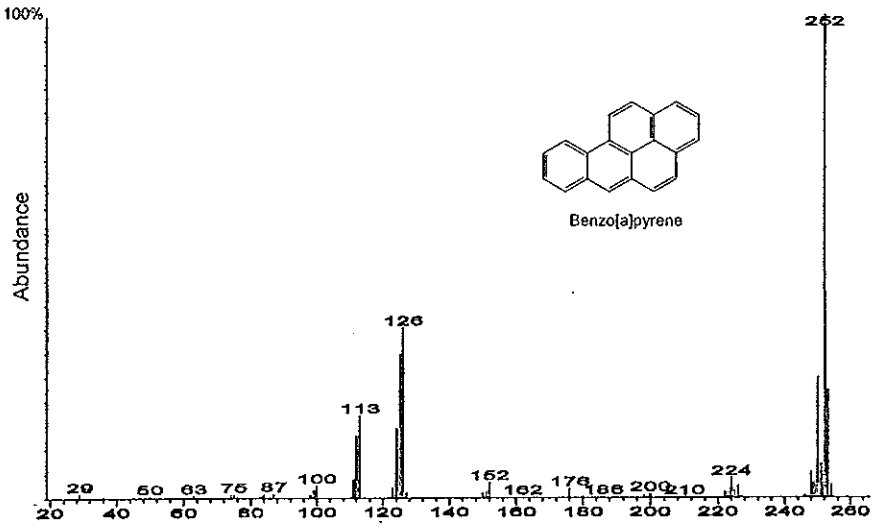
Log $K_{o/w}$: 6.12 mg L^{-1}

Vapor pressure: 5.6×10^{-9} mm Hg at 25°C

Solubility in water: 2.3×10^{-3} mg mL^{-1} at 17°C

Appearance: Yellowish crystals or powder

Mass spectrum (Hewlett Packard, 1998b):



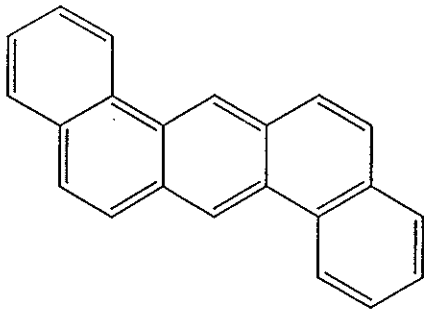
Benzo[*a*]pyrene, a crystalline yellow solid, is a polycyclic aromatic hydrocarbon (PAH) with five aromatic rings (Budavari *et al.*, 1989). It is not produced or used commercially but is commonly found in coal tar, automobile exhaust fumes (especially from diesel engines), tobacco smoke and charbroiled food resulting from the incomplete combustion of organic materials (IARC, 1973a). It is moderately persistent in the environment, easily binds to any particulate matter and could leach into raw water (Qiao *et al.*, 2006; Wu *et al.*, 2005; Zhang *et al.*, 2004). Two major sources of benzo[*a*]pyrene in drinking water, are the contamination of raw water supplied from natural and man-made sources and the leachate from coal tar and asphalt linings in water storage tanks, including distribution lines (U.S. EPA, 2006a). For health effects, this substance is an experimental carcinogen, mutagen, tumorigen, neoplastigen and teratogen (Borm *et al.*, 2005) for humans exposed to this compound by breathing or skin contact (DHFS, 2000). Therefore, the U.S. EPA has determined that benzo[*a*]pyrene is in classification of B2 (probable human carcinogen) and the guideline allows a maximum level of 0.01 μg L^{-1} in drinking water (U.S. EPA, 2006a).

2.2.6 Dibenzo[*a,h*]anthracene (ATSDR, 1993)

CAS No.: 53-70-3

Chemical formula: $C_{22}H_{14}$

Structure formula:



Synonyms: *1,2,5,6-dibenzo(a,h)anthracene,*

1,2:4,6-dibenzo(a,h)anthracene, 1,2:5,6-dibenzo(a,h)anthracene, DB(a,h)A, or DBA

Molecular weight: 278.35

Boiling point: 524°C

Melting point: 266-267°C

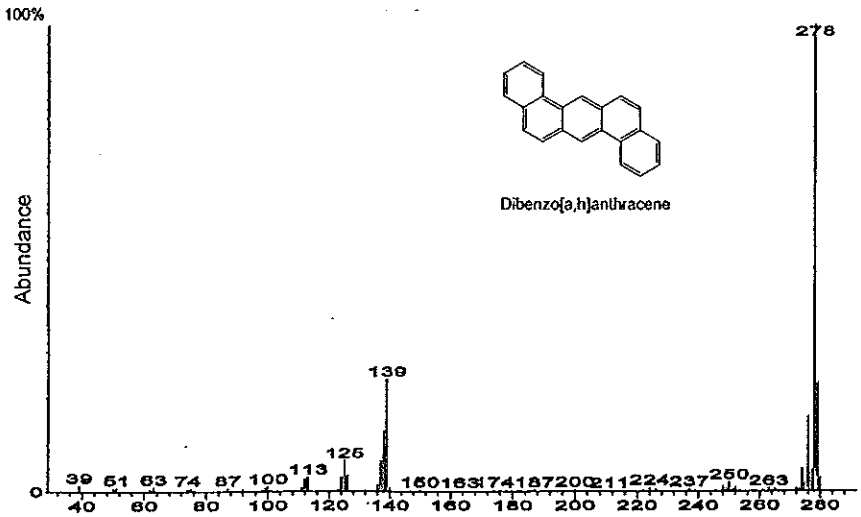
Log $K_{o/w}$: 6.84 mg L^{-1}

Vapor pressure: 1×10^{-10} mm Hg at 20°C

Solubility in water: 5×10^{-4} mg mL^{-1} at 20°C

Appearance: Colorless crystalline powder

Mass spectrum (Hewlett Packard, 1998b):



Dibenzo[*a,h*]anthracene is a polycyclic aromatic hydrocarbon (PAH) with five aromatic rings which exists as crystalline plates or leaflets and is insoluble in water (Budavari *et al.*, 1989). There is no commercial production and use for this compound but it occurs as a component of coal tars, shale oils, soots (IARC, 1985) and has been detected in gasoline engine exhaust, coke oven emissions, cigarette smoke, charcoal broiled meats and vegetation near the heavily traveled roads including surface water and soils near hazardous waste sites (ATSDR, 1993). If it is released into water resources, it is expected to be very strongly adsorbed to sediments and particulate matter (Faust, 1995). For health effects, there is no report on epidemiologic studies or case reports on the carcinogenicity of dibenzo[*a,h*]-anthracene in humans (White *et al.*, 1985). But there are some reports on animals on tumor production in animals by different routes of administration, having both local and systemic carcinogenic effects (ATSDR, 1993). Therefore, the U.S. EPA has assigned dibenzo[*a,h*]anthracene in a classification of B2 (probable human carcinogen) (U.S. EPA, 1995). However, there is no implemented maximum concentration level (MCL) of this compound for drinking water but the U.S. EPA recommended it to be included in water criteria of surface water used as a drinking water source for protection of aquatic life and human health, which limits that it should not exceed 0.1 $\mu g\ L^{-1}$ (García-Falcón *et al.*, 2004a; OJEC, 1998).

Chapter 3

Sample preparattion for SVOCs in Water

3.1 Introduction

Generally, the validity of results from chemical analyses initially depends on the quality and integrity of the sample that has been obtained. But in fact, even pure single substances used as standards rarely exceed 99.99% purity, and this still leaves the possibility of 10 $\mu g\ g^{-1}$ of another material being present (Mendham *et al.*, 2000). So the problems facing analytical chemists in analyzing organic compounds, especially SVOCs in water, are not only from the wide range of analyte types and their very low concentration but also from sample matrices that are frequently complex and unknown (Fifield and Haines, 1996). Although sophisticated analytical techniques are available it is still not possible to extract every bit of information even with a very small number of the samples (Braune *et al.*, 2005). It is also not possible to directly introduce the sample into the analytical instrument due to the presence of particles or too low concentration and complexity of sample (Baltussen, 2000). Therefore, the samples need specific treatment in order to make them compatible with analytical techniques. Particularly, sample preparation is hard to avoid.

Sample preparation is a process of transferring the analytes of interest from their original surroundings (sample matrices) into a form which is more suitable for the analytical instruments that are not typically suitable to handle the matrix directly (Baltussen *et al.*, 2002; Kataoka, 2003). Generally, most sample preparations involved in the separation techniques (Table 3.1) rely on the trapping or adsorbing of the analytes from the sample then the trapped or adsorbed analytes can be released with a small amount of organic solvent or thermal desorption, and subsequently, injected into the analytical instrument (Baltussen, 2000; Grob, 2004).

Table 3.1 Summary of separation techniques and phase system (Fifield and Haines, 1996; Fifield and Kealy, 2000)

Techniques	Phase system
Solvent extraction	Liquid-liquid
Solid phase extraction	Liquid-solid
Liquid (column) chromatography (LC)	Liquid-solid, Liquid-liquid
Gas chromatography (GC)	Gas-solid, Gas-liquid
Supercritical fluid extraction and chromatography	Supercritical fluid-Liquid or solid
Gel permeation chromatography (GPC)	Liquid-solid
Electrophoresis (CE)	Liquid
Capillary electrochromatography (CEC)	Liquid-solid
Thin layer chromatography (TLC)	Liquid-solid, Liquid-liquid
Paper chromatography	Liquid-liquid
Ion-exchange and size exclusion chromatography	Liquid-solid
Precipitation	Liquid-solid

One important step in the sample preparation process concerns an extraction which is often used to separate the analyte of interest from a sample matrix using variable procedures (Fifield and Haines, 1996) so that a few potential interference species are not carried through to the analytical separation stage (Kataoka, 2003; Majors, 2003; Mitra, 2004; Zief, 1982) and subsequencely provide a good yield and high selectivity.

The extraction is principally based on the selective partitioning of the analyte or interfering components between two immiscible phases. When a phase (A) containing a solute or analyte, X, is brought into contact with a second phase (B), the analyte partitions itself between two phases (Mendham *et al.*, 2000).

$$X_A \rightleftharpoons X_B \qquad (3.1)$$

The equilibrium constant expression referred to as the Nernst partition or distribution law (Fifield and Kealy, 2000), is given by

$$K_D = \frac{[X_B]}{[X_A]} \qquad (3.2)$$

where X is the concentration of analyte in each phase at constant temperature and K_D is the distribution constant or partition coefficient, which is independent of the total analyte concentration (Harvey, 2000). The extraction could be optimized so that the

distribution of analyte between two phases is forced to the right in equation 3.1 when the resulting value of K_D is large. It will indicate a high degree of extraction from phase A to B. Conversely, if K_D is small, less chemical X is transferred from phase A into phase B. If K_D is equal to 1, equivalent concentrations exist in each phase (Harvey, 2000; Stoker, 1993).

However, although many of the chromatographic instrumental techniques which are mostly used for the analysis of organic compounds, have matured and many laboratories still use techniques based on age-old methodologies with some degree of miniaturization but low levels of automation, whereas high throughputs are required (Majors, 2003). Therefore, new technologies of sample preparation with increasing selectivity and sensitivity of the assay are being developed or modified for future demand.

3.2 Conventional sample preparation techniques for SVOCs in water

The determination of SVOCs in water frequently involves the use of conventional techniques and a number of extraction techniques have been implemented. Common pretreatment methods that are commercially available for the extraction of SVOCs in liquid samples prior to chromatographic analysis are discussed in this section.

3.2.1 Liquid-liquid extraction (LLE)

Enrichment by liquid extraction is one of the oldest methods and it is still widely employed (Kataoka; 2003). The extraction process is based on selective partitioning of the analytes between two phases. The analyte is partitioned from one liquid phase into another liquid phase which is an immiscible phase, usually between an organic solvent and water. LLE was widely used in many standard procedures and can be applied to all types of samples include SVOCs in water (Table 3.2).

Table 3.2 Summary of LLE techniques for extraction of SVOCs in water (2001-present)

Analyte	Recovery	Detection limit	Analytical technique	Reference
PAHs	82-111%	0.007- 0.03 μg L^{-1}	GC-FID	(Rezaee *et al.*, 2006)
Phenolic compounds	-	5.6 - 20.0 mg L^{-1}	on-chip MEKC	(Wakida *et al.*, 2006)
Butylrhodamine B	69%	-	Microfluidic chip-LIF	(Cai *et al.*, 2006)
1,4-Dioxane	86-102%	0.2 ng mL^{-1}	GC-MS	(Park *et al.*, 2005)
Nitrophenol	-	0.45-0.98 μg L^{-1}	HPLC-UV	(Jiang *et al.*, 2005)
Haloacetic acid	54%	2 μg L^{-1}	HPLC-UV	(Wang *et al.*, 2005c)
PCBs	62-70%	0.11–0.24 ng L^{-1}	GC-FID	(Rossi *et al.*, 2004)
Herbicides	89-97%	0.4 μg L^{-1}	CE	(Zhou *et al.*, 2003)
Pesticides	75-104%	0.002-2.0 mgL^{-1}	GC-ECD	(Brito *et al.*, 2002)
Organochlorine pesticides	-	0.005-0.2 μg L^{-1}	GC-ECD	(Zhao and Lee, 2001)

LLE is used to separate a selective compound from a mixture or to remove unwanted impurities from a solution. Its achievement is based on the choice of solvents and the analyte characteristics, *i.e.*, solubility, polarity, ionic character and stability (Harvey, 2000; Stoker, 1993). Analytes distribute themselves between the aqueous and organic layers according to the relative solubility in each solvent or Nernst distribution law (Fifield and Kealy, 2000; Mitra, 2004), where the distribution coefficient, K_D, is equal to the analyte ratio in each phase at equilibrium as shown in equations 3.1 and 3.2. The extraction efficiency (E) for a one step extraction can be calculated by (Pettersson, 2004).

$$E = 1 - \frac{1}{1 + K_D\left(\frac{V_E}{V_S}\right)} \qquad (3.3)$$

where K_D is the distribution constant or partition coefficient and V_E and V_S are the volumes of the organic solvent and the water, respectively. The extraction efficiency can be enhanced by increasing the volume of solvent or by repetitive extraction with small portions of solvent. A powerful mean to improve the selectivity of the

extraction is to utilize a combination of solvents (Fifield and Kealy, 2000; Pettersson, 2004).

The simplest LLE technique is performed by shaking or stirring the sample with an appropriated organic solvent at room temperature without pH adjustment, that is, at about neutral pH (Mendham *et al.*, 2000). The method would typically use large volume of organic solvent (100–250 mL) with a similar volume of sample and the extraction would have to be repeated 2–3 times to achieve a high recovery (Smith, 2003). However, procedures with sample volumes up to 100 liters when the sample is very dilute have been published (Gbmez-Bellnchbn *et al.*, 1988; Hermans *et al.*, 1992). After drying the extracted sample with anhydrous salt (e.g. sodium sulphate), the solvent would be concentrated by evaporation. The resulting sample would frequently require a further clean-up stage. With some samples, the initial solvent extraction step results in the formation of an emulsion and the extraction process could become prolonged (Majors, 2003).

LLE is a versatile technique and requires only simple equipment but it often has several disadvantages, *i.e.*, tedious, time consuming, labor intensive, not easily automated, non-selective and expensive due to the use of large quantities of organic solvents which may have significant health hazards and disposal costs associated with their use (Mendham *et al.*, 2000; Popp *et al.*, 2003). To overcome some of the problems such as long analysis time, Farrell and Pacey (1996) investigated the recovery of several SVOCs using a new thermospray liquid-liquid extractor (TSLLE) as shown in Figure 3.1. The base system includes a multi-port extraction vessel, jacketed in a cooling flask, a dual-stage condenser for progressive cooling, several thermospray probes, and solvent/sample delivery systems. Aqueous mixtures of SVOCs were used to evaluate the TSLLE. For most compounds, recovery values of 80-100% were obtained during a single cycle less than 1 h.

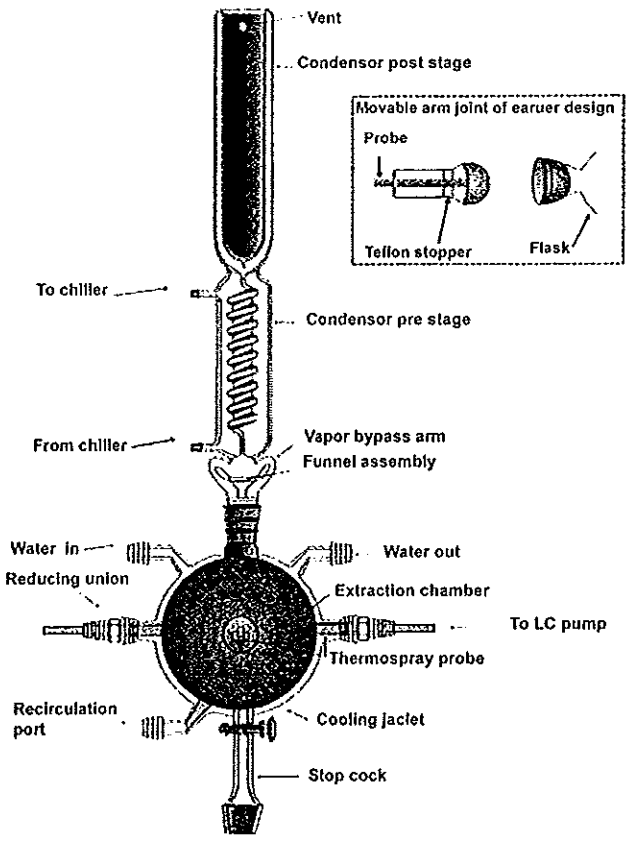


Figure 3.1 Sketch of the thermospray liquid/liquid extraction apparatus (Farrell and Pacey, 1996)

Although LLE is quite laborious off-line, this method can be applied as an on-line or fully automated systems and methods using segmented flow extraction combined with GC have been reported (Ballesteros *et al.*, 1990; Ballesteros *et al.*, 1993a; Fogelqvist *et al.*, 1986; Fujiwara *et al.*, 2000; Goosens *et al.*, 1992; Jiang *et al.*, 2005; Peng *et al.*, 2001; Peng *et al.*, 2000; Rice *et al.*, 1997; Roeraade, 1985; Synovec *et al.*, 1990; Vreuls *et al.*, 1999). Typically in these systems, an organic extracting solvent was pumped at a low flow rate and mixed with the aqueous sample stream to produce an aqueous to organic volume ratio. The mixture was passed through an extraction coil composed of a PTFE tube where analytes were transferred from the aqueous into the organic phase. Phase separation is achieved using a semi-permeable membrane or a sandwich-type phase separator. The organic phase is then led through an interface and passed onward into the detector while the aqueous stream was wasted. An example of this on-line LLE is shown in Figure 3.2. It

was applied for the chemiluminescence determination of atropine where a detection limit of 1 ng mL^{-1} was obtained (Fujiwara *et al.*, 2000).

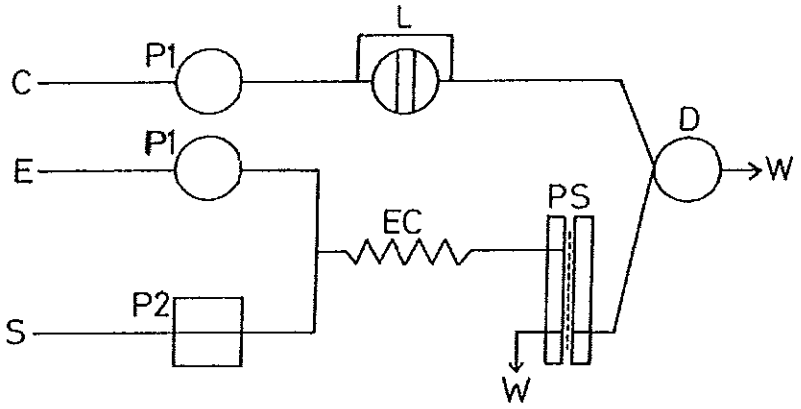


Figure 3.2 Flow diagram for the on-line solvent extraction/reversed micellar mediated Chemiluminescence determination of atropine: C, dichloromethane; E, dichloromethane; S, sample; L, luminescent reagent; EC, extraction coil; PS, phase separator; D, detector; W, waste; P1, plunger pump; P2, peristaltic pump (Fujiwara *et al.*, 2000)

More recently, an on-line LLE–GC system was combined with electron capture detector (ECD) for the determination of phenolic hydrolysis degradation products of *N*-methylcarbamates in milk (Ballesteros *et al.*, 1993b) or combined with atomic emission detection (AED) for the detection of several nitrogen, chlorine and sulphur containing pesticides in aqueous samples (Goosens *et al.*, 1995). These LLE-GC systems could enhance the extraction yield and improve the chromatographic behavior of the solutes, *i.e.*, RSD was in the range of 2-4%, recoveries were over 70% with detection limit at or below 0.1 μg L^{-1}.

One of the disadvantages often cited with the use of LLE is that analysts performing LLE have generally experienced difficulties on exposure to large volume of organic solvents and generation of dirty waste. To overcome these problems microfluicdic chips are being developed and implemented (Cai *et al.*, 2006; Malmstadt *et al.*, 2003; Oleschuk and Harrison, 2000a; Tokeshi *et al.*, 2000; Wang *et al.*, 2005d). For example, a microchannel fabricated quartz glass chip was used to separate analytes as shown in Figure 3.3.

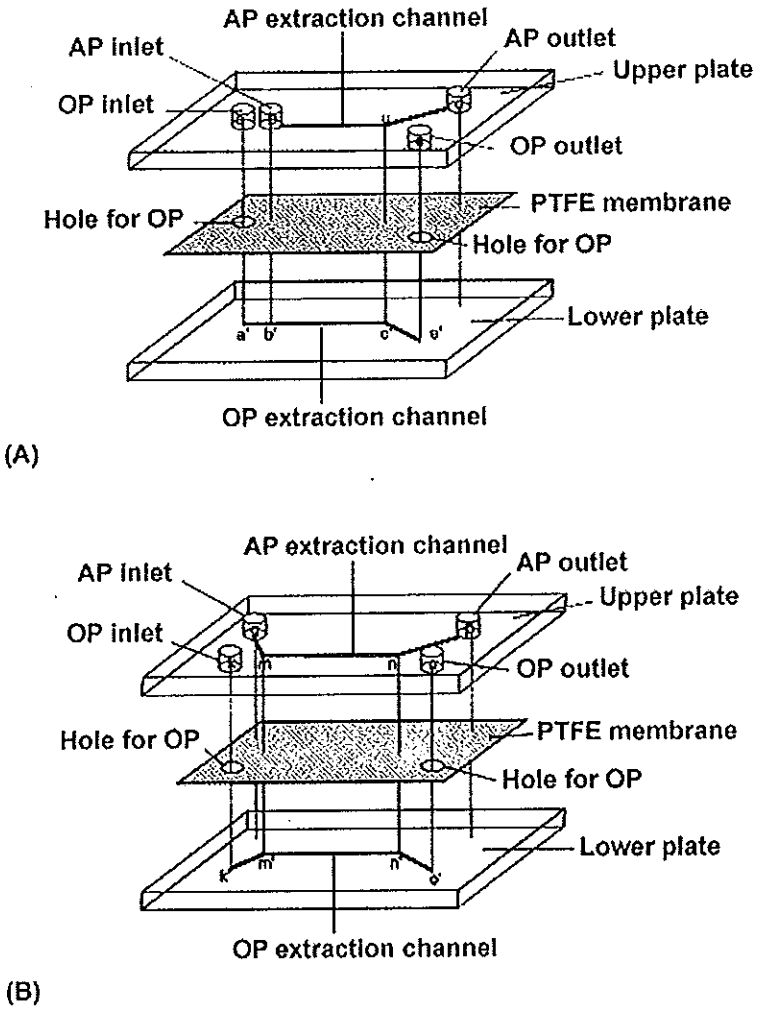


Figure 3.3 Schematic diagram of the microfluidic chip for liquid–liquid extraction with "{" shaped extraction channels (A) and "}" shaped extraction channels (B) (Cai *et al.*, 2006)

The sample was introduced into a microchannel (less than 1000 μm) to form a parallel two phase laminar flow producing a liquid–liquid aqueous–organic interface. Within the microchannel, the aqueous–organic interface did not attain the upper–lower arrangement produced by differences in specific gravity normally observed in LLE. In the microchannel environment, surface tension and frictional forces are stronger than specific gravity, resulting in an interface that is side by side and parallel to the sidewalls of the microchannel. The product was extracted from aqueous solution into the organic solvent within a short time (seconds) when the flow

was very slow or stopped, corresponding with molecular diffusion time. The extraction system required no mechanical stirring, mixing, or shaking (Tokeshi *et al.*, 2000). The advantages of microfluidic chip based solvent extraction systems are low consumption of sample and reagent, high extraction efficiency owing to enhanced interfacing area/volume ratio between the organic and aqueous phases as well as the significantly reduced diffusion distance in microfluidic systems.

Besides these developments LLE still requires several sample handling steps. It may also present the following problems to the analyst, *i.e.*, phase emulsions, the evaporation of large solvent volumes to concentrate analyte and non-reproducible extraction (Majors, 2003; Zief, 1982). In addition, in some case the analytes need to be derivatized before gas chromatographic analysis in order to increase their volatility thus it is either time consuming or possible lost of analytes during the procedure (Kataoka, 2003). Recent developments using a solid phase and membrane to physically separate the extraction liquid from the sample have demonstrated new possibilities (Calafat *et al.*, 2004; Crespin *et al.*, 1997; García-Falcón *et al.*, 2004b; Guo and Mitra, 2000; Jönsson and Mathiasson, 1999; Karwa *et al.*, 2005; Poole, 2003). So there is a tendency to replace LLE by other enrichment methods. This is related to the inconvenience of handling solvents, which is a negative factor when personal safety aspects are considered, particularly when systems have to be operated by non-chemists. In addition, it would be an improvement to avoid the use of solvents (Pettersson, 2004). This has also been one of the goals in the development of the new techniques for trace enrichment.

3.2.2 Solid Phase Extraction (SPE)

SPE is an increasingly useful sample preparation technique and widely used for trace enrichment of very dilute solutions such as natural waters where large sample volumes may have to be processed in order to yield sufficient concentrations of analyte sufficient for detection. It was first used about five decades ago but it did not really become a scientific technique until the 1970s. The growth in applications of this technique began in 1977 when the Waters Corporation introduced commercially available, prepackaged disposable cartridges/columns containing bonded silica adsorbents (Dressler, 1979; Frei and Brinkman, 1981). The term solid phase

extraction was established in 1982 by employees of the J.T. Baker Chemical Company (Zief, 1982). With SPE, many of the problems associated with LLE can be prevented such as incomplete phase separations, less quantitative recoveries, use of expensive or breakable specialty glassware, and disposal of large quantities of organic solvents. SPE provides more yield of quantitative extractions that are easy to perform, rapid, and can be performed off-line or online. The off-line experiment is simple and highly flexible (Kharat *et al.*, 2002) whereas on-line SPE can be automated and provides a high sample throughput (Alnouti *et al.*, 2005; Barrett *et al.*, 2005; Calderoli *et al.*, 2003; Columé *et al.*, 2001; Visser *et al.*, 2003). Furthermore, SPE is a multi stage separation technique providing greater opportunity for selective isolation than LLE, thus, the solvent use, lab time and potentials for the formation of emulsions are reduced (Fritz, 1999; Fritz and Macka, 2000; Huck and Bonn, 2000; Wells, 2000). SPE is often used to extract SVOCs analytes not only in liquid samples but also can be used with solids that are pre-extracted into solvents and these are summarized in Table 3.3.

Typically, SPE is a sample preparation technique which is based on the partitioning of compounds between a liquid (sample) phase and solid (extraction) phase where the intermolecular forces between the phases influences retention and elution (Supelco, 1998a). Both the analytes and the interferences of the samples are retained on the adsorbent by different mechanisms. Retention may involve non-polar, polar or ionic interactions (Fritz, 1999). The wide range of SPE adsorbents available provides a wide range of interactions, *i.e.*, organic compounds are adsorbed by a solid adsorbent made of bonded silica or polymers. Large enrichment factors (typically 100–1000) are possible and selectivity can be achieved by choosing an appropriate adsorbent material for analytes that do not adsorb impurities and/or matrix compounds (Poole *et al.*, 2000). Then SPE allows desorption of analyte by a different solvent. Figure 3.4 shows the concept of SPE. Various SPE products are now available such as column cartridges, disks and well plates (Poole, 2003). The propylene columns are packed with 100, 200, or 500 mg of an adsorbent sandwiched between two 20 μm polyethylene frits and have capacities of 1, 3, or 6 mL, respectively (Simpson, 2000). Adsorbents with average particle diameters of 40 μm and pocessing a narrow size distribution of 32-60 μm produce the optimum efficiency

(Fritz, 1999). The primary decision for analysts to achieve an extraction by SPE is the selection of the adsorbent to optimize since adsorbents are available in a wide variety of chemistries, types and sizes. Selecting the most suitable product for each application and sample is important (Supelco, 1998a). Therefore, if more selectivity and reduced process times of factors of six to nine is required, SPE can be a good alternative to LLE (Fifield and Kealy, 2000; Font *et al.*, 1993; Fritz, 1999; Fritz and Macka, 2000).

Table 3.3 Summary of SPE techniques for extraction of SVOCs in water (2001-present)

Analyte	Recovery	Detection limit	Analytical technique	Reference
PAHs	-	1-5 ng L^{-1}	GC-MS	(El-Beqqali *et al.*, 2006)
atrazine and simazine	83–104%	33 and 9 ng L^{-1}, respectively	HPLC-UV	(Zhou *et al.*, 2006)
Organochlorine pesticides	82-104%	0.008-0.012 μgL^{-1}	GC-ECD	(Shukla *et al.*, 2006)
PAHs	87-115%	0.9-58.6 ng L^{-1}	HPLC-PAD	(Liang *et al.*, 2006)
Phenolic compounds	96–106%	-	CE	(Blanco *et al.*, 2005)
Phenoxy acid herbicides	80-120%	1–4 ng L^{-1} (surface water) 4–12 ng L^{-1}(sewage water)	GC-MS	(Pereiro *et al.*, 2004)
Endocrine disruptors	29-111%	0.001 µg L^{-1} (tap water) 0.036 µg L^{-1} (river water)	GC-MS	(Brossa *et al.*, 2003)
Aromatic compounds	90-101%	-	GC-MS	(Yu *et al.*, 2003)
Pesticides	100-120%	0.02-0.13 µg L^{-1}	GC-ECD	(López-Blanco *et al.*, 2002)
Dihidropyridine	90-95%	-	HPTLC	(Kharat *et al.*, 2002)
Organochlorine pesticides	92%	0.01 - 0.1 µg L^{-1}	GC-ECD	(Columé *et al.*, 2001)

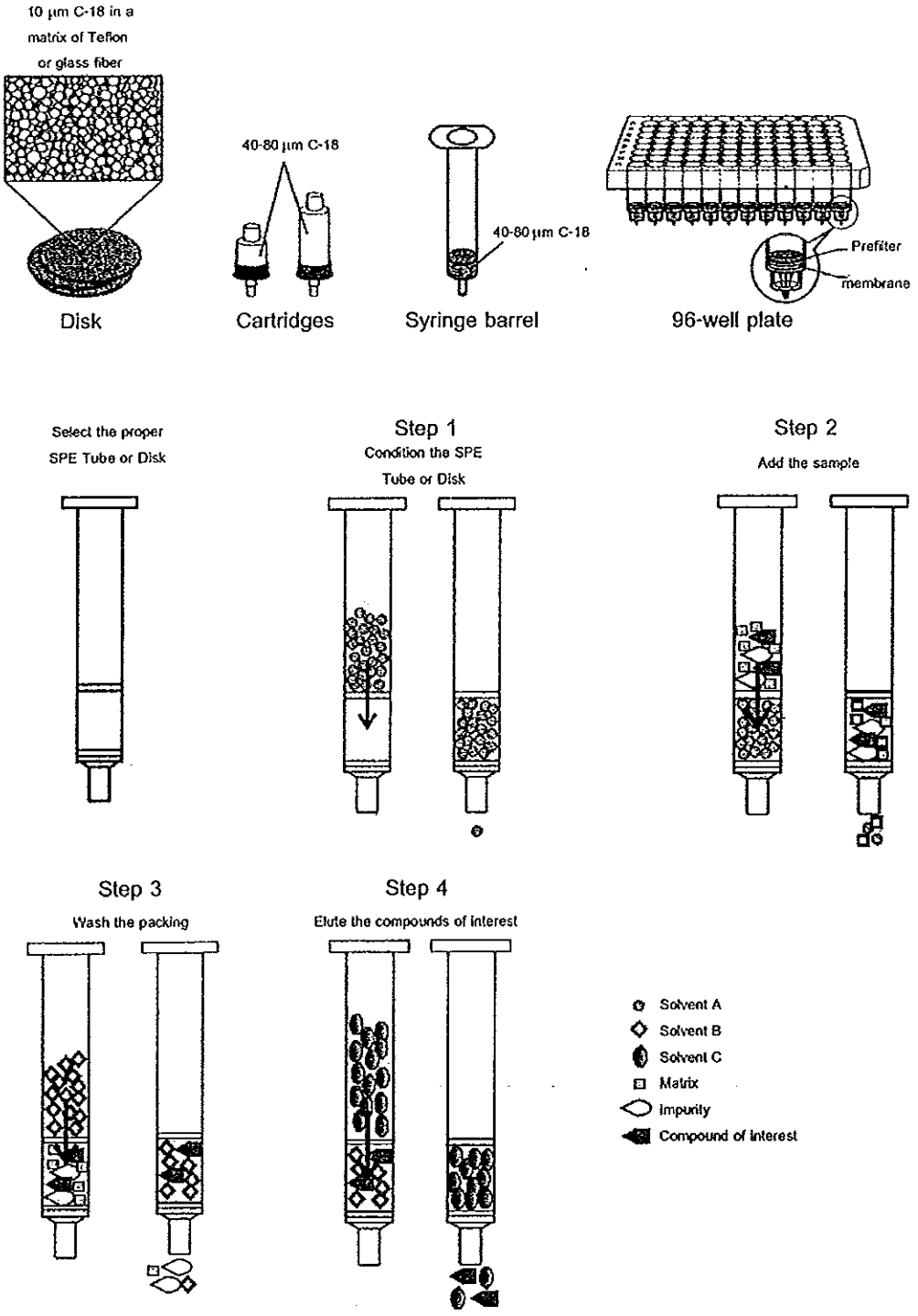


Figure 3.4 Concept of solid phase extraction (SPE): types and procedure for use (Supelco, 1998a)

Generally, SPE consists of four steps (Figure 3.4) (Poole *et al.*, 2000). Column preparation or prewash, sample loading (retention or sorption), column postwash, and sample desorption (elution or desorption), although some of the recent advances in sorbent technology reduce or eliminate column preparation procedures. The prewash step is used to condition the stationary phase, if necessary, and the optional column postwash is used to remove undesirable contaminants (Simpson, 2000). Usually, the compounds of interest are retained on the adsorbent while interferences are washed away. Analytes are recovered via an elution solvent (Thurman and Mills, 1998). However, adsorbent particles in SPE cartridges do act as depth filters toward particulate matter when the sample solution is not truely homogeneous. Particulate matter can become lodged in the spaces between the adsorbent particles or in the intra-particulate void volume or pore space within adsorbent particles. The filtering of particulate matter is generally disadvantage to the analysis and can lead to plugging of the extraction adsorbent or channeling of the flow through the adsorbent (Hagen *et al.*, 1990; Thurman and Mills, 1998). Fritz (1999) summarized the limitation of a plugging problem in SPE which depends on the concentration, type, and size of the particulates in the sample, the pore size of the adsorbent and the surface area of the adsorbent bed. Therefore, the performing SPE extraction and other analytical procedures must be concerned with the potential for the analyte's association with particulate and colloidal matter contaminating in the sample. If the sample is not filtered, particulates can partially or entirely elute from the adsorbent, leading to both a dissolved and particulate result when the sample is analyzed (Simpson, 2000; Smith, 2003). In addition to concern about the potential for suspended solids in the water sample plugging the SPE adsorbent and analytes of interest adsorbing onto particulates, loss of the analyte may occur if small particulates pass through the pores of the adsorbent bed (Poole *et al.*, 2000). To avoid these problems and ensure consistent results, sample particulate matter should be removed by filtration prior to SPE analysis.

Besides the use of cartridges, alternative forms of adsorbent bed have been developed, *i.e.*, SPE disks. These include Empore extraction disks (3M, Inc., St. Paul, MN) (Hagen *et al.*, 1990) SPEC disc (Ansys, Inc.; Irvine, CA) and Speedisk (J.T. Baker, Phillipsburg, NJ) (Thurman and Mills, 1998). These disks have relatively

large cross-sectional areas compared to packed-bed cartridges. This reduced bed-mass results in low void volumes, thus, minimizing solvent consumption in the rinsing and elution steps, improving selectivity and facilitating high solvent flow rates when large volumes of sample are to be processed (Fifield and Kealy, 2000; Liška, 2000). In addition, the extraction disks also eliminate the problems which are encountered in the use of cartridges, *i.e.*, low flow due to blockage by particles, the inadequate and variable packing density of cartridges and non-specific matrix adsorption (Poole, 2003).

SPE disks are used much like filter paper in a filtration apparatus. At least three different disk devices are commercially available. The particle-loaded membranes consist of 8–10-mm adsorbent particles suspended in a web of PTFE microfibrils. The membranes have a homogeneous structure containing about 80% (w/w) or more of adsorbent particles formed into circular disks 0.5 mm thick with diameters from 4 to 96 mm, where a 47 mm disk is mostly used (Supelco, 1998a). Particle-embedded glass fiber disks contain 10–30-mm adsorbent particles woven into a glass-fiber matrix available in a wide range of sizes (Poole, 2003).

The original disks were designed for use with standard vacuum filtration apparatus. After the disk is loaded into the apparatus and the apparatus placed on the vacuum flask, the disk is conditioned using aliquots of each solvent prior to loading the water sample. Then the filtration apparatus containing the disk is transferred to a vacuum flask containing a test tube for elution with approximately 5 to 10 mL of eluent (Simpson, 2000). Although the most frequently used disk size is the 47 mm, which is suitable for standard methods (0.5–1 L water sample volumes). The use of small diameter SPE disk (4.6 mm) has been reported but they were used to couple in-line with an analytical system in order to determine SVOCs in water as shown in Figure 3.5 (Barceló *et al.*, 1993; Bernal *et al.*, 1997; Columé *et al.*, 2001; Lacorte and Barceló, 1995; Lim *et al.*, 2004; López *et al.*, 1998; Marti and Ventura, 1997; Quayle *et al.*, 1997; van der Hoff *et al.*, 1996; Viana *et al.*, 1996).

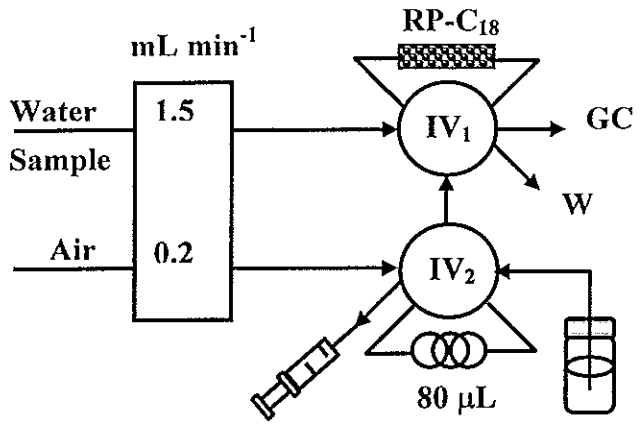


Figure 3.5 Scheme of the continuous preconcentration and elution steps for the determination of organochlorine phosphorous (OCPs) in waters. IV: injection valve, W: waste, GC: gas chromatograph. (Columé *et al.*, 2001)

Advances in SPE, microfluidics and miniaturization hold a great promise in terms of sample throughput advantage. Miniaturization of analytical processes into microchip platforms designed for micro total analytical systems (µ-TASs) is a new and rapidly developing field. For example, a microfabricated analytical microchip device was developed using a porous monolith sorbent with two different surface chemistries, a hydrophobic and ionized surface (Fritz, 1999; Fritz and Macka, 2000; Karwa *et al.*, 2005; Rossi and Zhang, 2000). The monolithic porous polymer was prepared by in situ photo-initiated polymerization within the channels of the microfluidic device and used for on-chip SPE as shown in Figure 3.6. Use of the device for sorption and desorption of various analytes was demonstrated (Boos and Fleischer, 2001; Dahlin *et al.*, 2005; Hibara *et al.*, 2002; Karwa *et al.*, 2005; Oleschuk *et al.*, 2000b; Poole, 2003; Sayah *et al.*, 2000; Wolfe *et al.*, 2002; Yu *et al.*, 2001).

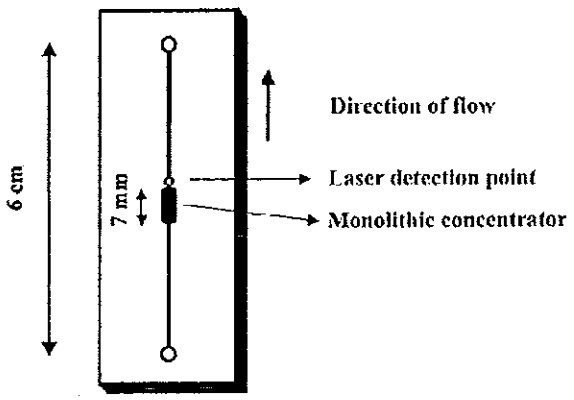


Figure 3.6 Microfluidic chip layout (Yu *et al.*, 2001)

However, the greatest disadvantage of the SPE technique is that it is difficult to simultaneously extract polar and non-polar compounds and sometimes this requires development of two separate procedures, *i.e.*, extraction and clean up (Alnouti *et al.*, 2005; Kataoka, 2003; Pichon, 2000; Poole, 2003). For example, when analyzing pesticides from a large group of compounds with widely different structures and biological activities, the extraction process needs adsorbents of more than one type such as C-18 for non-polar compound, followed by a florisil for the polar compounds so that it can effectively isolate the compounds of interest from others (Baltussen *et al.*, 2002; Huck and Bonn, 2000; Liška, 2000; Majors, 2003).

3.2.3 Solid phase microextraction (SPME)

In order to overcome the disadvantages associated with the use of LLE and SPE such as large solvent use in LLE and channeling in SPE, a new approach which is based upon sorption has been developed (Baltussen *et al.*, 2002; Supelco, 1998b). It is introduced as a solvent free sample preparation or microscale extraction technique based on a sorptive principle (Pawlizyn, 1999) that relies on the partition equilibrium of target analytes between a polymeric stationary phase, a coated fused silica fiber, and the sample matrix (Peñalver *et al.*, 1999). It does not rely on the temporary storing of the analytes on a limited number of adsorptive sites (Zygmunt *et al.*, 2001). Although the molecular interaction is much weaker than in the case of

adsorbents, leading to a reduced retention power and reduced enrichment capacity but systems based on sorption show much lower catalytic activity and enable reactive substances to be enriched (Baltussen *et al.*, 1999a). In addition, SPME does not require organic solvents like LLE and SPE which are expensive and may be harmful to health and the environment. Furthermore, SPME has been shown to be a highly selective and sensitive technique being very simple, fast, easily automated and portable (Peñalver *et al.*, 1999; Supelco, 1998b). Therefore, it has potentially gained use in the extraction of many environmental analytes (Grebel *et al.*, 2006).

An SPME device was developed by Arthur and Pawliszyn in 1990 (Arthur and Pawliszyn, 1990) and commercialized in 1993 by the Supelco company (Lord and Pawliszyn, 2000) (Figure 3.7). It consists of a fused silica fiber with an outer diameter of typically 150 μm which is coated with a layer of an immobilized sorbent, typically 5-100 μm thick (Baltussen, 2000). The small size of the SPME fiber and its cylindrical shape allow the fiber to be fixed in the needle of a syringe-like device. The fiber can be exposed by depressing the syringe plunger into a gaseous or aqueous sample for analyte extraction and into the heated zone of a gas chromatographic injector or liquid chromatographic interface for desorption (Supelco, 1998b). The concept of SPME is shown in Figure 3.8.

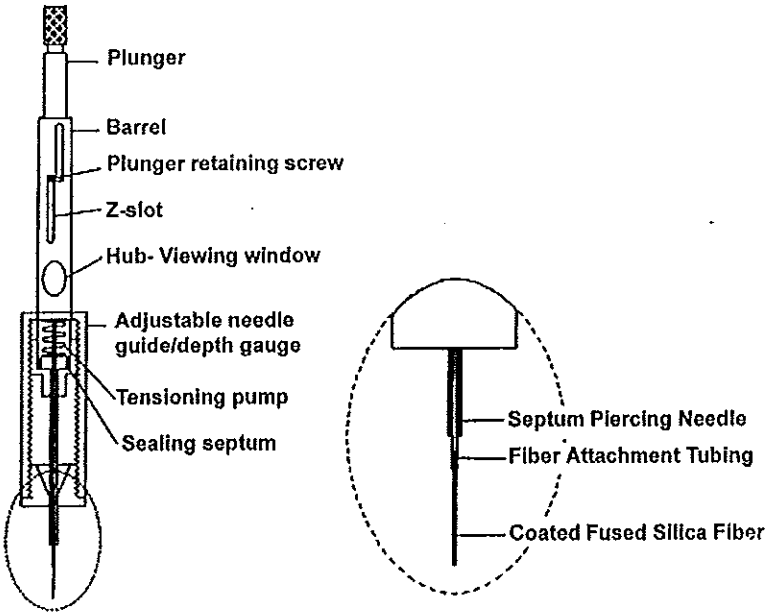


Figure 3.7 Design of the first commercial SPME device made by Supelco (Lord and Pawliszyn, 2000)

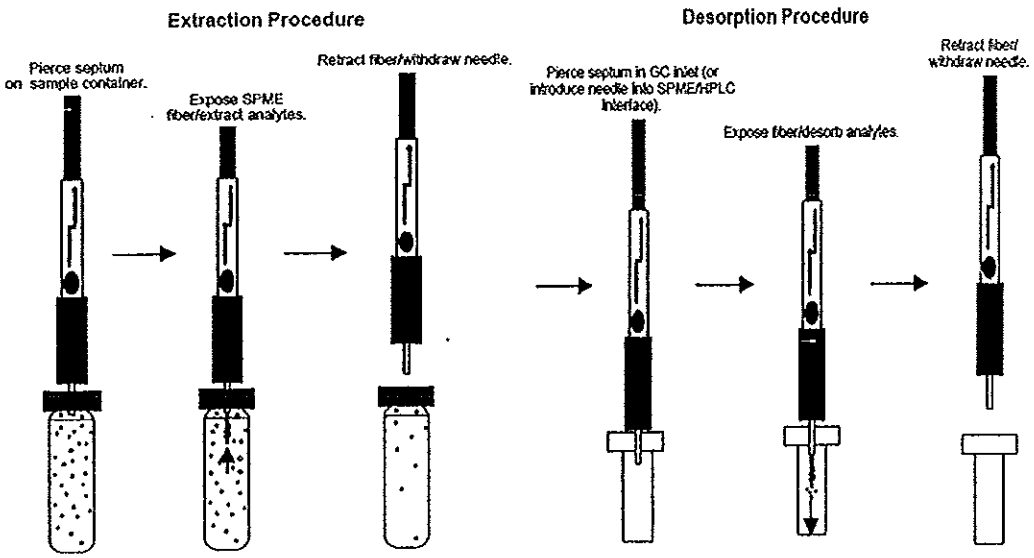


Figure 3.8 Concept of SPME (Supelco, 1998b)

The SPME process comprises two steps. First, the target analytes are extracted from a sample matrix by exposing a coated fiber to the sample for a period of time. The fiber is then removed from the sample and the retained analytes are desorbed in an analytical instrument in order to be separated and quantified (Pawlizyn, 1999). The entire process is very simple and can be automated and coupled to GC, HPLC or CE (Figures 3.8-3.10).

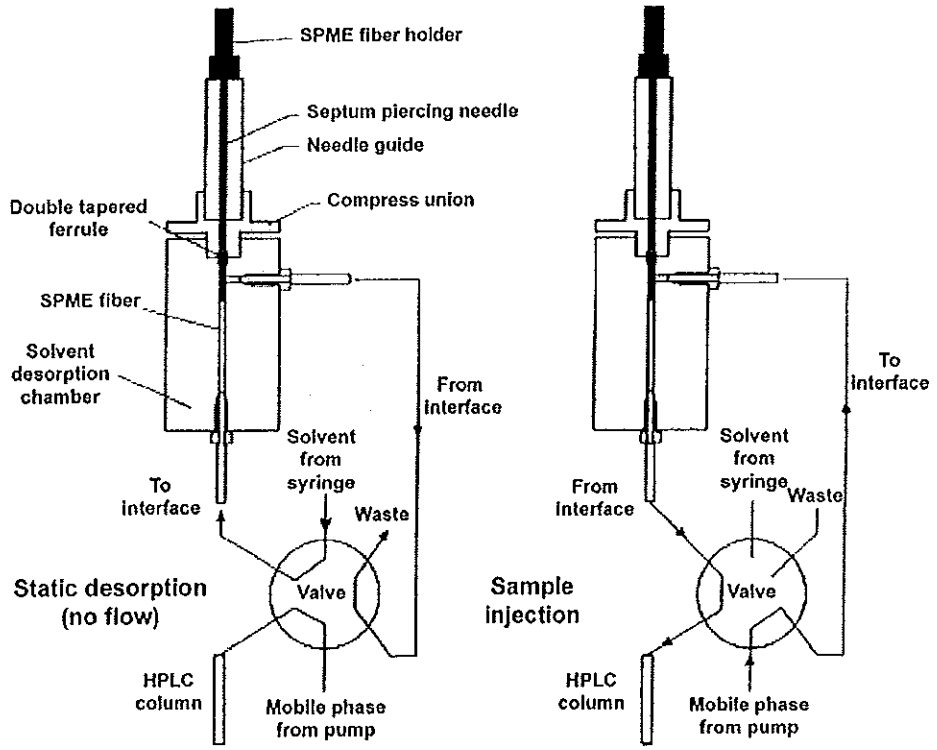


Figure 3.9 Schematic diagram of the Supelco SPME–HPLC interface (Supelco, 1998b)

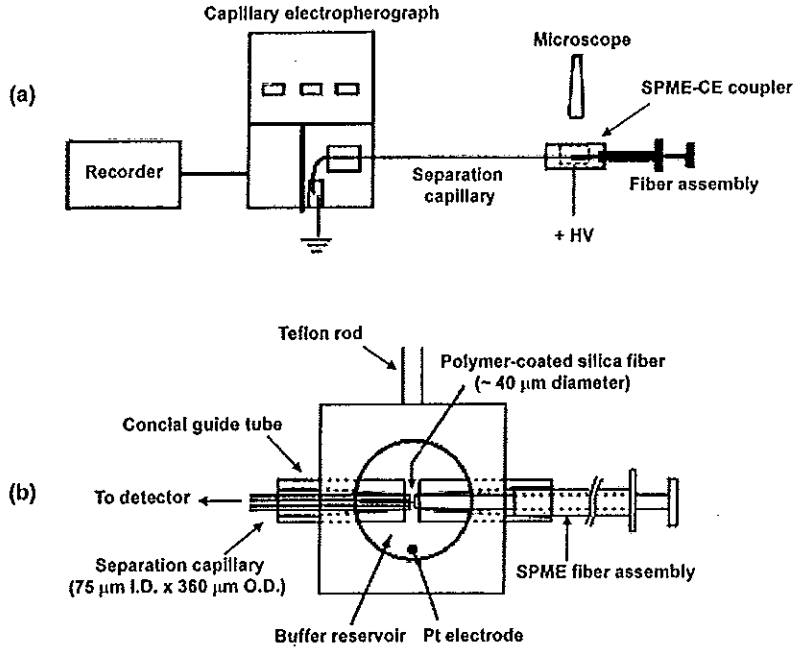


Figure 3.10 Schematic of the SPME–CE system (a) and interface (b) (Whang and Pawliszyn, 1998)

Typically, SPME extraction is considered to be completed when the analyte concentration has reached distribution equilibrium between the sample matrix and the fiber coating. In practice, this means that once equilibrium is reached, the extracted amount is constant within the limits of experimental error and it is independent of any further increase of extraction time. The equilibrium conditions can be described as (Lord and Pawliszyn, 2000; Louch *et al.*, 1992; Pawlizyn, 1999)

$$n = \frac{K_{fs} V_f V_s C_o}{K_{fa} V_f + V_s} \qquad (3.4)$$

where n is the number of moles extracted by the coating, K_{fs} is a fiber coating/sample matrix distribution constant, V_f is the fiber coating volume, V_s is the sample volume, and C_0 is the initial concentration of a given analyte in the sample.

When the sample volume is very large, Equation 3.4 can be simplified to

$$n = K_{fs} V_f C_o \qquad (3.5)$$

In this equation, the amount of extracted analyte is independent of the volume of the sample. Therefore, in practice there is no need to collect a defined sample prior to analysis as the fiber can be exposed directly to the ambient air, water, production stream, etc. The amount of extracted analyte will correspond directly to its concentration in the matrix without being dependent on the sample volume. When the sampling step is eliminated, the whole analytical process can be accelerated and errors associated with analyte losses through decomposition or adsorption on the sampling container walls will be prevented. This advantage could be enhanced practically by developing portable field devices on a commercial scale (Pawlizyn, 1999). Numerous applications using SPME for the determination of SVOCs, and priority pollutants in aqueous samples, have been reported as summarized in Table 3.4. Reported detection limits cover a very wide concentration range, from ng L^{-1} (ppt) to mg L^{-1} (ppm) level.

Another mode besides sampling directly in the aqueous phase is the extraction in the headspace. This was described theoretically by Zhang and Pawliszyn (Zhang and Pawliszyn, 1993) and Ai (Ai, 1998). In headspace-SPME, the volatilized analytes are extracted and concentrated on the SPME coating. The analytes will partition strongly into the SPME fiber and equilibration times can be reduced

substantially because diffusion coefficients are higher in the gas than in the liquid phase (Zhang and Pawliszyn, 1993). In addition, samples containing high molecular weight or particulate material can be analyzed with the greatest accuracy. Moreover, fiber lifetime is also extended because these unwanted compounds do not come into contact with the fiber (Llompart *et al.*, 1998).

Table 3.4 Summary of SPME techniques for extraction of SVOCs in water (2001-present)

Analyte	Recovery	Detection limit	Analytical technique	Reference
Amines	89-99%	3.5 - 5 μg L^{-1}	CE	(Fang *et al.*, 2006)
PAHs	80–110%	4–5 ng L^{-1}	GC-MS	(Hsieh *et al.*, 2006)
Phenolic compounds	88- 103%	0.47 - 9.01 μg L^{-1}	GC-FID	(Zhou *et al.*, 2005)
PAHs	-	0.05-0.16 μg L^{-1}	GC-FID	(Mohammadi *et al.*, 2005)
Herbicides	83-113%	1.2–3.4 mg L^{-1}	HPLC-UV	(Huang *et al.*, 2004)
PAHs	-	3-29 ng L^{-1}	GC-MS	(King *et al.*, 2004)
Pesticides	45-160%	0.01 μg L^{-1}	GC-MS	(Sakamoto and Tsutsumi, 2004)
Organochlorine pesticides	39-94%	< 0.081 ng L^{-1}	GC-ECD	(Cai *et al.*, 2003)
Aromatic amines	55-104%	0.6-17 μg L^{-1}	HPLC-UV	(Chang *et al.*, 2003)
Organophosphorous pesticides	-	2 - 8 μg L^{-1}	GC-MS	(Tomkins and Ilgner, 2002)
Phthalates	-	0.005-0.04 μg L^{-1}	GC-MS	(Luks-Betlej *et al.*, 2001)
Organophosphorous pesticides	75 -103%.	0.049 - 0.301 μg L^{-1}	GC-FPD	(Yao *et al.*, 2001)

Although SPME can be applied to determine SVOCs it has been primarily used for VOCs (Chang *et al.*, 2003; González-Toledo *et al.*, 2001; Huang *et al.*, 2004; Krutz *et al.*, 2003; Peñalver *et al.*, 2002; Popp *et al.*, 2000). Recently polydimethylsiloxane (PDMS), a popular coating that has high affinity of sorptive interaction between the coating and the non-polar compounds, was used to extract

SVOCs in water (Supelco, 1998b). However, because of the wide polarity range of SVOCs, from very apolar (PAHs, PCBs) to polar (some pesticides) (Peñalver *et al.*, 1999), PDMS is not applicable for more polar compounds and poor recovery is obtained (Supelco, 1998b).

To improve the capacity of SPME fibers for a wide range of SVOCs, several new SPME coatings have been introduced (DeBruin *et al.*, 1998; Martos and Pawliszyn, 1998). To date, only seven kinds of SPME coatings are commercially available, including polydimethylsiloxane (PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB), polyacrylate (PA), carboxen/polydimethylsiloxane (CB/PDMS), carbowax/divinylbenzene (CW/DVB), carbowax-templated resin and divinylbenzene/carboxen/polydimethyl siloxane (DVB/CB/PDMS). However, most of these fibers are generally prepared by simple physical deposition or partial crosslink of the polymer coating onto the surface of the fused-silica fibers. The lack of proper chemical bonding between the polymer coating and fused-silica fiber surface may be responsible for the low thermal and chemical stability. Additionally, selectivity for individual or specific analytes obtained on these fibers is limited (Hu *et al.*, 2006). Thus, future advancements in SPME technology should greatly depend on the development of new selective coatings (Mullett and Pawliszyn, 2003). Recently, some new types of fibers, such as polypyrrole (Xiao *et al.*, 2001), molecularly imprinted polymer (MIP) (Koster *et al.*, 2001), restricted access material (RAM) (Mullett *et al.*, 2002) and immunoaffinity coatings (Yuan *et al.*, 2001) were investigated. They provided good selectivity towards target analytes. Although these coating materials have significantly increased trapping capacity, the sorption mechanism is lost because these materials are no longer pure polymeric sorbents (Eisert and Levsen, 1996; Krutz *et al.*, 2003; Magdic and Pawliszyn, 1996). Therefore, the application of these fibers was still limited due to the difficulty and high cost of fiber preparation (Hu *et al.*, 2006).

Another problem with SPME concerns its detection limit. Although most compounds can be monitored below the desired 1 μg L^{-1} (ppb) level for surface water by SPME, the limit for drinking water (ca. 0.1 μg L^{-1}) cannot be reached for many analytes although high sensitive GC–ECD or GC–MS is applied (Baltussen, 2000). This lack in sensitivity is the most important disadvantage of SPME and is

partly due to the sorbents having significantly lower analyte capacity than typical adsorbents in SPE due to the extremely small amount of sorbent coated onto the fiber, up to 0.5 μL, and also the limit of the fiber length which varies between only 9 and 11 mm. Thus, higher capacity is restricted by mechanical problems and thermal stability of the fiber film (Lipinski, 2000). Therefore, it is hard to overcome the sensitivity problem (Baltussen *et al.*, 2002) and today sorption SPME is still not widely accepted despite its clear instrumental advantages, *i.e.*, simplicity and being solventless (Baltussen *et al.*, 1999b; Lord and Pawliszyn, 2000).

In addition, since SPME is an equilibrium sampling technique, long equilibration time is also a problem. In order to increase the speed of reaching equilibrium, the sample is almost always stirred during extraction (Motlagh and Pawliszyn, 1993). Although this is a rather straightforward approach, it is not ideally suitable for automated sampling because of the large number of stir bars needed and the time to reach equilibrium depends on the nature of the analytes, with equilibrium times ranging from 2 to 60 min (Grebel *et al.*, 2006; Supelco, 1998b). Therefore, the efficiency of SPME technique is still not enough to provide the good recovery and short analysis time required.

3.2.4 Stir bar sorptive extraction (SBSE)

Another technique of sorptive sample enrichment, with a larger volume of sorbent than in SPME, is known as SBSE. It was introduced in 1999 for trace enrichment of organic compounds from aqueous food, biological, and environmental samples (Baltussen *et al.*, 1999b). The theory is similar to SPME, therefore, the amount of analyte which is extracted at equilibrium can be calculated with the same equation as shown in Equation 3.4 (Baltussen, 2000).

The sample enrichment device consists of a magnetic stir bar or stainless steel encased in a glass sheath (Figure 3.11).

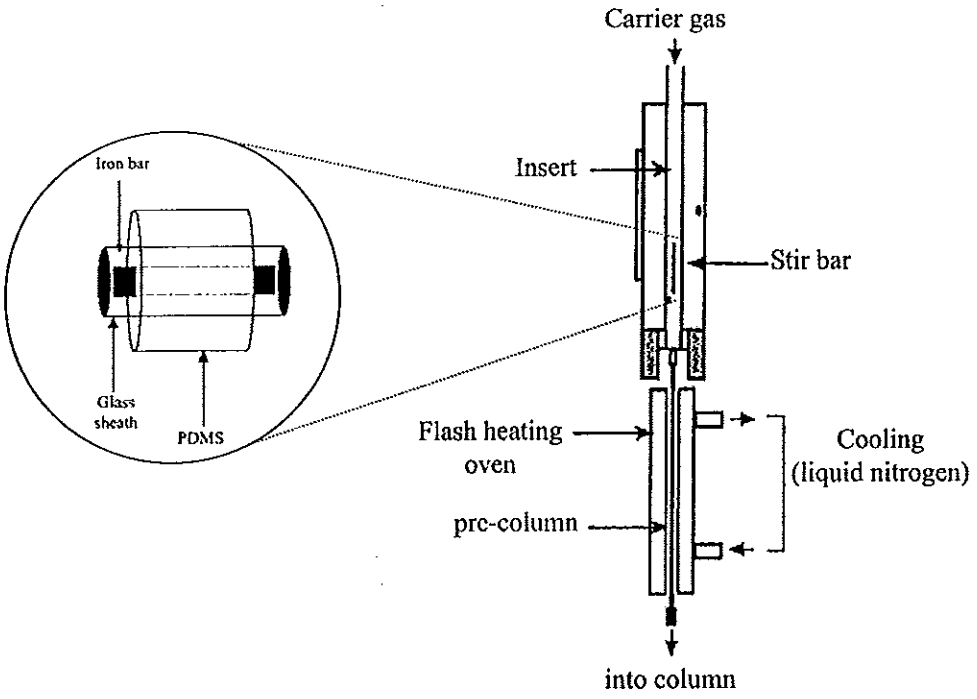


Figure 3.11 Schematic representation of a stir bar applied for SBSE and desorption unit (Redrawn from (Vercauteren *et al.*, 2001))

The glass is coated with a sorbent. The length of stir bar is typically 10 to 40 mm. The sorbent coating varies from 0.3 to 1 mm, resulting in sorbent phase volumes of 55-220 µL (Baltussen *et al.*, 1999b; García-Falcón *et al.*, 2004a). Liquid samples are extracted by placing the device in a flask or vial containing the sample. A magnetic stirrer is used to set the stir bar in rotation. This enhances the mass transport in the system and also increases the extraction rate (León *et al.*, 2003). After an extraction has been carried out, the stir bar device is manually removed from the sample container, optionally rinsed with pure water and finally dried by wiping with a tissue. Finally, the analytes are desorbed either thermally using a dedicated desorption unit (Ochiai *et al.*, 2002) or by extraction with a suitable solvent (Kolahgar *et al.*, 2002). In contrast to SPME where desorption is performed in the injection port or inlet of a gas chromatograph, SBSE is used in combination with a thermal desorption system. Because more extraction phase is used, the desorption process is slower than that for a SPME fiber. Therefore, the desorption system combined with cold trapping is

required (Baltussen, 2000). The whole process has been automated and two systems are commercially available, *i.e.*, the TDS-A classic thermal desorption system and a specially designed Twister desorption unit (Tienpont *et al.*, 2002). The systems can be mounted on gas chromatographs equipped with a programmed temperature vaporizing inlet. The programmed temperature vaporizing injector is operated as a cryotrap for cryogenic refocusing of the thermally desorbed analytes. Both systems allow fully automated control of all desorption, trapping and injection conditions, including temperatures, flows and split or splitless modes (David *et al.*, 2003).

Since a larger volume of sorbent is used in SBSE, ranging between 55-219 μL, a greater amount of analyte will be extracted, usually about 100-300 times more than extracted with SPME, and the limit of detection is also lowered, and can be down to sub-ng L^{-1} level (Serodio and Nogueira, 2004). SBSE has been successfully applied with a variety of SVOCs in water as summarized in Table 3.5. This technique when combined with thermodesorption–GC-MS enabled detection limits down to the low ng L^{-1} range because of the much lower phase ratio (volume of the water phase divided by the volume of the PDMS phase) compared to SPME (Popp *et al.*, 2001).

SBSE could also be combined with liquid chromatography (García-Falcón *et al.*, 2004a; Niehus *et al.*, 2002; Popp *et al.*, 2004; Popp *et al.*, 2001). For example, García-Falcón *et. al.* (2004a) used SBSE followed by high-performance liquid chromatography with a fluorescence detector to determine eight PAHs in water samples. Analytical method parameters such as linearity (r^2>0.991), precision (<9%), detection (0.5–7.3 ng L^{-1}), quantitation (1.0–22 ng L^{-1}) limits and relative recovery of about 100% were obtained.

Table 3.5 Summary of SBSE techniques for extraction of SVOCs in water (2001-present)

Analyte	Recovery	Detection limit	Analytical technique	Reference
Benzophenone (BP) and derivatives	98.5%	0.5–1 pg ml^{-1}	TD–GC–MS	(Kawaguchi *et al.*, 2006b)
PAHs	-	Sub ng L^{-1}	TD–GC–MS	(Roy *et al.*, 2005)
OH-PAHs	15.4-46.9%	0.27–25 ng L^{-1}	TD–GC–MS	(Itoh *et al.*, 2005)
PCBs	28% (PCB 209) and 93% (PCB 1, PCB 52, PCB 77)	0.05–0.15 ng L^{-1}	TD–GC–MS	(Popp *et al.*, 2005)
Pesticides	58.5–132.0%	0.2–20 ng L^{-1}	TD–GC–MS	(Nakamura and Daishima, 2005)
Pyrethroid pesticides	81.8-105.0%	1.0–2.5 ng L^{-1}	LVI–GC–MS	(Serodio and Nogueira, 2005)
PAHs	62-97%	0.1–1.2 ng L^{-1}	HPLC–ED	(Popp *et al.*, 2004)
PCBs	62.1-86.7%	0.3-1.8 ng L^{-1}	TD-GC-MS	(Montero *et al.*, 2004)
PAHs	43-57%	0.5-7.3 ng L^{-1}	HPLC-FD	(García-Falcón *et al.*, 2004a)
PAHs	20.1-97.2%	0.3–2 ng L^{-1}	HPLC–ED	(Popp *et al.*, 2003)
Endocrine disruptors	42 - 96%	0.01–0.24 ng L^{-1}	LVI–GC–MS	(Peñalver *et al.*, 2003)
PAHs	54-100%	0.4–5 ng L^{-1}	HPLC–ED	(Niehus *et al.*, 2002)
Pesticides	8-84%	0.0001-6 mg L^{-1}	LC-MS	(Blasco *et al.*, 2002)
PAHs	58-100%	0.2–2 ng L^{-1}	HPLC–ED	(Popp *et al.*, 2001)
PCBs	50-60%	10 ng L^{-1}	TD-GC-MS	(Benijts *et al.*, 2001)

Besides SBSE, the stir bar technique has also been applied to headspace sorptive extraction (HSSE) (Bicchi *et al.*, 2000; Ochiai *et al.*, 2001; Tienpont *et al.*, 2002) as shown in Figure 3.12. The stir bar coated with sorbent is normally hung above sample level to adsorb evaporated analyte of interest which is then thermally desorbed for analysis.

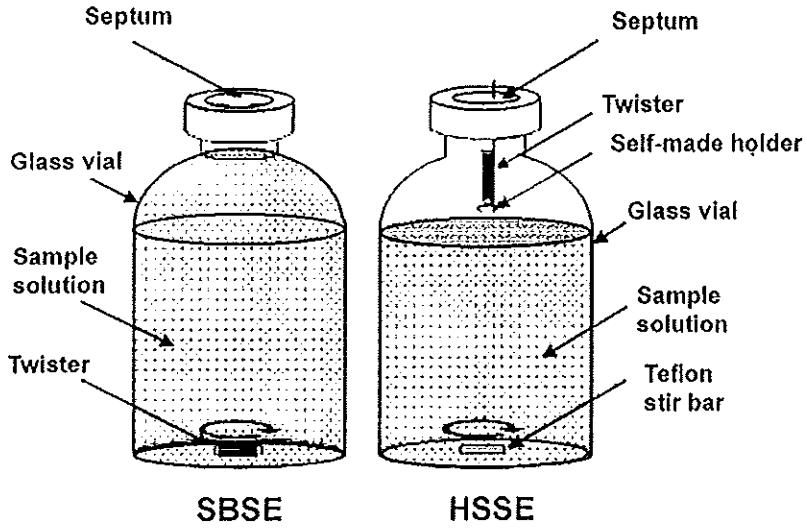


Figure 3.12 Diagram of SBSE and HSSE set-ups (Ochiai *et al.*, 2001)

To date, the only sorbent which has reportedly been used for coating the stir bar, is polydimethylsiloxane (PDMS) (Bicchi *et al.*, 2003), however, the use of stir bars coated with polar sorbents is predicted for the future (Baltussen *et al.*, 1999b). Using PDMS, the primary mechanism of interaction with organic solutes is via absorption or partitioning into the PDMS coating such that the distribution constant between PDMS and water ($K_{PDMS=W}$) is proposed to be proportional to the octanol-water partition coefficient (K_{OW}) as shown in the Equation 3.6.

$$K_D = \frac{[X]_B}{[X]_A} = K_{PDMS/W} \approx K_{OW} \qquad (3.6)$$

According to the theoretical development for the SBSE technique given by Baltussen *et al.* (Baltussen *et al.*, 1999b);

$$K_{OW} \approx K_{PDMS/W} = \frac{[X]_{PDMS}}{[X]_W} = \frac{m_{PDMS}}{m_W} \times \frac{V_W}{V_{PDMS}} = \beta \frac{m_{PDMS}}{m_W} \quad (3.7)$$

where $[X]_{PDMS}$ and $[X]_W$, m_{PDMS} and m_W, are the analyte concentration and the analyte mass in the PDMS and water phase, respectively. While V_{PDMS} and V_W represent the volume of the PDMS sorbent and water phase, respectively. Therefore, the parameters for determining the mass of an analyte which are recovered by SBSE using the PDMS sorbent are the partition coefficient of the analyte K_{OW} and the phase ratio β of the volume of the water phase to the volume of the PDMS coating on the stir bar.

Baltussen *et al.* (1999b) has theoretically compared the recovery by SBSE (using a stir bar which was assumed to be coated with a 100 µL volume of PDMS) to recovery by SPME (having an assumed coating volume of 0.5 µL of PDMS) (Baltussen *et al.*, 1999a). The extraction of a 10 mL sample of water was demonstrated and the results are as shown in Figure 3.13. With SPME, a poor recovery was obtained from analytes with low K_{OW} values while SBSE gave a higher value. A small volume of the PDMS sorbent used in SPME resulted in a large phase ratio (V_W/V_{PDMS}) as implied in equation 3.7. Therefore, the theoretical extraction efficiency will reach 100% for analytes with high octanol–water partition coefficient (log K_{OW} greater than 2.7) (Baltussen *et al.*, 2002). However, since the volumes of PDMS used in SBSE is larger than in SPME, a longer time is required to reach equilibrium because more analyte mass will be transferred to the PDMS sorbent phase (Vercauteren *et al.*, 2001). Therefore, the extraction efficiency remains a balance between sorbent mass and sample volume. Besides, it is possible that co-concentration of undesirable matrix components from solution may occur due to the nonselective sorptive capability of the PDMS sorbent. Since SBSE produces analyte accumulation in the sorbent but not sample cleanup, the study of matrix effects has to be considered (Benijts *et al.*, 2001; Ochiai *et al.*, 2002).

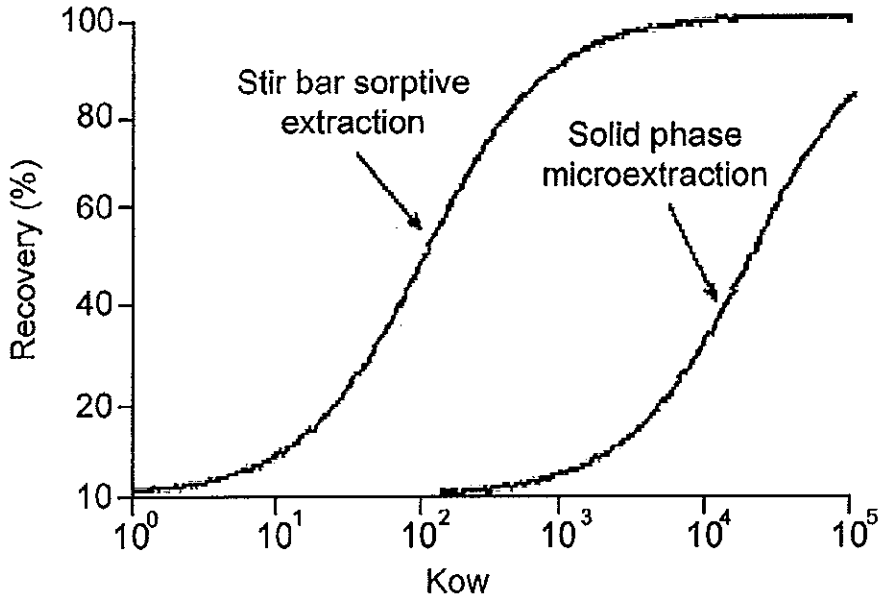


Figure 3.13 Theoretical recovery of analysis in SBSE and SPME from a 10 mL water sample as a function of their octanol-water partitioning constant. Volume of PDMS on SPME fiber: 0.5 µL: volume of PDMS on SBSE stir bar: 100 µL (Baltussen *et al.*, 1999a)

3.3 Alternative sample preparation techniques for SVOCs in water

Although many conventional sample preparation methods are still in use, the trend in recent years has been changed towards efficient green chemistry. The goals include the ability to use smaller initial sample sizes even for trace analyses, greater selectivity in extraction, increasing of potential for automation or for in-line/on-line methods, reducing manual operations and more environmental friendly approach with less waste by the use of small volumes or no organic solvents. These are being achieved in different ways and are still the subject of active research. This work lead to the introducing of newly developed techniques, *i.e.*, membrane extraction (ME) and capillary microextraction (CME) for analysis of SVOCs.

3.3.1 Membrane extraction (ME)

The application of ME as a sample preparation tool in analytical chemistry was first introduced by G. Audunsson, at the Department of Analytical

Chemistry, Lund University, Lund, Sweden (Nilvé *et al.*, 1989). Several review papers that provide background information and applications have been published (Jönsson and Mathiasson, 2000; Ketola *et al.*, 2002; van de Merbel, 1999). Membrane extraction is a family of techniques that requires very little or no solvent and provides remarkable clean-up efficiency.

Generally, a membrane can be defined as a selective barrier between two phases. When a driving force is applied across a membrane, the transport of analyte from one phase (the donor phase or feed side) to the other (the acceptor phase or permeate side) occurs (van de Merbel, 1999). The rate of extraction is referred to as a flux (Figure 3.14) of analyte from the donor to acceptor and this will be proportional to the concentration difference (Δc) of the diffusing species through the membrane. Thus,

$$\Delta C = \alpha_D C_D - \alpha_A C_A \qquad (3.8)$$

where C_D and C_A are the concentration in the donor (sample) and acceptor phases, and α_D and α_A are the fractions of the analytes in the donor and acceptor phases, respectively. The extraction conditions are typically selected when the value of α_D is close to 1 and α_A is very small. C_A is zero at the beginning of the extraction and increases during the process, to greater than C_D which is equal to the total concentration in the extracted sample.

Normally, the extraction in this technique can be evaluated in terms of extraction efficiency (E) which is the fraction of analyte collected in the acceptor:

$$E = \frac{n_A}{n_S} \qquad (3.9)$$

where n_S and n_A are the number of moles of analyte in the sample and analyte collected in the acceptor, respectively. This parameter is similar to recovery but not identical as observed in SPE because the extraction efficiency is a function of flow rate and other physical parameters (Jönsson and Mathiasson, 2003). Most of the high extraction efficiency is obtained at the low donor flow rates because a low flow rate will increase the residence time of an analyte molecule in the donor channel. However, in practice, it is often relevant to maximize the enrichment concentration rather than the extraction efficiency so that it could provide the largest instrument

signals for a given extraction time. Therefore, the enrichment concentration by membrane extraction is based on the change of flow rate of either donor or acceptor phase (Jönsson and Mathiasson, 2003; Kuosmanen *et al.*, 2003; Wang *et al.*, 2005b; Wang and Mitra, 2005c).

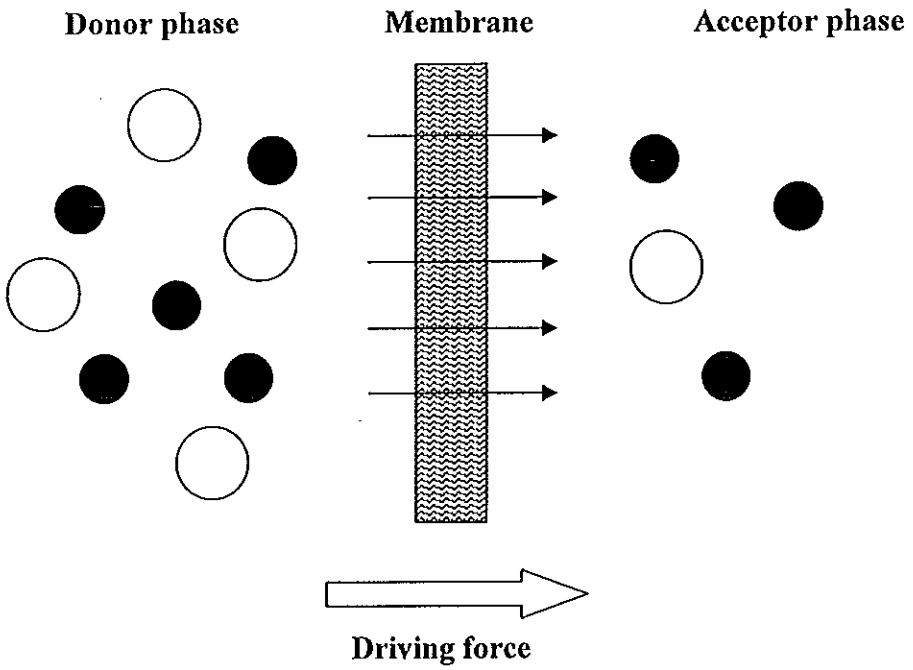


Figure 3.14 Schematic of a membrane system (van de Merbel, 1999)

Typically, the driving forces for analyte movement across the membrane involve, *i.e.*, (i) a concentration difference which leads to a molecule flux, (ii) an electric potential difference which leads to an electrical flux and (iii) pressure difference which leads to a volume flux (van de Merbel, 1999). Very often, more than one of these driving forces is presented in a membrane extraction process although one of them will dominate. In most cases, the driving force is a concentration gradient (Smith, 2003). Extraction is achieved when some interest species are transported to a larger extent than the others and ideally, one component is completely transported from the donor to the acceptor phase, while all other components are totally retained. In addition, the extraction can be enhanced by removing the analyte from the acceptor phase by many reactions, *i.e.*, ionization, complexation, or derivatization so that the free solution concentration of the analyte species in the acceptor phase is reduced

(Smith, 2003). The systems based upon ME can be automated readily and connected on-line and in-line to chromatography systems and other instruments. A wide polarity range of analytes can be extracted (van de Merbel, 1999).

Membranes can be categorized as porous and non-porous, and based on structure or geometry as flat sheet, spiral and hollow fiber (Calabro *et al.*, 2002; Jönsson and Mathiasson, 2000; Matsuyama *et al.*, 2001; Müller *et al.*, 2003; Srinivasan *et al.*, 1997). A wide variety of membrane materials can be used such as polypropylene, polysulfone or a cellulose derivative (Cordero *et al.*, 2000). The separation by the porous membranes is not only based on a difference in size, but also ionic compounds with the same charge as the membrane ions are included (van de Merbel, 1999). For a non-porous membrane, it consists of a liquid or polymer film in which a molecule must actually dissolve in order to be able to pass through. In this case, the efficiency of membrane to transport the particular compound is largely dependent on the partition coefficients between the different parts of a membrane separation system. Only compounds which are easily extracted from the donor phase into the membrane and also easily extracted from the membrane into the acceptor phase will be transported (Jönsson and Mathiasson, 2000).

In order to increase the membrane area, hollow fiber membranes can be used instead of the planar forms. Typically, hollow fiber membranes are shaped like tubes (200 to 500 um I.D.), allowing fluids to flow inside as well as on the outside (Pinto *et al.*, 1999; Sysoev *et al.*, 2001). The diffusion of analyte through the hollow fiber membrane is much larger therefore, the recovery per unit time is much higher which leads to improved detection limit (Jönsson and Mathiasson, 2000; van de Merbel, 1999).

For instrumentation, in flow systems, membrane holders for membrane extraction are usually constructed of two blocks of inert material with a machined groove in each. The blocks are clamped together with a membrane between them, thus, a flow through channel is formed on each side of the membrane as shown in Figure 3.15. For sample preparation use, channel volumes are typically in the range 10–1000 μL (Jönsson and Mathiasson, 2000). With proper modification, the membrane units shown in Figure 3.15 are in principle applicable to all versions of ME.

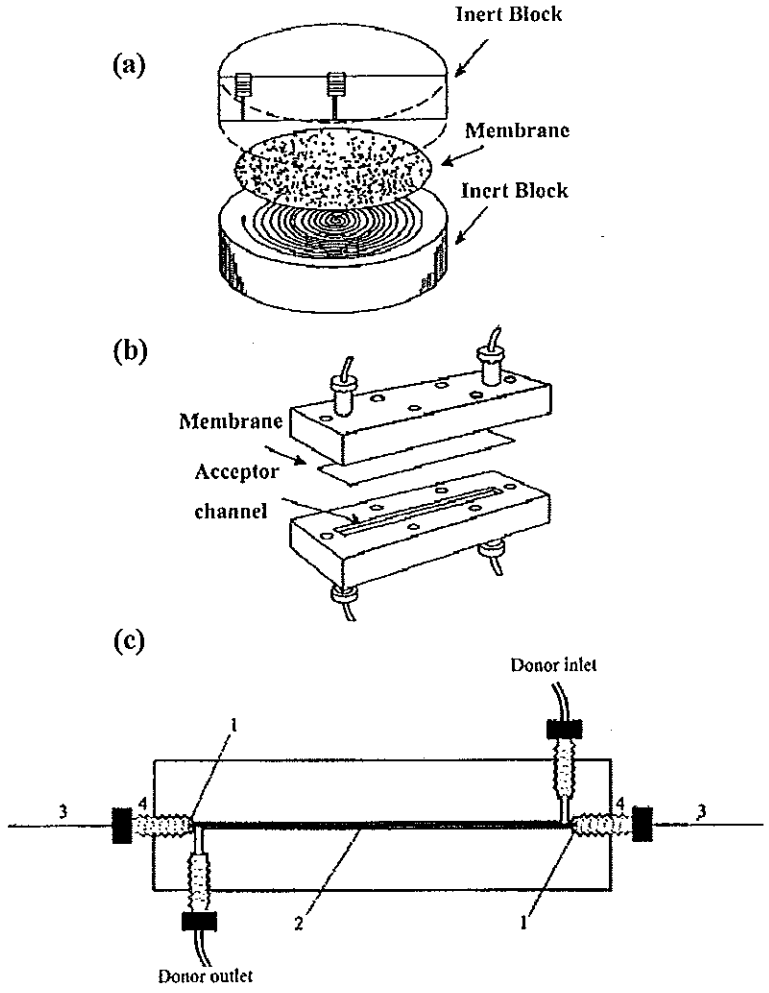


Figure 3.15 Different membrane modules for flow system. (a) flat membrane module with spiral channel, (b) flat membrane module with 10 uL channel volume and (c) hollow fiber module with 1.3 uL acceptor channel(1=O-rings, 2=polypropylene hollow fiber, 3=fused silica capillaries, 4=male nuts) (Jönsson and Mathiasson, 2000).

Generally, there are two ways to design a hollow fiber membrane unit (Figure 3.16) (Kou *et al.*, 2001). The membrane can be introduced into the sample or the sample can be introduced into the membrane. For the first design (Figure 3.16a-d), the ratio of membrane surface area to sample is quite low. Therefore, the sample usually needed stirring in order to enhance analyte diffusion (Jönsson and Mathiasson, 2000). In another design, the aqueous sample is either made to "flow over" (Figure

3.16a) or "flow through" (Figure 3.16f) the hollow fiber while the stripping of carrier (gas or organic solvent) flows countercurrent on the other side. The contact surface per volume is much higher than the previous one. In separation, the flow through mode provides higher extraction efficiency than the flow over mode because tube side volume is smaller than the shell side volume which results in a higher surface per volume ratio for aqueous sample (Ketola *et al.*, 2002; Kotiaho *et al.*, 1991; Srinivasan *et al.*, 1997).

For applications, ME has been used in combination with LC, GC, CE and MS separation systems for analysis of SVOCs in water as shown in Table 3.6. The on-line coupling to an LC system is the most straightforward. Transferring a part of the acceptor phase to an injection loop and injecting it is in principle sufficient. If the acceptor volume is relatively large compared to the injection volume, a major part of the enriched analyte fraction is lost (Jönsson and Mathiasson, 2000). Therefore, this approach is only attractive if analyte concentrations are not too low (Shen *et al.*, 1996). For trace analysis, it is better to either use a miniaturized membrane system with a low acceptor volume which can be completely injected (Lindegard *et al.*, 1994) or concentrate the entire acceptor phase on a preconcentration column. In the latter case, it should be realized that the analytes of interest are always in their ionized form, which means that the pH of the acceptor phase will have to be adjusted in order to neutralize and allow trapping on a hydrophobic phase (van de Merbel, 1999). In many cases, this is also performed on-line; examples are the determination of chlorinated phenols (Knutsson *et al.*, 1996) and phenylurea herbicides (Nilvé *et al.*, 1994) in natural water samples, using a polymer phase and an octadecylsilica phase, respectively.

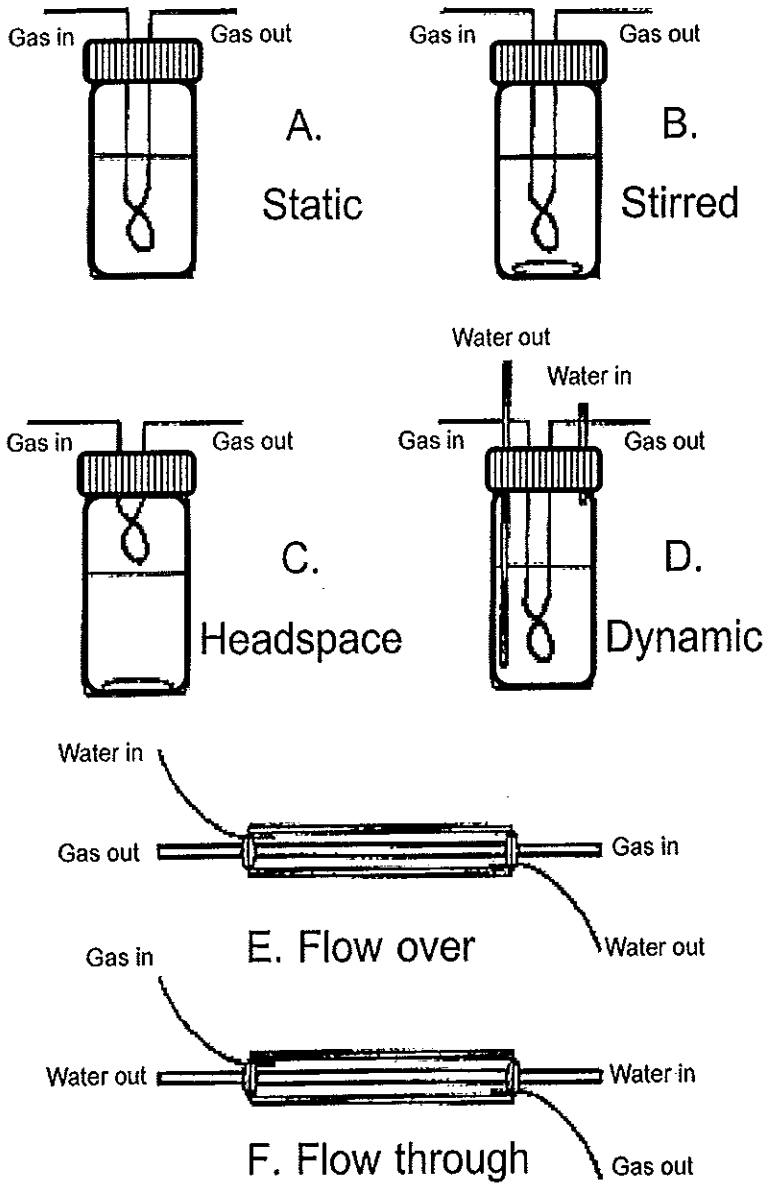


Figure 3.16 Different configurations of hollow fiber membrane extraction unit (Smith, 2003)

Table 3.6 Summary of ME techniques for extraction of SVOCs in water (2001-present)

Analyte	Recovery	Detection limit	Analytical technique	Reference
Organochlorine pesticides	68-116%	1–20 ng L^{-1}	GC-ECD, GC-MS	(Barri *et al.*, 2006)
Phenolic herbicides	50-130%	6.0 - 8.0 ng L^{-1}	HPLC	(Berhanu *et al.*, 2006)
2,4,6-Trichlorophenol	100–120%	25-30 µg L^{-1}	ISLM–FFIA	(Tudorache and Emnéus, 2005)
Phenols	42% (2-chlorophenol) 98% (2,4-dichlorophenol)	0.01-0.6 µg L^{-1}	GC-MS	(Schellin and Popp, 2005)
Haloacetic acid	-	0.02-2.69 µg L^{-1}	HPLC	(Kou *et al.*, 2004)
Organotin compounds	63–94% (deionised water) 52–89% (sea water)	0.5-1.5 µg L^{-1}	GC-FID	(Cukrowska *et al.*, 2004)
Organochlorine compounds	85-108%	0.001–0.008 µg L^{-1}	GC-MS	(Basheer *et al.*, 2004)
Chlorophenols	70–121%.	0.02–0.09 µg L^{-1}	HPLC	(Liu *et al.*, 2003b)
Sulfonylurea herbicides	85-108%	10–50 ng L^{-1}	HPLC	(Liu *et al.*, 2003a)
Organophosphorus pesticides	66-94%.	0.05 µg L^{-1}	GC-PFPD	(Xu *et al.*, 2003)
Sulfonylurea herbicides	83-95%	0.05 µg L^{-1}	HPLC	(Chao *et al.*, 2002)
Fungicides	-	0.25 - 0.5 µg L^{-1}	HPLC	(Sandahl *et al.*, 2002)
Phenolic compounds	90- 106%	0.5-20 µg L^{-1}	FIA-MIMS	(Alberici *et al.*, 2001)

Coupling of ME to packed column GC has been performed simply by injecting part of the aqueous acceptor phase into the GC column (van de Merbel, 1999). More recently, in order to on-line couple the membrane unit to a capillary GC system, an interface was used for the removal of water and the transfer of the analytes into an organic solvent (Matz *et al.*, 1999; Shen *et al.*, 1998). More suitable for direct coupling to GC is the use of an entirely organic acceptor phase, which has been performed with silicone membranes (van de Merbel, 1999). Another way is to a sorbent trap can interface the membrane to the GC. In this case, the analytes that have permeated across the membrane are carried by a gas stream to the trap for preconcentration. The trap is then heated rapidly to desorb the analytes into the GC as a narrow injection band (Guo and Mitra, 1999a:1999b: 2000).

For complex samples, a mass spectrometer (MS) is adapted to couple to GC for separation and detection. Detection limits of the membrane-based techniques are typically in the ppt to ppb range (Mitra, 2004). A membrane extraction system has also been coupled on-line to CE (Pálmarsdóttir *et al.*, 1997). A miniaturized hollow fiber membrane with a very low inner volume (1.3 mL) was used to enrich the analyte (the basic drug bamphase, buterol) in a volume compatible to direct injection into the CE capillary via the double-stacking technique. Although the membrane unit was connected on-line to the CE system, the method involved a manual step, because the CE capillary had to be placed in the electrode vessel after injection of the enriched analyte fraction (Thordarson *et al.*, 1996). In another approach, ME has been interfaced to MS and called membrane introduction (inlet) mass spectrometry (MIMS). MIMS was first reported in 1963 by Hoch and Kok (1963) for measuring oxygen and carbon dioxide in the kinetic studies of photosynthesis, with the membrane being placed in the vacuum compartment of the MS. All permeates enter into the ionization source of the instrument directly as shown in Figures 3.17 and 3.18.

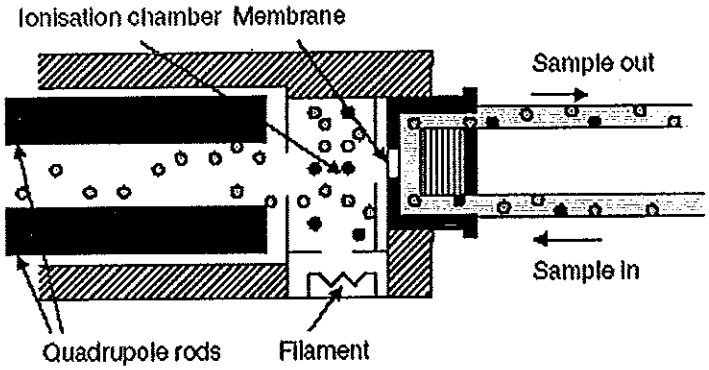


Figure 3.17 Schematic diagram of a membrane introduction mass spectrometer. The water or air sample is circulated over the membrane and the analytes pass through the membrane into the ionization chamber of the mass spectrometer (Kotiaho *et al.*, 1991).

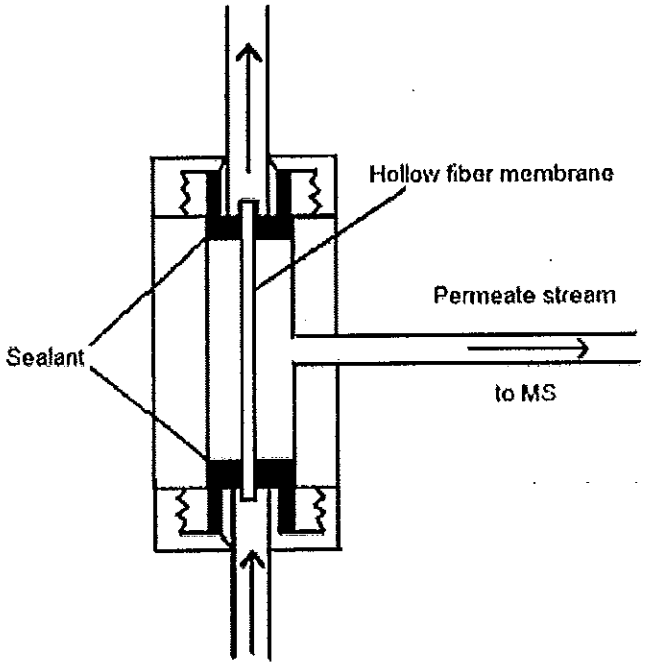


Figure 3.18 In the flow-through geometry, the sample flows across the inner surface of the membrane while the outer surface is exposed to the mass spectrometer vacuum (LaPack *et al.*, 1990).

In most instances, the sample matrix is excluded to a very high degree. Traditionally, a semipermeable membrane, usually silicone rubber, is placed between the liquid or gaseous sample and the vacuum of the mass spectrometer. Analytes of interest pass across the membrane into the mass spectrometer by a three step process called pervaporation. Pervaporation is a term used to describe the extraction of organic compounds from an aqueous matrix to a gas phase through a semipermeable membrane. The three steps of pervaporation are (a) adsorption of the analyte on the surface of the membrane, (b) diffusion of the analyte through the membrane under the force of a concentration gradient, and (c) evaporation of the analyte into the vacuum of the mass spectrometer. The extraction is derived by the concentration gradient across the membrane which governed by Fick's first law (Kotiaho *et al.*, 1991; Srinivasan *et al.*, 1997).

The analysis by MIMS is very fast with typically analysis times in the range of 1 to 6 minutes and usually requires less than 1 mL of sample, and also using ME in this application can eliminate the need for any sample preparation compared to conventional methods (Allen *et al.*, 2001; Bauer, 1995; Bocchini *et al.*, 2001). However, this technique has not been used for the routine analysis for SVOCs in water because the membrane can not be operated at temperatures much higher than 70°C before bubble formation in front of the membrane cause highly unstable signals (Ketola *et al.*, 2002). Moreover, at temperatures above 100°C the signals fall almost to baseline because the large volumetric expansion of water as it starts to boil (Bier *et al.*, 1990). Therefore, the low inlet temperature limits the vaporization of the SVOCs from the membrane surface and results in long membrane response time (> 5 min) for such compounds. It indicates that MIMS is not the ideal answer to every analytical problem. The technique will never replace chromatographic techniques which are well established and have been used with great success for decades. Samples that contain very complex mixtures of analytes will still require chromatographic separation to untangle the complex web of overlapping spectral masses that would be impossible to solve using MIMS techniques (Bauer, 1995).

Therefore, this thesis focused an the effort to develop ME system for multi residue SVOCs analysis by in-line coupling to GC-MS. GC-MS is one of the most attractive and powerful techniques for routine analysis of some ubiquitous

organic pollutants due to its good sensitivity and high selectivity and versatility (Santos and Galceran, 2003).

3.3.2 Capillary microextraction (CME)

CME or "in-tube solid phase microextraction", is a form of open tubular trapping that was pioneered by Kaiser and Rieder (Kaiser and Rieder, 1989), Grob and co-workers (Grob and Schilling, 1985; Grob *et al.*, 1990; Grob and Habich, 1985), Roerraade and Blomberg (Bicchi *et al.*, 1989; Blomberg and Roeraade, 1987; Burger and Munro, 1986; Burger and Munro, 1987; Roeraade and Blomberg, 1989). The principle of extraction is similar to SPME (Equation 3.4 and 3.5), but the extraction device is a piece of fused silica GC capillary column instead of a fiber. Typically PDMS, a sorptive phase, was utilized for dynamic enrichment (Blomberg and Roeraade, 1988; Burger and Munro, 1986). The coated lengths of fused silica columns with an inner diameter of 0.3-0.5 mm are commonly employed. A typical film thickness of 10-15 μm is used (Bicchi *et al.*, 1989) but in some cases high capacity CME or open tubular traps with extremely thick films of 100 μm (Roeraade and Blomberg, 1989) or even 165 μm (Burger and Munro, 1986) are preferred. In dynamic sampling process, the absorptive action is based on the partition law (Nardi, 2003b). Organic compounds in aqueous samples are directly extracted from the sample into the internally coated stationary phase of a capillary column and then desorbed for analysis by introducing a stream of mobile phase or static desorption with organic solvent (Kataoka, 2003) and also occasionally, it can be desorbed in the injector of GC (Grob and Habich, 1985). Desorbed compounds are finally injected into the column for analysis.

The main advantage of CME is that complete water removal from the trap can be obtained by purging with a short plug of gas through the capillary while long drying times, such as in solid phase extraction on cartridges or disks, are required (Baltussen *et al.*, 1998). In addition, there is no need for cryotrapping or other focusing techniques as in SBSE (Baltussen, 2000), thus, it is more economical. By using a piece of bonded-phase capillary GC column for sorption, a larger amount of stationary phase and a more robust film are obtained compared to SPME. These differences result in higher enrichment factors and longer extractor life. Also many

capillary GC stationary phases are commercially available, therefore, CME enables easy changing of the extraction-phase polarity, which extends the application range of the method (Wang *et al.*, 2004). For example, Eisert and Pawlizyn used Omegawax were able to extract several phenylurea pesticides from aqueous samples. The sample was analyzed by CME coupled with HPLC-UV. Low detection limits were obtained at approximately 10 μg L^{-1} (Eisert and Pawliszyn, 1997). In 2002, Mullette *et al.* reported the use of a molecular imprinted polymeric material for the selective determination of propranolol in biological samples. The results showed good recovery because of the high affinity of the adsorbent for the target analytes (Mullett *et al.*, 2002). Recently, in oeder to achieve ultra trace levels (pg L^{-1}), Kabir *et al.* (2004) developed a surface-bonded sol-gel polytetrahydrofuran coating in order to determine polar and non-polar compounds using CME coupled with GC-FID. Low detection limits were obtained (260-1000 pg L^{-1}) with RSD < 12%. For SVOCs analysis, CME can be an attractive enrichment technique in aqueous samples and several reports have been published as shown in Table 3.7.

Table 3.7 Summary of CME techniques for extraction of SVOCs in water (2001-present)

Analyte	Recovery	Detection limit	Analytical technique	Reference
Fluoroquinolones (FQs)	above 81%	7-29 ng L^{-1}	LC/MS/MS	(Mitani and Kataoka, 2006)
Estrogens	above 86%	2.7-11.7 ng L^{-1}	LC/MS/MS	(Mitani *et al.*, 2005)
Hexachlorocyclohexanes (HCHs)	-	2–12 ng L^{-1}	GC–ECD	(Wang *et al.*, 2005a)
PAHs	-	0.15-3.07 µg L^{-1}	HPLC	(Kim *et al.*, 2004)
Polar and non-polar compounds	-	260-1000 pg L^{-1}	GC-FID	(Kabir *et al.*, 2004)
Phenols and polycyclic aromatic hydrocarbons (PAHs)	52.2- 80%	< 6 µg L^{-1}	GC-FID	(Olejniczak *et al.*, 2003)
Polar pesticides	-	0.01-1.2 µg L^{-1}	HPLC–ESI-MS	(Wu *et al.*, 2002)
Endocrine disruptors	-	0.1-4.0 µg L^{-1}	HPLC	(Kataoka *et al.*, 2002)
Aromatic compounds	89.6- 96.4%	0.4-10 µg L^{-1}	HPLC	(Xiao *et al.*, 2001)
Propranolol	-	0.32 mg L^{-1}	HPLC	(Mullett and Pawliszyn, 2001)
Carbamates	15 - 34%	0.02-0.3 µg L^{-1}	HPLC	(Gou and Pawliszyn, 2000)
Carbamate pesticides	97.3-100%	1 µg L^{-1}	HPLC	(Gou *et.al.*, 2000)

Figures 3.19 and 3.20 illustrate examples of system which CME can be coupled on-line or in-line with HPLC and GC (Baltussen *et al.*, 2002; Djozan and Amir-Zehni, 2004; Gou *et. al.*, 2000; Gou and Pawliszyn, 2000; Olejniczak *et al.*, 2003; Wang *et al.*, 2004). In both systems, CME shows suitability for automation, *i.e.*, extraction, desorption and injection can be performed continuously using a standard autosampler. These automated procedures not only shorten the total analysis time, but will also be more accurate and precise than manual techniques (Djozan and Amir-Zehni, 2004; Nardi, 2003b). In addition, no carryover is observed after the desorption step because the capillary can be thoroughly washed. Therefore better reproducibility can be achieved (Gou *et al.*, 2000; Xiao *et al.*, 2001).

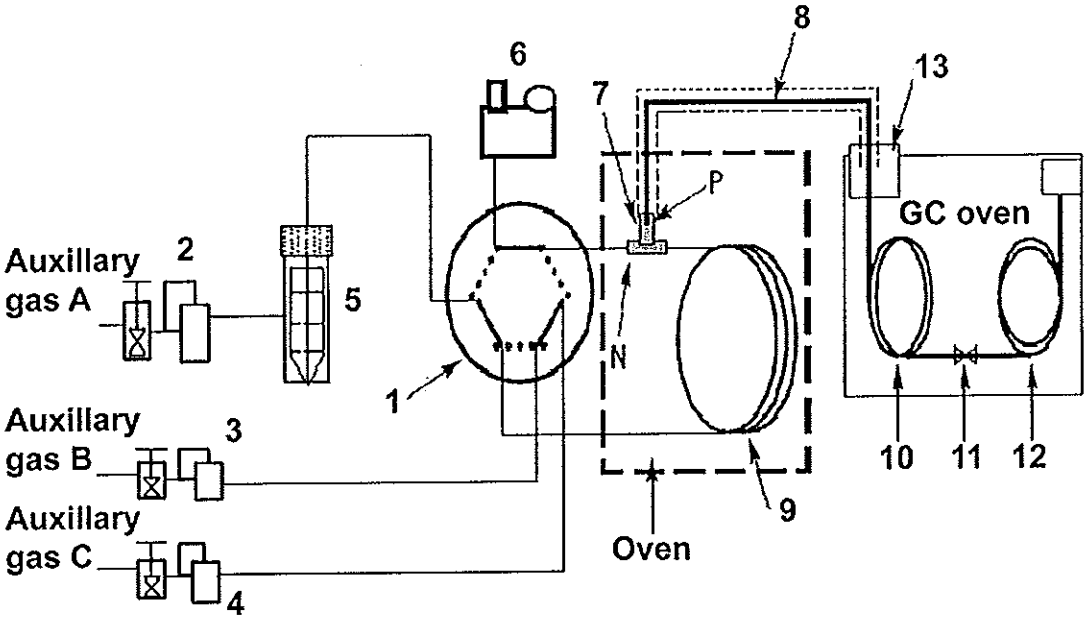


Figure 3.19 Schematic diagram of the on-line in-tube SPME system coupled with the high resolution GC system. (1) six-port valve, (2) flow controller for sampling, (3) flow controller for desorption gas, (4) flow controller for auxiliary gas, (5) sample vial, (6) mini water-circulating pump, (7) micro tee piece, (8) capillary transfer, (9) capillary extractor, (10) precolumn, (11) press-fit or micro union, (12) analytical column, (13) on-column injector (Wang *et al.*, 2004).

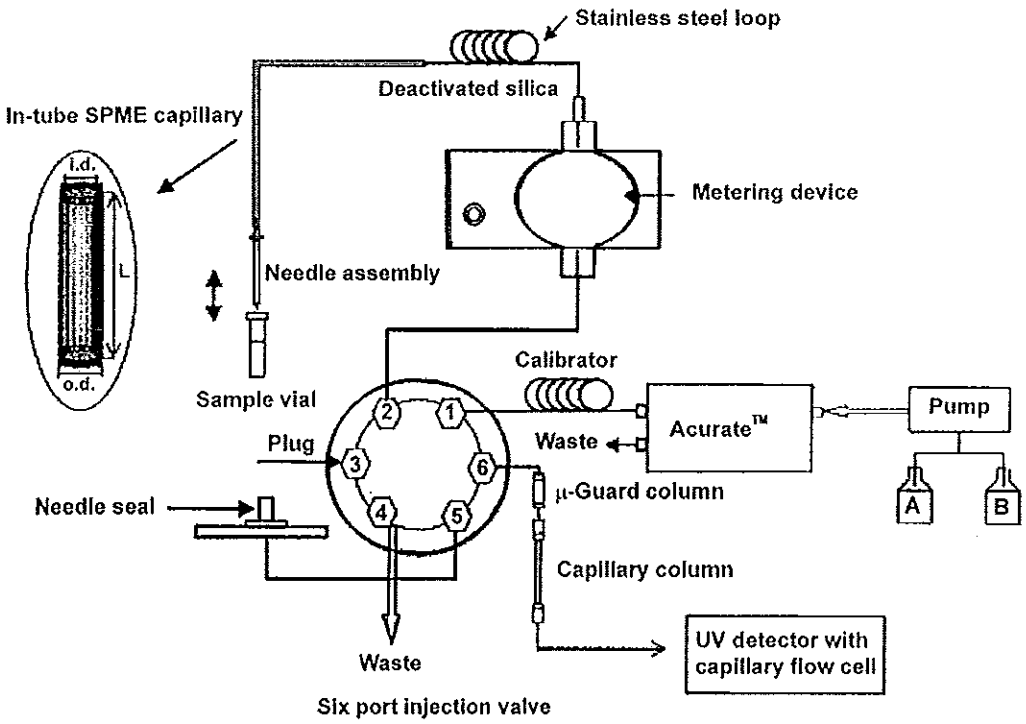


Figure 3.20 Schematic of in-tube SPME/capillary LC as implemented on the Hewlett-Packard 1100 LC system (Gou and Pawliszyn, 2000).

However, the extraction efficiency in CME techniques depends upon many factors, one of which is film in the thickness. It should be considered in contrast with SBSE. Due to the thin coat of film capillary the column, it will offer a low retention power for the trapping of multi residue compounds from aqueous samples, particularly in the case of more polar compounds that do not partition strongly into the stationary phase. Also the classical coating techniques will not be suitable to coat thicker film because the deposited film will quickly rearrange into droplets due to drainage and rayleigh instability when a fluid of a certain density floats above a fluid of lesser density (Bartle *et al.*, 1987). Then the problems associated with dynamic coating could possibly occur (Roeraade, 1985; Roeraade and Blomberg, 1989). Therefore CME with a thinner film (< 10 μm) should be considered for enrichment of trace compounds in aqueous samples because a thin film thickness can reduce high bleed and carry over (Globig and Weickhardt, 2005; Raghani and Schultz, 2003; Tan *et al.*, 1999). In this thesis, a new choice of CME was developed and presented.

Chapter 4

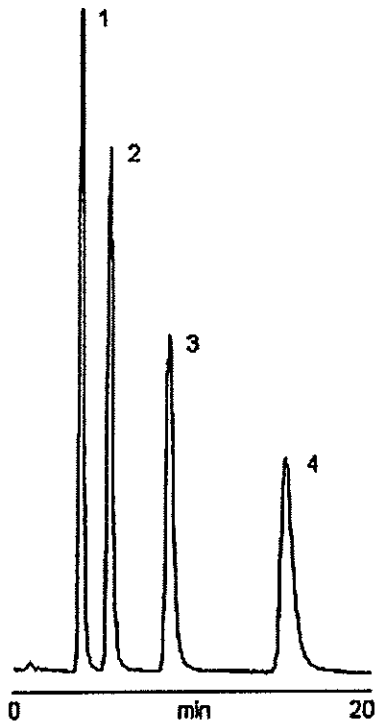
Chromatographic analysis

4.1 Principle

There are many compounds contaminated in water, organic compounds more so than inorganics, and these are present at the ultra to trace level (10^{-10} – 10^{-12}) (Townshend, 1995). Their chemical and physical properties are often very closely related which makes it very difficult to discriminate a single compound from others that are also present in the sample. Therefore, the selection of sample preparation (discussed in Chapter 3) and analytical technique depends on the class of target compounds to be analyzed.

Chromatography is one well-known family of analytical techniques that are generally used for the separation of mixtures. Most common methods are gas chromatography (GC) and liquid chromatography (LC) that can be combined with a wide range of detectors (Grob, 2004; Meloan, 1999; Mendham *et al.*, 2000; Mitra, 2004; Townshend, 1995). Chromatographic analysis exploits the differences in partitioning adsorption behavior between a mobile phase and a stationary phase in order to separate the components in a mixture. The technique employs the injection of a sample or a mixture which contains the analyte, into the mobile phase which is in a stream of gas or solvent passing through the stationary phase. The stationary phase, a material in the chromatographic column will retard the passage of the components of the sample. 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. The time required for a component to pass through the system is called the *retention time* (Harrison and Mora, 1996) and the information obtained from the analysis is

contained in the chromatogram that indicates the type, concentration or mass profile of the sample components (Poole and Schuette, 1985) as shown in Figure 4.1.



Column: PLRP-S 100Å 3µm, 150x300µm
Eluent: 60% Water : 40% ACN
Flow Rate: 5.0µl/min
Detector: UV

KEY
1. Methyl paraben
2. Ethyl paraben
3. Propyl paraben
4. Butyl paraben

Figure 4.1 Example of a chromatogram (Polymer Laboratories Ltd., 2005)

4.2 Column efficiency

The ideal chromatographic process is one in which the component of a mixture form narrow bands which are completely resolved from other peaks or zones in a reasonable time (Gudzinowicz, 1967). Typically, there are two main theories of chromatography, the rate and plate theories (Grob, 1985). In the rate theory, the retention is a measure of the speed at which a substance moves in a chromatographic system. When the compounds are eluted with the eluent, the retention is usually measured as the retention time; R_t or t_R as shown in Figure 4.2. It is the time between injection and detection, which is the run time of the compounds divided by the run time of the eluent front.

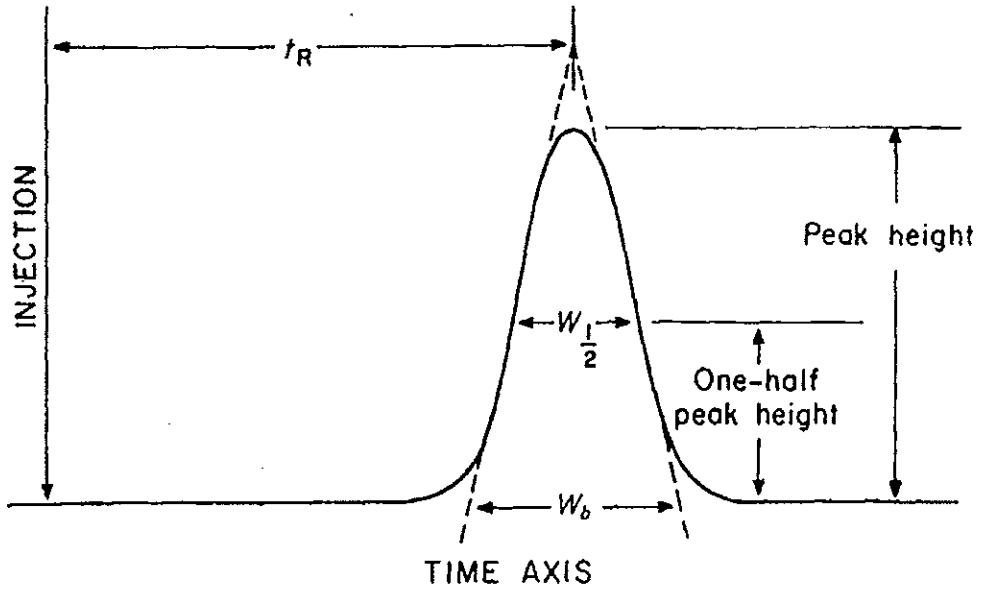


Figure 4.2 Measurement of t_R, W_b and $W_{1/2}$

The retention of a compound often differs considerably between experiments and laboratories due to variations of the eluent, the stationary phase, temperature, and the setup. Therefore, it is important to compare the retention of the test compounds to that of one or more standard compounds under absolutely identical conditions.

Plate theory is another chromatographic theory developed to describe the chromatography system in equilibrium. As the solute moves through the particles of the stationary phase, some molecules will travel by slightly longer path than others. This multiple path effect also contributes to band broadening. The effect of these factors on the efficiency of a separation is often expressed in the van Deemter equation as shown in Equation below.

$$H = A + \frac{B}{u} + C\bar{u} \qquad (4.1)$$

where H is the height equivalent to a theoretical plate (HETP), A is the eddy diffusion term, and represents the band broadening that occurs because of random choices of multiple paths available for analytes in packed columns. The $B/\bar{u}$ is the longitudinal diffusion term, representing the diffusivity of the analyte in the mobile phase and the $C\bar{u}$ term is mass transfer rate term, which refers to the resistance to mass transfer of the analyte between the two phases.

The equation indicates that the highest efficiencies will be achieved with stationary phases of small size (small value of A and $C\bar{u}$) and thin coating of liquid (small value of $C\bar{u}$). Therefore, in this study a gas chromatography technique with a 30 m open tubular column (0.25 mm I.D., 0.25 μm film thickness) was employed. In addition, a liquid phase in this column is coated on fused silica with no packing material packs, thus all molecules in the chromatograph would followed the same path and the multiple path term (A) could be eliminated which is known as the Golay equation (Townshend, 1995). Values of H can be plotted against the flow rate of mobile phase for a given solute producing a hyperbolic curve showing the optimum flow rate for highest efficiency as shown in Figure 4.3.

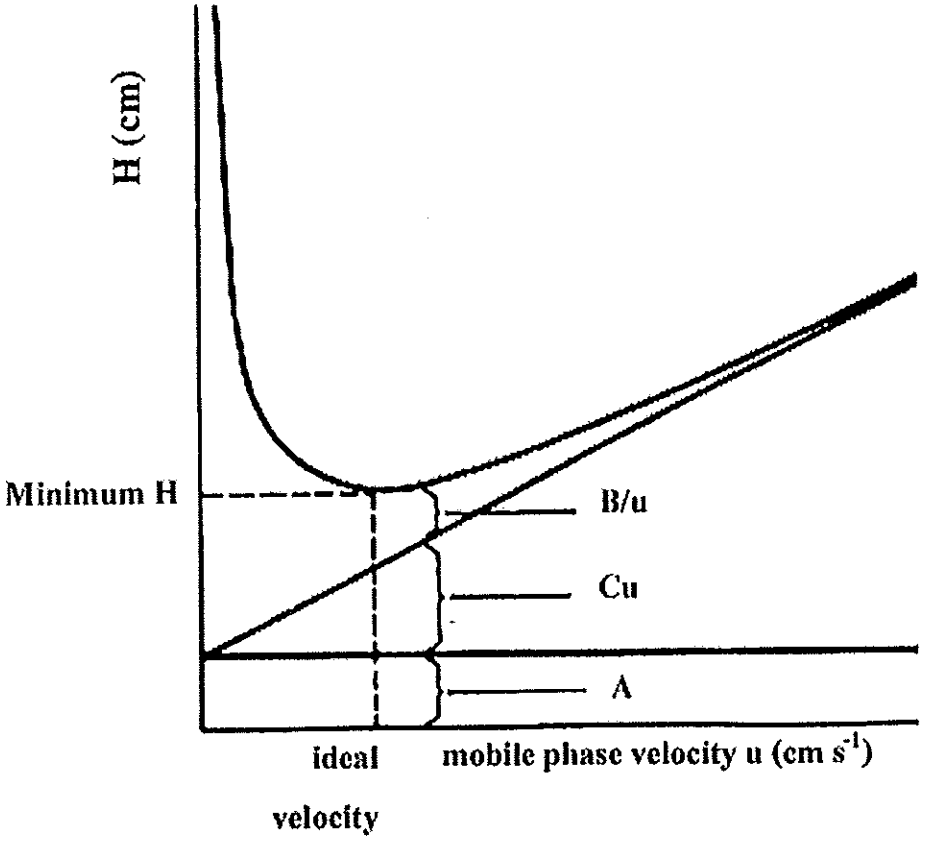


Figure 4.3 van Deemter plot (Fifield and Kealy, 2000)

The optimum flow rate which indicates the highest column resolution will give the lowest H or HETP.

$$HETP = \frac{L}{N} \tag{4.2}$$

where L is length of column in centimeters and N is the number of theoretical plates. The plate number N of a column may be determined directly from a chromatogram as shown in Figure 4.2 by the relationship between the retention time and peak width.

$$N = \left(\frac{4t_R}{W_b}\right)^2 \tag{4.3}$$

where t_R is the retention time and W_b is the base peak width as shown in Figure 4.2 Another common way to determine N makes use of the relationship

$$N = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2 \tag{4.4}$$

where $W_{1/2}$ is the width at half the peak height.

In this study, Equation 4.4 was used to calculate N at various flow rates. The value N is then substituted in Equation 4.2 to obtain HETP. A plot between HETP and carrier gas flow rate was carried out in order to find the optimum carrier gas flow rate at minimum HETP.

4.3 Qualitative analysis

Qualitative analysis by chromatography techniques involves the identification of unknowns that could be interpreted in many available methods, *i.e.*, a plot of logarithm of retention time versus carbon atom, kovats retention index, comparing retention data (Grob, 2004), or using a selective detector (Günzler and Williams, 2002). A plot of logarithm of retention *versus* carbon number is a method that shows a linear dependence exists between the logarithm of the retention times for compounds in homologous series and the number of carbon atoms in the molecule. A typical series of plots is shown in Figure 4.4. If the retention time of an unknown sample falls between the seven- and eight-carbon straight chained alkanes it is

impossible for unknown to be a straight chained alkane since fractional carbon atoms are not allowed in a molecule. However, although this technique can eliminate a number of potential materials, it cannot identify unknown components if a series of plots is not a homologous or pseudo homologous series (Debbrecht, 2004).

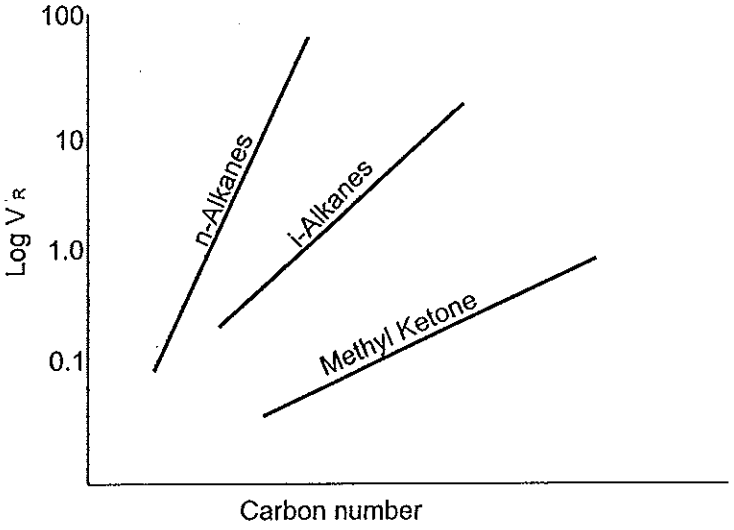


Figure 4.4 Logarithm of adjusted retention time versus carbon number (Wehrli and Kováts, 1959)

In 1959, Wehrli and Kováts introduced Kovats retention index system that is another concept of the retention index to help confirm the structure of organic molecules (Wehrli and Kováts, 1959). This method is based on a scale that is defined by the elution order of the normal alkanes. There is a large amount of reference data available that provides retention index data for a wide range of compounds, therefore, the identification is simple (Figure 4.5). The retention index (I_x or KI) can be calculated by the following equation (Günzler and Williams, 2002).

$$I_x = 100_z + 100\left(\frac{\log(t'_{R_x} - t'_{R_z})}{\log(t'_{R_{z+1}} - t'_{R_Z})}\right) \qquad (4.5)$$

where I_x is the retention index of the component X

z is the carbon number of the n-alkane eluting before X

z+1 is the carbon number of the n-alkane eluting after X

t'_R is the adjusted retention time

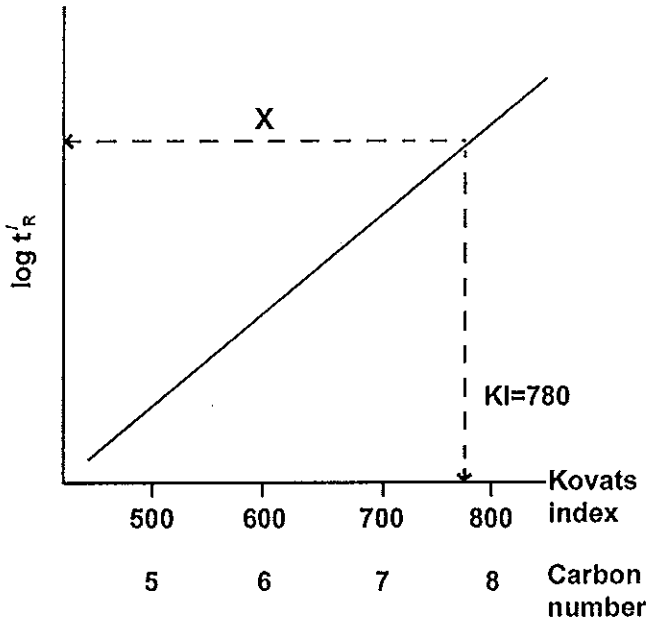


Figure 4.5 Plot of logarithm of adjusted retention time versus Kováts index (Debbrecht, 2004)

However, in some cases it is not possible to identify an eluted solute alone due to the requirement of the unambiguous identification of some solute that do not match the reference index (Debbrecht, 2004).

Retention data is now the most frequently used method to identify analytes by comparing adjusted retention time (t_R) of an unknown sample with that of a known sample or standard. The retention time is the time elapsed from injection of the sample component to the recording of the peak maximum. The retention volume is a product of retention time and flow rate of the carrier gas. Generally, the adjusted retention time or the adjusted retention volume and the relative retention are used in qualitative analysis. Adjusted retention time (volume) is the difference between retention time (volume) of the sample and an inert component (usually air) or some non retained component (e.g. methane). However, this method cannot confirm the presence of a single analyte molecule when its retention time is not unique, *i.e.*, due to other compounds that could have the same retention time. Therefore, a high selective detector should be considered before analysis. For example, an electron capture detector that is selective to molecules having high electronegativity, *i.e.*, halogen (Harvey, 2000; Matisová and Dömötörová, 2003).

Selective detection such as mass spectrometry, fourier transform infrared spectroscopy or atomic emission detection are additional methods that is presently used to support the identification for complex sample. These detectors are not only more useful for selective quantitation of a small number of chemicals known to be present in a complex mixture but also are useful for identification purposes (Günzler and Williams, 2002).

4.4 Quantitative analysis

Quantitative analysis of a component in chromatography using different detectors is based on a detector response, *i.e.*, peak area or peak height that is linear with respect to the concentration of analyte over a wide range of concentrations (Mendham *et al.*, 2000). The accuracy of analysis is limited by the overall error encountered, including errors due to the sampling technique, possible sample decomposition, detector and recorder performance, integration, calculations, etc. With a good technique, precision and accuracy can be obtained by employing standards with known purities. Results of an analysis are reported as the average ± the standard deviation of the replicates (Zweig and Sherma, 1982).

For measurement of the mass or concentration of analytes, quantitation may be carried out by calibration, which means the relationship between the content of the sample and response of the assay method. Chemical standards which are pure substances are used to calibrate and validate all or part of the methodology of a chemical analysis (Rubinson, 1987). There are several methods of standardization such as normalization, external standard, internal standard or standard addition (Mendham *et al.*, 2000).

4.4.1 Normalization

The most basic method is called the "normalization" technique in which the areas of all peaks recorded are added to give a total area which is then normalized to 100%. The ratio of the individual areas to the total area gives the component's area percent. Depending upon the sample mixture and detector being used, the area percents can sometimes be related to either the component's weight or

molecular percent in the mixture (Gudzinowicz, 1967). By normalization, the peak area percent of each component would be calculated. For example,

$$Total\ area = A_a + A_b + A_c$$

where A_a, A_b and A_c are the respective area of component a, b and c.

$$\%a = (100 \times A_a) / total\ area$$

$$\%b = (100 \times A_b) / total\ area$$

$$\%c = (100 \times A_c) / total\ area$$

The above method would provide useful information when all components had similar detector responses and were eluted from the same column (Gudzinowicz, 1967).

4.4.2 External standard calibration

An external standard calibration is the most commonly used method where the standard is analyzed separately from the unknowns being tested. A series of standard samples are prepared at various concentrations over the desired range and analyzed. A calibration curve is then generated with peak response plotted versus standard concentration. Peak responses of each analyte are then determined and compared to the curve to generate the analyte concentration as shown in Figure 4.6 (Harris, 1995). This method is best for analyte in liquid samples where the analytes are soluble in the sample matrix and the matrix has no effect on the analyte response. The main difficulty with external standard calibration is that it does not compensate for any variability due to variation in the analyte matrix (Ardrey, 2003; Fifield and Kealy, 2000).

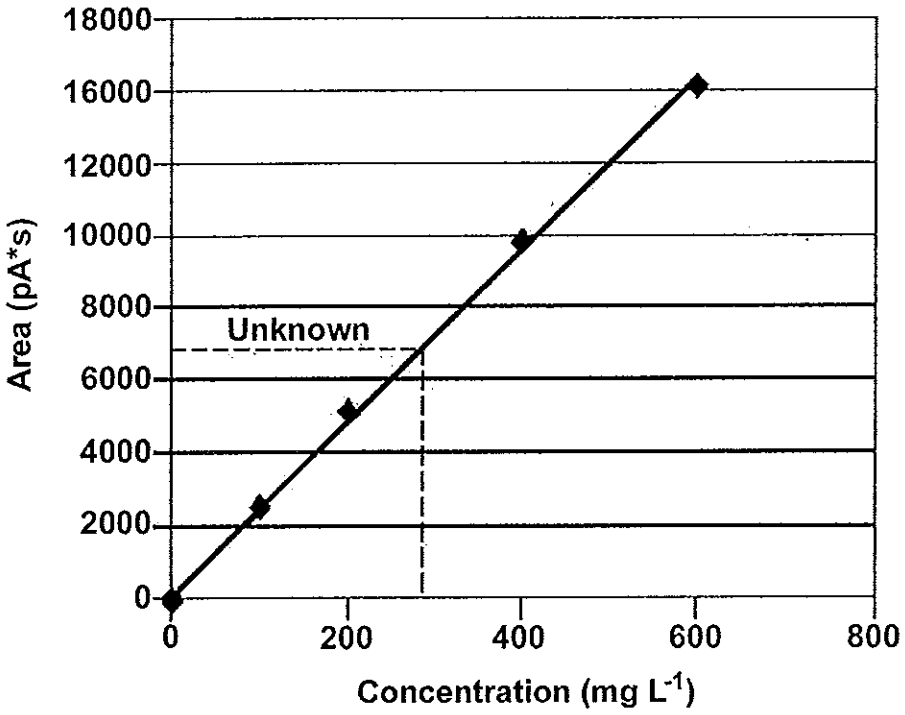


Figure 4.6 External calibration curve

4.4.3 Internal standard calibration

An internal standard calibration is a method which is used to eliminate the problem when all components of a multicomponent mixture are not normally eluted normally, their height and area of chromatographic peak are affected not only by the amount of sample but also by fluctuations of the carrier gas flow rate, the column and detector temperatures (Zweig and Sherma, 1982). To prepare the calibration curve, internal standard is added to each replicate sample and blank, and then analyzed chromatographically. The peak responses of the analyte and internal standard are recorded. The ratio of areas of analyte to internal standard is plotted versus the concentrations of the known standards. For the analytes, this ratio is calculated and the actual analyte concentration is determined from the calibration graph as shown in Figure 4.7.

Although the internal standard calibration can compensate for some errors in external standard quantitation, there are several difficulties in method development such as choosing an appropriate internal standard that must be available in extremely pure form and never appear in the sample of interest, and also does not

interfere in either the extraction or the chromatographic analysis of the analyte. In addition, it must be structurally similar to the analytes so that it undergoes similar extraction and chromatography, otherwise, the compensation will be lost (Grob, 2004; Harris, 1995; Mitra, 2004; Zweig and Sherma, 1982).

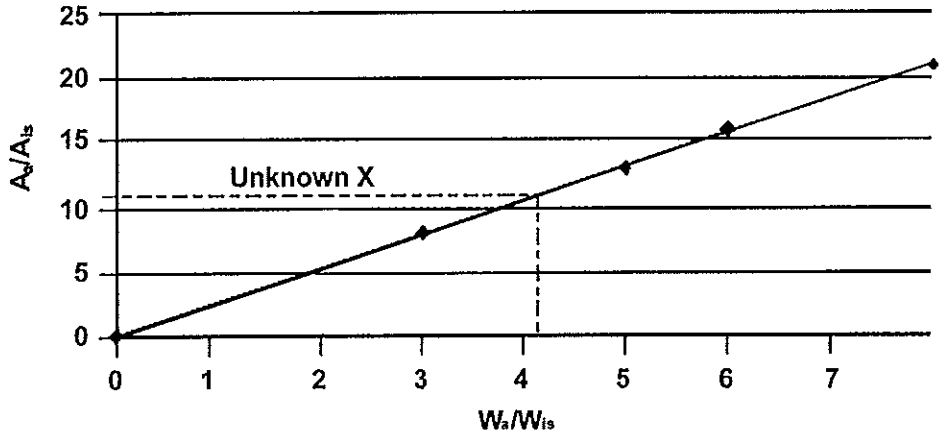


Figure 4.7 Internal standard calibration curve, A_a, A_{is} = Peak area of analyte and internal standard respectively, W_a, W_{is} = amount of analyte and internal standard respectively

4.4.4 Standard addition calibration curve

The complication of matching the matrix of the standards to that of the sample can be conducted by standardization in the sample. Therefore, a standard addition calibration method is used to minimize that problem and compensate for variation in analyte recovery and peak response at the lower limit of detection (Harris, 1995; Harvey, 2000). To prepare the calibration curve, a series of increasing known quantity of standard is added directly to the samples. They are analyzed and recorded by plotting between response and concentration of spiked sample. Then the actual concentration of analyte in the sample can be determined by extrapolating the calibration curve as shown in Figure 4.8 (Harvey, 2000). For this method, analyte response must be linear throughout the range of concentrations used in the calibration curve.

In practice, the sample is divided up into several equal portions, then added to an increasing level of standard. The samples are analyzed and peak response

versus the final concentration is plotted. The final concentration of the standard is the concentration of standard after it is added to the sample. The original concentration is then determined by extrapolation to the x-axis. Alternatively, a single additional sample can be prepared and the original concentration of analyte can be determined from following equation:

$$\frac{original\ concentration\ of\ analyte}{final\ concentration\ of\ analyte\ (sample + standard)} = \frac{area\ from\ original\ sample}{area\ from\ (sample + standard)}$$

To calculate the original concentration of the sample in Figure 4.7, the final (diluted) concentration of the sample X is expressed in terms of the initial concentration of the sample. Then the initial concentration of the sample is determined (Harris, 1995).

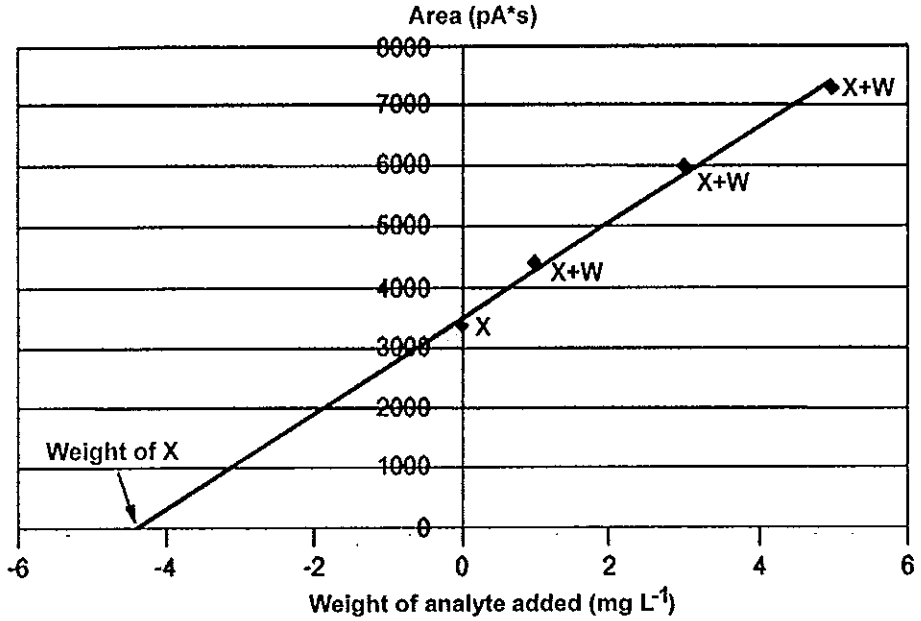


Figure 4.8 Standard addition calibration curve

4.5 Method validation

Once the chromatographic method has been developed, it is necessary to validate before being implemented. Validation is the process of verifying that a procedure yields acceptable results. In addition, it ensures the writing procedure has sufficient detail so that different analysts or laboratories following the same procedure

obtain comparable results (Harvey, 2000). In general, methods for regulatory submission must include studies on linearity, accuracy, precision, detection limit and robustness (Green, 1996).

4.5.1 Linear dynamic range (LDR, Linearity)

The linearity (Figure 4.9) is defined by the range over which the analytical signal is directly proportional to the amount of analyte present in various concentrations. If the linear range is exceeded, the introduction of more analyte continues to produce an increase in response but no longer is this directly proportional to the amount of analyte present (Ardrey, 2003; Grob, 2004). This is referred to as the dynamic range of the detector. At the limit of the dynamic range, the detector is saturated and the introduction of further analyte produces no further increase in response. It is important when obtaining precise results that the signals from the samples to be determined should lay on the linear part of the calibration graph since elsewhere within the dynamic range a small change in detector response corresponds to a relatively large range of concentrations. Acceptability of linearity data is often judged by examining the correlation coefficient of the linear regression line from the response versus concentration plot. A correlation coefficient of > 0.99 is generally considered as evidence of acceptable fit of data to the regression line (Green, 1996; U.S. EPA, 2003a).

4.5.2 Limit of detection (LOD)

The limit of detection (Figure 4.9) is defined by the smallest quantity of an analyte that can be reliably detected and produces a signal that exceeds the signal observed from a blank by a certain factor. The acceptable factor used is usually between 2 (Harris, 1995) or 3 times the standard deviation for the measurement of the blank (Long and Winefordner, 1983). In addition LOD can be considered from the intensity of signal from the analyte compared to that from the noise in term of signal to noise ratio (S/N) when is generally accepted as ≥ 2 or 3 (American Chemical Society Committee on Environmental Improvment, 1983). Standard deviation depends upon the number of replicate measurements which normally the International

Union of Pure and Applied Chemistry (IUPAC) recommends to be 20 for the blank (Long and Winefordner, 1983).

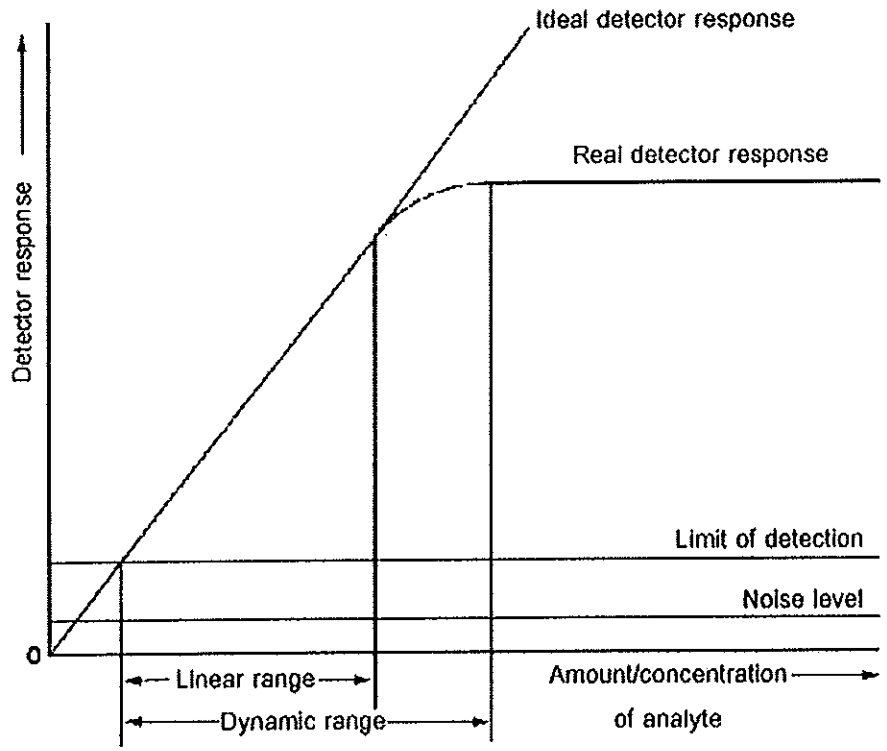


Figure 4.9 Detector response curve showing (a) 'ideal' behavior, (b) 'real' behavior, (c) its linear range, (d) its dynamic range, (e) the noise level, and (f) the limit of detection at three times the noise level.

4.5.3 Recovery

Recovery is a term used to establish the accuracy of a method. It is estimated from the recovery of spiked analytes from the matrix of interest. The calculation of recovery is described by the following formula (U.S. EPA, 2003a).

$$Recovery = \%R = \frac{C_s - C_u}{C_n} \times 100 \qquad (4.6)$$

where C_s represents a measured concentration of the spiked sample aliquot, C_u is a measured concentration of the unspiked sample aliquot and C_n is a nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot.

The International Conference on Harmonization (ICH) quideline recommeds to determine recovery from data which are collected from a minimum of three concentration levels cover specified range, *i.e.*, 80-120% of the target concentration for assay test, 70-130% for content uniformity testing and 50-150% of the target concentration for impurity test; such as 0.1-2.5 wt % (Green, 1996; Swartz and Krull, 1997). The mean value of acceptable recovery is considered over the range of 70-130% and can be used as a guideline in evaluating method performance (U.S. EPA, 2003a).

4.5.4 Precision

The precision of chromatographic method is the measure of agreement or closeness of analyte concentrations to each other when the analyses were performed using identical conditions. It is generally the measurement of the amount of scatter in the results obtained from multiple analyses of a sample. Mathematically it is calculated and expressed as standard deviation (SD) (Grob, 2004).

$$SD = \left[\sum \frac{(\bar{x} - x_1)^2 + \ldots + (\bar{x} - x_n)^2}{n-1} \right]^{1/2} \qquad (4.7)$$

Or expressed in term of the relative standard deviation (RSD)

$$RSD = \frac{SD}{\bar{x}} \times 100 \qquad (4.8)$$

The acceptable RSD depends on the type of chemical analysis, *i.e.*, trace analysis, minor component analysis and major component analysis that are also variable among the wide variety of analytical methods. Typically, the RSD of ±10-30% is allowed for trace analysis, ±3-10% for minor component and ±0.1-5% for major component or purity assay (Umbreit, 2004). In practice, the allowance for RSD to evaluate the chromatographic separation in trace level has been recommended by U.S. EPA that it should be less than or equal to 20% for every analyte and for every level of calibration (U.S. EPA, 2003a).

4.6 Chromatographic analysis for SVOCs in water

The increasing number of SVOCs pollutants in water calls for extensive monitoring programs. The requirements, both in terms of time and costs of most traditional analytical methods (*e.g.* chromatographic methods) often constitute an important impediment for their application in these programs (Rodriguez-Mozaz *et al.*, 2006). Methods using GC or LC coupled to an appropriate detector and using suitable sample handling procedures provide detection limits typically in the range of 0.001–1 μg L^{-1} (ppb) (Meloan, 1999; Poole and Schuette, 1985). At these levels, target or unknown compounds can be determined or identified by means of their retention and spectral characteristics. Principally, most of the analytes can be determined by both techniques. LC is often a method of choice when polar, non-volatile or thermolabile compounds are to be analyzed (Liška and Slobodnik, 1996) but LC has benefits less than GC due to insufficient separation power of common LC columns for the analysis of very complex mixtures (Townshend, 1995) while GC provides a great separation efficiency, high speed of analysis and the availability of a wide range of highly sensitive detectors. Lately on-line coupling of LC with mass spectrometer (MS) has become an attractive tool for analysis of SVOCs in water sample but its performance still lacks that obtained by GC-MS due to the lack of a universal LC-MS system for all types of compounds (Santos and Galceran, 2002; Townshend, 1995). Therefore, GC which is present in almost all major chemical laboratories continues to play an important role in the identification and quantification of ubiquitous SVOCs in water. Most U.S. EPA methods for SVOCs analysis use GC with various detectors, *i.e.*, GC-FID or ECD for phenols (Method 8041), GC-ECD for phthalates (Method 8061A), GC-NPD for amines (Method 8070A), GC-ECD for chlorinated pesticides (Method 8081A), GC-ECD for PCBs (Method 8082), GC-FID for PAHs (Method 8100), GC-ECD for chlorinated hydrocarbons (Method 8121), GC-NPD or FPD for organophosphorus compounds (Method 8141A), GC-ECD for chlorinated herbicides (Method 8151A), GC-MS for SVOCs/base neutral acids (BNA) (Method 8270C) and dioxin (Method 8280) (U.S. EPA, 2003a).

Typically the separation by GC is suitable for almost any mixture of components that exhibits reasonable volatility. The immediate quantitative analysis of components present at pg L^{-1} (ppq) levels is possible to obtain (O'Neill, 1993) because

GC can be coupled to a wide variety of selective and sensitive detectors. Especially, the hyphenated systems which include mass spectrometry can provide powerful means for obtaining rapid and extensive information on separated analytes (Grob, 2004; Santos and Galceran, 2002; Singh *et al.*, 1998).

The combination of gas chromatography with mass spectrometry (GC–MS) was first reported in 1958 and was made available commercially in 1967 (Gohlke, 1959; Ryhage, 1964). Since then, it has become increasingly utilized and accepted as a routine technique. Virtually all compounds that pass through a GC capillary column can be ionized and the full analytical capabilities of the mass spectrometer utilized. Mass spectra from an analyte are obtained by using either electron (EI) or chemical ionization (CI). Then the MS fragmentation pattern can provide unambiguous component identification by comparison with library spectra (Ardrey, 2003; Santos and Galceran, 2002).

As SVOCs represent an important class of hazardous organic chemicals that are ubiquitous in the environment, particularly in water due to their high persistence and bioaccumulation. A large number of publications have been reported on the analysis by GC–MS (Basheer *et al.*, 2002; Brossa *et al.*, 2003; El Beqqali *et al.*, 2006; Itoh *et al.*, 2005; Kawaguchi *et al.*, 2006a; Llorca-Porcel *et al.*, 2006; Olivella, 2006; Santos and Galceran, 2003; Serôdio and Nogueira, 2004). Therefore, it is currently a well-known technique that is applied to the analysis of a significant number of pollutants in water samples. The compounds most commonly analyzed include polycyclic aromatic hydrocarbons (PAHs) (El Beqqali *et al.*, 2006; Itoh *et al.*, 2005; King *et al.*, 2004; Olivella, 2006; Singh *et al.*, 1998), pesticides (Basheer *et al.*, 2002; McLaughlin and Johnson, 1997; Nakamura and Daishima, 2005), polychlorinated biphenyls (PCBs) (Singh *et al.*, 1998), phenol (Kawaguchi *et al.*, 2006a; Kawaguchi *et al.*, 2005; Montero *et al.*, 2005) as well as other endocrine disrupting chemicals such as phthalates and short ethoxy alkylphenol etoxilates (Brossa *et al.*, 2003; Serôdio and Nogueira, 2004). In addition, GC–MS is the technique of choice for the analysis of emerging pollutants such as polybrominated diphenyl ethers (PBDEs) or polychlorinated alkanes (Barceló, 2003; Richardson, 2002).

For the separation of SVOCs, GC capillary columns are typically made of fused silica, chemically bonded with various stationary phases in order to achieve a range of different selectivity toward complex samples. Liquid phases which were immobilized or crosslinked stationary phase, allowed high temperature operation with low bleeding, so labile and/or less volatile compounds could be analyzed and detectors could be operated at high sensitivity (Muccio, 1995). Currently there are two general types of capillary columns, conventional (0.10 - 0.25 mm I.D.) and wide-bore capillary columns (0.53 mm I.D.) for suitably determining the retention time of SVOCs (Santos and Galceran, 2002). Most analyses have been based on the nature of the analytes to be separated. A non-polar stationary phase like DB-1 (or OV-1, HP-1) and DB-5 (or HP-5) is suitable for non-polar compounds such as PCBs, PAHs and some pesticides (El Beqqali *et al.*, 2006; Itoh *et al.*, 2005; King *et al.*, 2004; Olivella, 2006). For more polar compounds like organophosphorous compounds stationary phase like OV-17 (or DB-1701) can be applied (Tahboub *et al.*, 2005; Tomkins and Ilgner, 2002; Zambonin *et al.*, 2004; Zulin *et al.*, 2002). However, at present for the separation of SVOCs, the preferred column to use is a HP-5MS (5% diphenyl-95% dimethylsiloxane) since it has low bleeding, robustness and has been used to analyze a wide range of compounds analysis (Agilent, 2004).

Therefore, in this work the development of reliable and suitable sample preparation techniques were performed with GC-MS and HP-5MS column to achieve the analysis of SVOCs in water.

Chapter 5

Development of an in-line system for the analysis of 4,4′-DDT in water

5.1 Introduction

Persistent Organic Pollutants (POPs) are toxic chemicals that can persist in the environment and bioaccumulate if they move up through the food chain. POPs have been linked to adverse effects on human and animals health, such as cancer, damage to the nervous system, reproductive disorders, and disruption of the immune system (Jones and Voogt, 1999). Some POPs are semivolatile organic compounds (SVOCs) whose contamination in water plays a major role in environmental problem. SVOCs originate from many sources, *i.e.*, electroplating/metal shop, fire fighting training area, pesticide mixing area, wood preservative and landfill. The last is the most likely source for releasing these pollutants into the water (Deuren *et al.*, 2002).

Typically, the analysis of SVOCs in aqueous sample is performed by a combination of various extractions and analytical methods, *i.e.*, gas chromatography (GC) or liquid chromatography (LC) since these techniques are well known for their good separation performance (Cai *et al.*, 2003; Fiedler *et al.*, 2002; Frías *et al.*, 2001). The common extraction method employed with liquid samples is liquid-liquid extraction (LLE) which has been the main method for enrichment of organic pollutants from aqueous solutions. Although it is still being widely used, this method requires large amount of toxic organic solvents, time consuming and labor intensive (Popp *et al.*, 2003). Microextractions, *i.e.*, solid phase extraction (SPE) and solid phase microextraction (SPME), have been reported to replace the LLE (Lipinski, 2000; Mukherjee and Gopal, 1996) since these methods use only a small amount of solvent to extract analytes from moderate amount of samples. SPME is particularly

suitable for direct injection. Although the microextraction techniques can provide good selective extraction and clean up but there are many disadvantages, e.g. SPE is easily clogged if the sample has large amounts of suspension particles and SPME is quite fragile and needs skill to operate. Moreover, its adsorption capacity is limited by the length of the coated fiber.

An alternative approach for the analysis of trace organic compounds in aqueous matrix is to use a membrane inlet unit coupled to analytical instruments, especially, mass spectrometer. This membrane inlet mass spectrometer (MIMS) has been used to determine volatile organic compounds (VOCs). High sensitivity and detection limits at low or sub-ppb concentrations can be obtained for many compounds (Ferreira *et al.*, 2002; Mendes *et al.*, 1996; Soni *et al.*, 1995). However, the analysis of SVOCs using this technique is much less common (Aggerholm and Lauritsen, 2001; Guo and Mitra, 2000; Mendes and Eberlin, 2000; Mendes *et al.*, 1996). This is because under normal conditions SVOCs do not evaporate from the membrane surface into the mass spectrometer and also do not dissolve very well in the membrane that is normally used (Basheer *et al.*, 2002; Lauritsen and Ketola, 1997; Riter *et al.*, 2002). In order to facilitate SVOCs analysis in aqueous samples, a new versatile and advantageous design of membrane inlet system is developed in this work by coupling it to a gas chromatograph-mass selective detector and using a simple thermal desorption unit to enhance the extraction efficiency.

Current researches on SVOCs are focusing on pesticides that are considered to be endocrine disruptor (Richardson, 2002). Among the SVOCs, 4,4'-Dichlorodiphenyl trichloroethane (4,4'-DDT) has been listed in the top 20 hazardous substances in 2005 (ATSDR, 2006). It is very persistent in the environment, can accumulate in the food chain and is probably a human carcinogen. Although it has been banned since 1970s, it is still used in countries where mosquito-borne malaria is a larger health problem than DDT's potential toxicity, particularly in Asia and the Pacific region including Thailand (Ome and Kegley, 2004). Therefore, it was chosen to be a representative of SVOCs for this study.

5.2. Chemicals and materials

5.2.1 Standard chemicals (Certified solutions with purity 99 - 100%, Restek Company.,USA) : 4,4′- Dichlorodiphenyltrichloroethane (4,4′-DDT), 1000 $\mu g\ ml^{-1}$

5.2.2 General solvents and chemicals

- Methanol (CH_3OH, Analytical reagent grade, Merck, USA)
- Ultra pure water (H_2O, produced in the laboratory using a Maxima water purifier, ELGA, England)

5.2.3 Materials

- Micro porous polypropylene support coated with 1-μm thick film of polydimethylsiloxane, was obtained from Applied Membrane Technology, Inc., USA. , 0.290 mm O.D. × 0.240 mm I.D.
- Teflon tubing, 15 cm × 0.10 in I.D. × ⅛ in O.D. (Alltech Associates, Inc., USA)
- Swagelok® T-union ($^1/_8$ in) (Restek Company, USA)

5.2.4 Samples

Several types of water samples, bottled water (locally produced, unregistered and registered) and tap water, were sampled in Hat Yai City, Thailand, and then analyzed for 4,4′-DDT by the membrane inlet system coupled to a gas chromatograph-mass spectrometer (MI-GC-MS). If it was undetectable (lower than the detection limit), a standard addition method was applied for the analysis to confirm the absence of 4,4′-DDT.

5.3 Instruments and apparatus

5.3.1 Gas chromatography-mass spectrometry system (GC-MS)

- Gas chromatograph model 6890 Series (Agilent Technologies, USA)
- Capillary Column: HP-5MS, 30 m × 0.25 mm I.D. × 0.25 μm film thickness of 5% diphenyl and 95% dimethylpolysiloxane (Agilent Technologies, USA)
- Helium Carrier Gas: Ultra High Purity, 99.99999% (Thai Industrial Gases Public Company Limited, Thailand)
- Mass Selective Detector (MSD) model 5973 (Agilent, Technologies, USA)
- Computer system model KAYAK (Hewlett Packard, USA)

5.3.2 Apparatus

- Syringe 10 μl (Agilent Technologies, USA)
- Volumetric flask 10 mL (Pyrex, USA)
- Amber glass bottle with PTEF- screw cap 5 mL (Pyrex, USA)
- Micro liter pipette (Gilson, France)
- A thermo stated laboratory-built heating box (10″ × 13″ × 12″)
- Peristaltic pump (Gilson, France)
- 10 port inject valve (Valco Instrument Co. Inc., USA)
- Sample loop 2 mL (Valco Instrument Co. Inc., USA)

5.4 Experiments

5.4.1 Preparation of 4,4′-DDT standard stock solutions

The standard stock solution of 4,4′-DDT was prepared from the standard chemical (5.2.1) at a concentration of 100 mgL^{-1}. The solution was dissolved in methanol and diluted to 10 mL in a volumetric flask. It was then transferred into an amber glass bottle with PTFE-lined screw cap to protect from light and stored as 4°C as recommended by the manufacturer.

5.4.2 Preparation of 4,4′-DDT spiked aqueous solutions

The spiked aqueous solutions were prepared by spiking 4,4′-DDT standard stock solution (5.4.1) into 300 mL of ultra pure water to give the desired 4,4′-DDT concentrations. The concentration of 1.0 mgL^{-1} was used to optimize all analysis parameters.

5.4.3 Analysis

A laboratory-built membrane inlet unit was constructed from a 15 cm Teflon tube (0.10 in I.D. × ⅛ in O.D.) and two Swagelok® T-unions (⅛ in) as shown in Figure 5.1. A hollow fiber membrane, micro porous polypropylene support coated with a 1-μm thick film of polydimethylsiloxane, was obtained from Applied Membrane Technology, Inc., USA. Three strands of 15 cm (0.290 mm O.D., 0.240 mm I.D.) hollow fiber membrane were inserted inside the Teflon tube. Each end of the tube was connected to one port of the Swagelok® T-union. The side ports of the two T-unions were used as the inlet and outlet of the sample that flowed through the hollow fiber lumen. The vertical ports were for the flow of the stripping gas (Helium gas, UHP grade, Thai Industrial Gases Public Company Limited, Thailand). Epoxy resin was used to glue the ends of the hollow fibers to the T-unions. This epoxy acted as a seal, preventing intermixing of the sample and stripping gas. The system was set up in a flow-though mode as stripping gas flowed outside the membrane surface while the sample solution flowed inside the lumen of the membrane. An aqueous sample was delivered through an MI unit using a peristaltic pump while stripping gas outside the fiber was flowed in the opposite direction. The sample was introduced into the GC-MS system using a 10-port valve (Figure 5.2).

A thermostated laboratory-built heating box (10″ × 13″ × 12″) was used as the desorption unit. It was designed to cover the MI unit and the ten port valve (Figure 5.3) so the pervaporation process could occur at constant temperature until the permeated analyte was injected to the GC-MS.

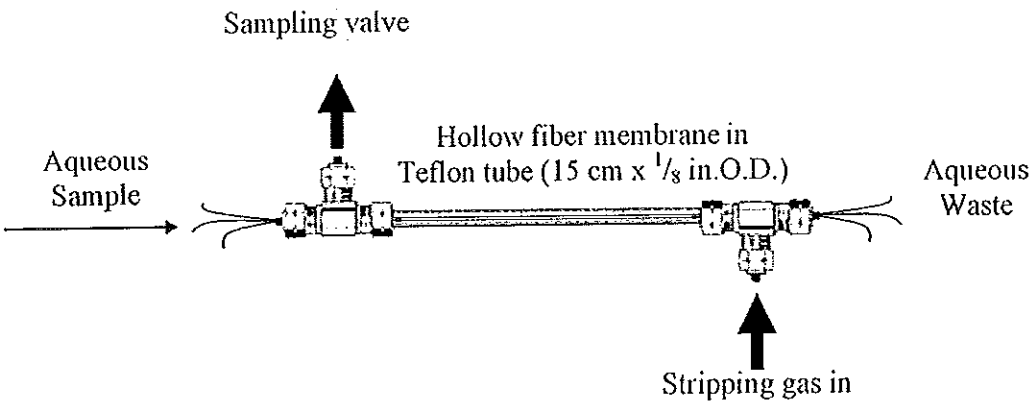


Figure 5.1 A laboratory-built membrane inlet unit

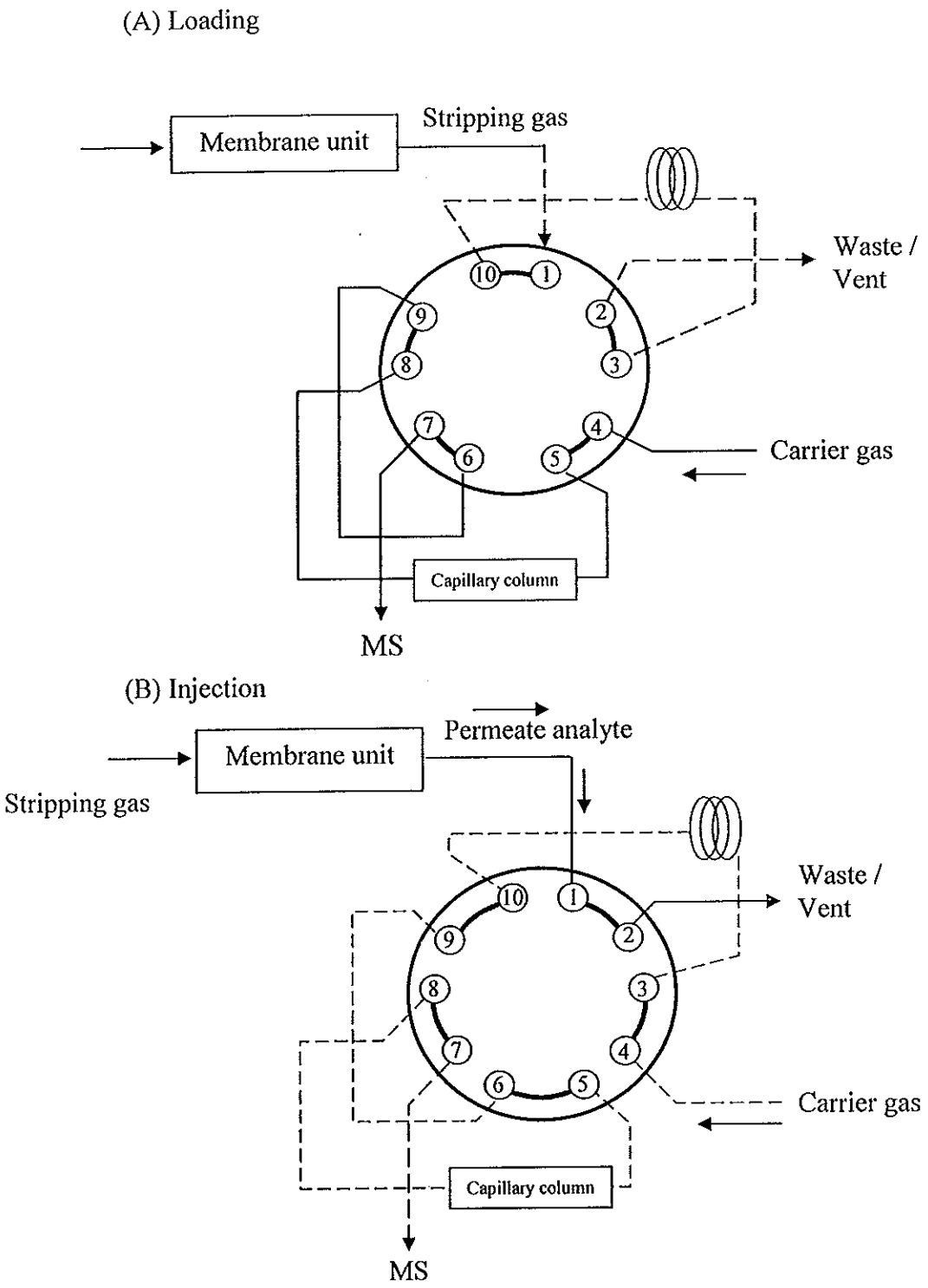


Figure 5.2 Schematic diagram of sample introduction part using a 10 port valve; (A) loading and (B) injection

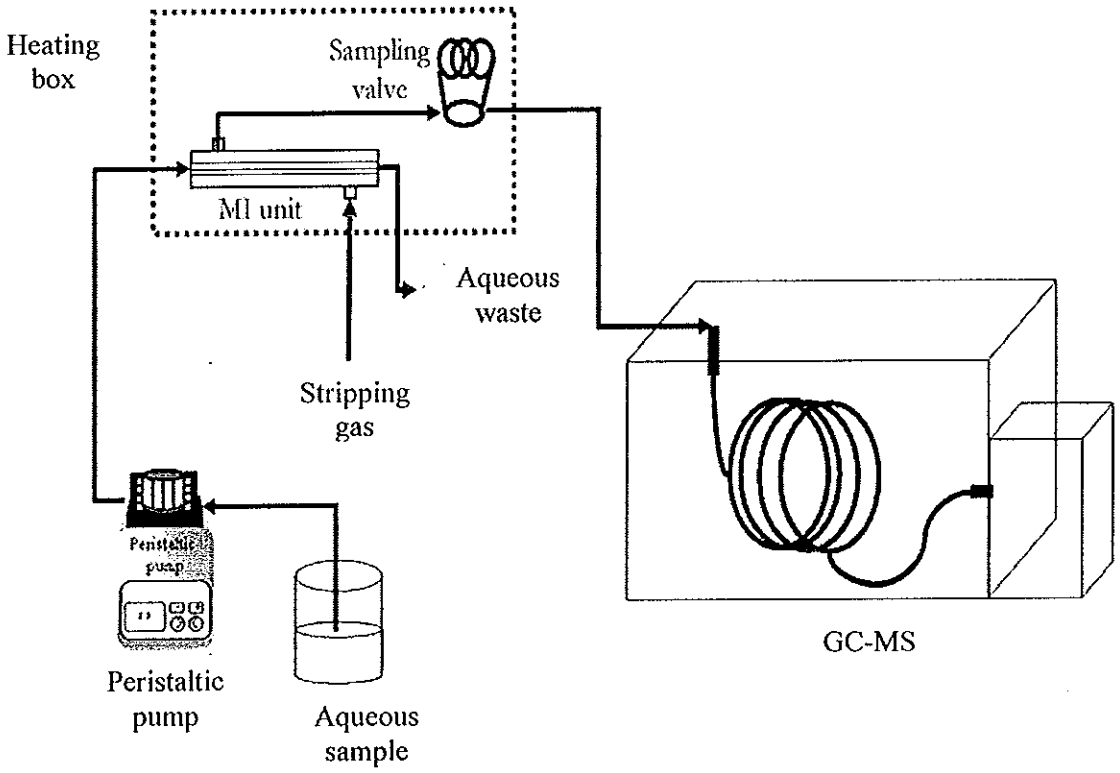


Figure 5.3 Schematic diagram of the membrane inlet unit coupled to a gas chromatograph-mass spectrometer (GC-MS)

5.5 Results and Discussion

5.5.1 Optimization of the analysis conditions of GC-MS

An HP-5890 gas chromatograph with 5973 mass selective detector and HP-5MS (30 m × 0.25 mm I.D. × 0.25 μm film thickness of 5% diphenyl and 95% dimethylpolysiloxane) capillary column were used in this study. Sample injection was made in the splitless mode. The mass spectrum of 4,4′-DDT was determined by injecting permeated analyte into the GC-MS. To optimize the analysis conditions of the GC-MS system for trace analysis of 4,4′-DDT in the water sample, the following parameters were studied. The carrier gas flow rate, column temperature and injector temperature. The temperature of the transfer line, MS source and MS quadrupole temperature were maintained at 230°C, 150°C and 280°C respectively following the recommendation by the HP5973 Mass Selective Detector Hardware Manual (Hewlett Packard, 1998a).

5.5.1.1. Carrier gas flow rate

A spiked aqueous solution at a concentration of 1.0 mgL^{-1} was pumped through the MI unit and analyzed by the GC-MS system. The optimum carrier gas flow rate was investigated by varying the flow rate of helium carrier gas from 0.3 to 1.2 mL min^{-1} with an increment of 0.1 mL min^{-1}. Column efficiency could be determined by the retention time (t_R) and peak width (W) from the chromatogram of 4,4′-DDT (see 4.2). The number of theoretical plates (N) at each flow rate was calculated using the plate theory equation; $N = 5.54\ (t_R/W½)^2$. The height equivalent to a theoretical plate (HETP) was then obtained by substituting N in the equation; HETP = L/N where L was the length of chromatographic column (30 m). A plot of HETP *vs* carrier gas flow rate, the van Deemter plot, would provide the optimum carrier gas flow rate, *i.e.*, the best efficiency for separation at minimum HETP (Grob, 2004). Figure 5.4 shows the relationship between the HETP and carrier gas flow rate. The van Deemter graph of 4,4′-DDT gives the minimum HETP at 0.8 mL min^{-1}.

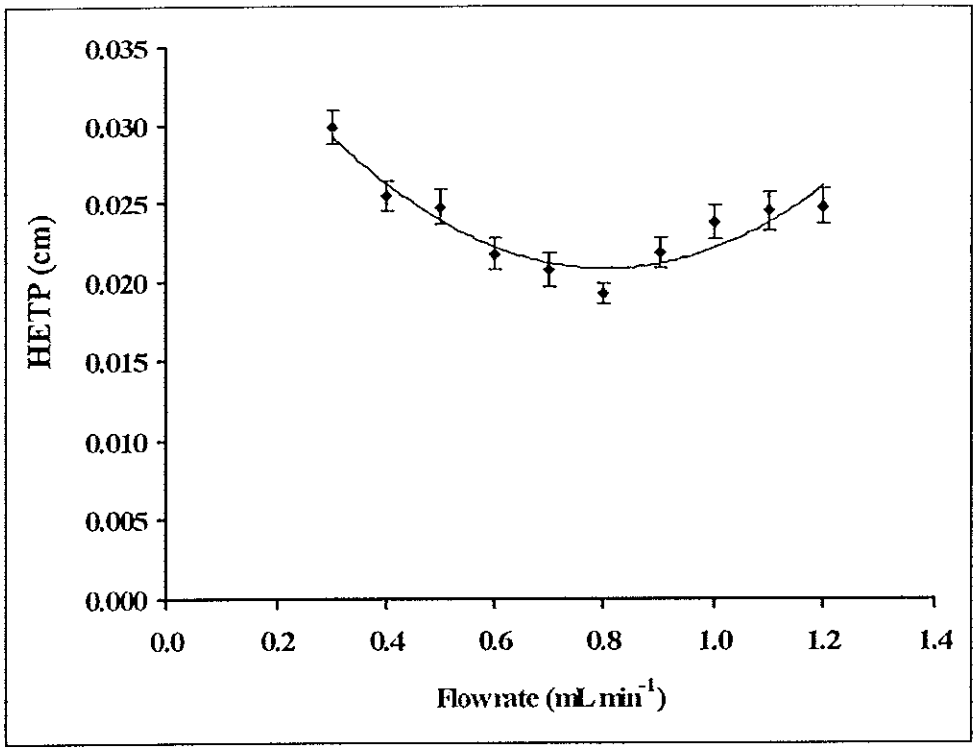


Figure 5.4 van Deemter plot of 4,4'-DDT

5.5.1.2. Column temperature

In order to obtain good resolution and response as well as a short analysis time in a GC-MS system, suitable separation conditions are required. Column temperature is one of the main variables affecting the separation. The analysis of 4,4'-DDT has mostly used temperature programming (Basheer *et al.*, 2002; Chao *et al.*, 2006; Frías *et al.*, 2001; Mukherjee and Gopal, 1996; Pandit *et al.*, 2001; Tahboub *et al.*, 2005; Takase *et al.*, 2003) and this required quite a long period of time (> 10 min) to analyze and cool the column down to its initial temperature before another analysis could take place. This would be difficult to operate in an in-line system where the results are required in a short period of time. Therefore, isothermal conditions were chosen for in this work. The spiked aqueous solution at a concentration of 1.0 mg L^{-1} was pumped through the MI unit and the evaporated analyte was stripped out of the membrane surface by helium gas and injected into GC-MS system. The optimum

column temperature was investigated by varying the temperature from 150, 180, 220, 250, 280 and 300°C.

Figure 5.5 shows the peak response of 4,4′-DDT at various column temperatures. The peak height increased when the column temperature increased. Since 4,4′-DDT is a semivolatile organic compound that has quite a high boiling points (185°C) (Ome and Kegley, 2004), therefore, a high temperature is needed to separate it from the column. After investigation, the column temperature at 300°C was chosen as optimum temperature because it gave the highest response. Temperatures above 300°C were not investigated because it was near the maximum acceptable temperature of the HP-5MS column (325°C) and this could degrade the column performance. Therefore, to ensure the high abundance and maintain the lifetime of the column, the optimum temperature should not be over this value (Grob, 1985).

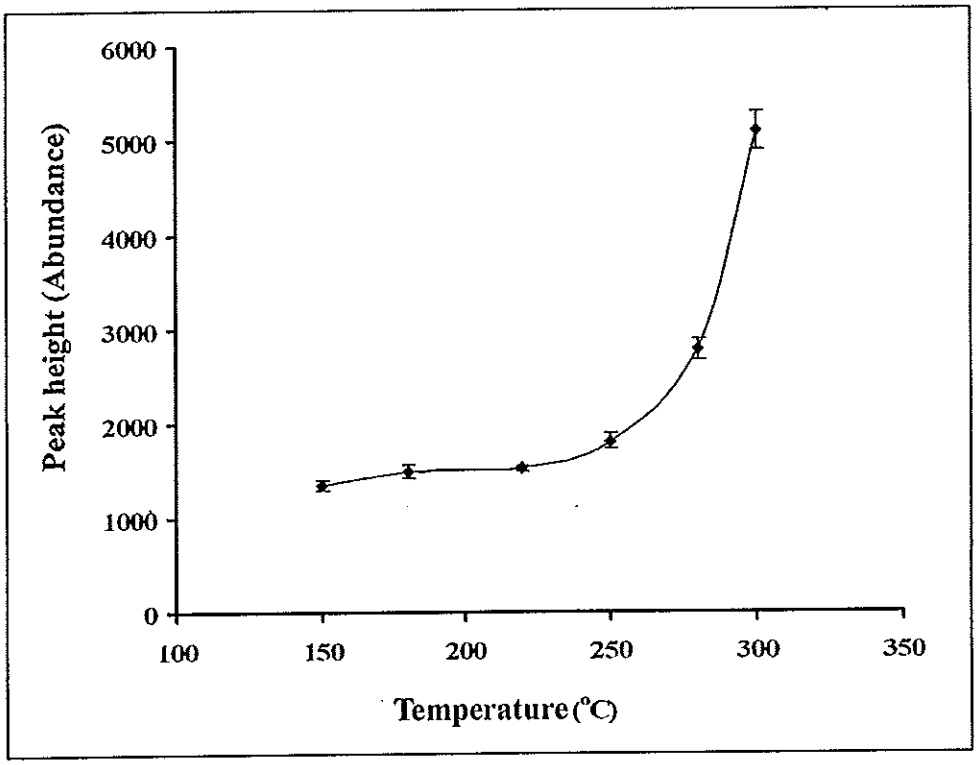


Figure 5.5 Effect of column temperature on 4,4′-DDT analysis

5.5.1.3. Injector temperature

The injector temperature is another important parameter needed to be studied. It should be set high enough so that there is enough thermal mass and energy to vaporize the injected sample without causing decomposition of the analyte (Grob, 2004). Setting the column temperature and carrier gas flow rate at the optimum conditions obtained earlier, the injector temperature was investigated by varying the temperature from 210°C to 270°C with an increment of 10°C. Figure 5.6 shows the peak response of 4,4′-DDT at various injector temperatures. The results show that the peak height increased when the temperature increased. This was because high temperatures could enhance the vaporization of analyte. When the temperature was higher than 250°C, the peak height decreased because the analyte may be degraded to other forms, *i.e.*, DDE or DDD, which is catalyzed by the glass surface of inlet liner at a temperature higher than its boiling point (260°C for 4,4′-DDT) (Agilent, 2004; Besbelli, 1992; McEven and Stephenson, 1979). Therefore, the MSD gave the highest abundance of 4,4′-DDT at 250°C and this was chosen to be an optimum injector temperature.

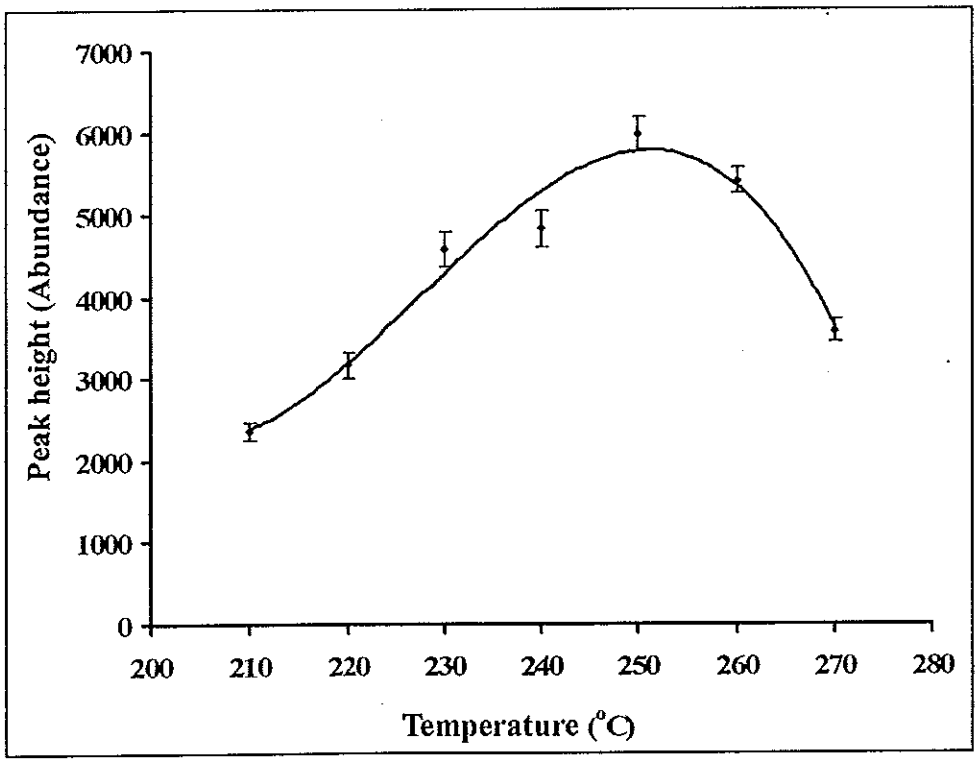


Figure 5.6 Effect of injector temperature on 4,4′-DDT analysis

5.5.2 Optimization of the extraction by the membrane inlet system

Parameters that can affect the extraction process, *i.e.*, sample and stripping gas flow rates, desorption temperature and loading time were optimized based on the justification of two criteria, namely high sensitivity and short analysis time. All data were obtained under SIM acquisition mode. Extraction performance was determined by comparing the response from ultra pure water and bottled water samples (0.1-1.0 mgL^{-1}).

5.5.2.1. Sample and stripping gas flow rate

The pervaporation rate of the analyte can be limited by poor mixing at the sample membrane interface which can cause the formation of a layer of analyte depletion, resulting in low response and longer response time (Guo and Mitra, 1999a). This effect is commonly reported when using linear flow on flat membranes (LaPack *et al.*, 1990). Therefore, the counter current flow is preferred since at a higher degree of flow, it can reduce the thickness of the mass transfer boundary layer and in addition, there is no change of liquid - gas phase while it allows only the component to permeate from the feed to the permeate side (Ribeiro Jr. and Borges, 2004). Therefore, the effect of flow such as the sample and stripping gas flow rates on the response was investigated.

Ultra pure water spiked with 0.5 mgL^{-1} of 4,4'-DDT, was pumped through the MI unit at various flow rates from 1 to 7 mL min^{-1} while the stripping gas flow rate was maintained at 4 mL min^{-1}. The results show that the peak response increased with sample flow rate (Figure 5.7). This is because a high sample flow rate could minimize a layer of depletion on the membrane surface by allowing a larger amount of analyte to contact the membrane per unit time (Kuosmanen *et al.*, 2003). However, at flow rates higher than 6 mL min^{-1}, a decreased response was obtained. This was probably because at too high a flow rate the time was too short for the analyte to permeating through the membrane. Therefore, the optimum value of sample flow rate was 6 mL min^{-1}. This optimum sample flow rate corresponded well with typical flow rates used in MIMS, *i.e.*, below 10 mL min^{-1} (Guo and Mitra, 1999a; LaPack *et al.*, 1990).

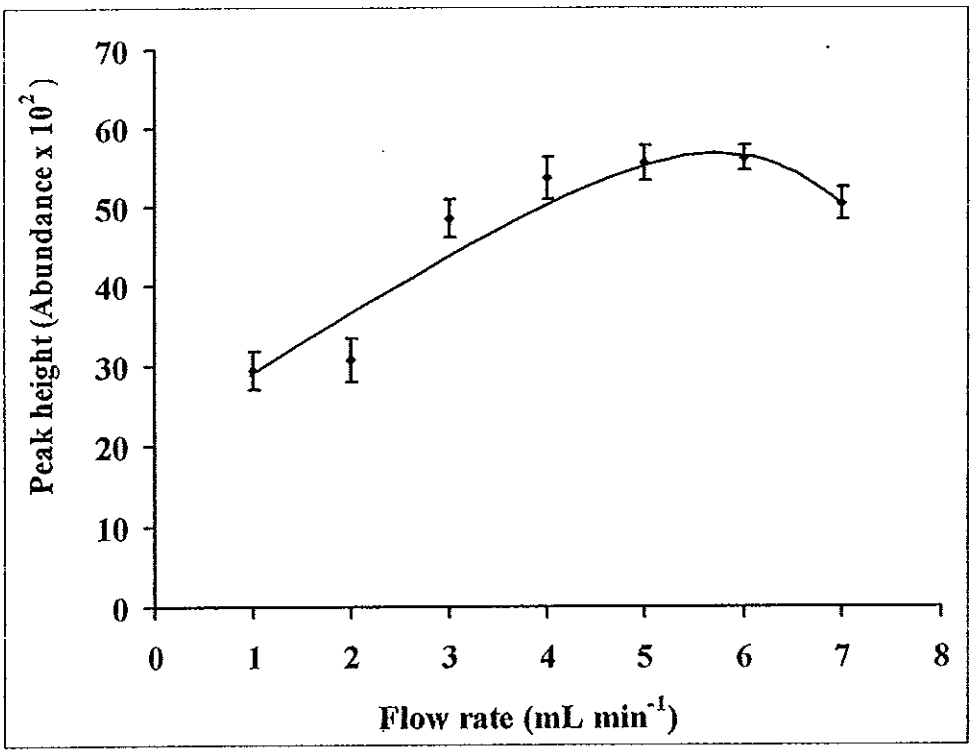


Figure 5.7 Effect of aqueous sample flow rate to peak response of 4,4'-DDT (0.5 mg L^{-1}) by fixing stripping gas flow rate at 4 mL min^{-1}

By considering the equation for extraction efficiency (E) (Kuosmanen *et al.*, 2003).

$$E = 1 - \frac{F_D}{(F_D + F_A K)} \qquad (5.1)$$

where F_D is the volume flow rate of the sample phase, F_A is the volume flow rate of the stripping gas phase and K is the partition coefficient, it can be seen that when the sample flow rate is fixed, the extraction can be improved by increasing the stripping gas flow rate. Therefore, the stripping gas flow rate was then optimized at the optimum sample flow rate (6 mL min^{-1}).

Helium was used to strip permeate the analyte to the GC-MS at 3, 4, 5, 6, 8 and 10 mL min^{-1}. Figure 5.8 shows that when the sample flow rate is fixed the extraction can be improved by increasing the stripping gas flow rate. The response increased with stripping gas flow rate until 9 mL min^{-1} then started to decrease due to high dilution from the increase of stripping gas at high flow rate.

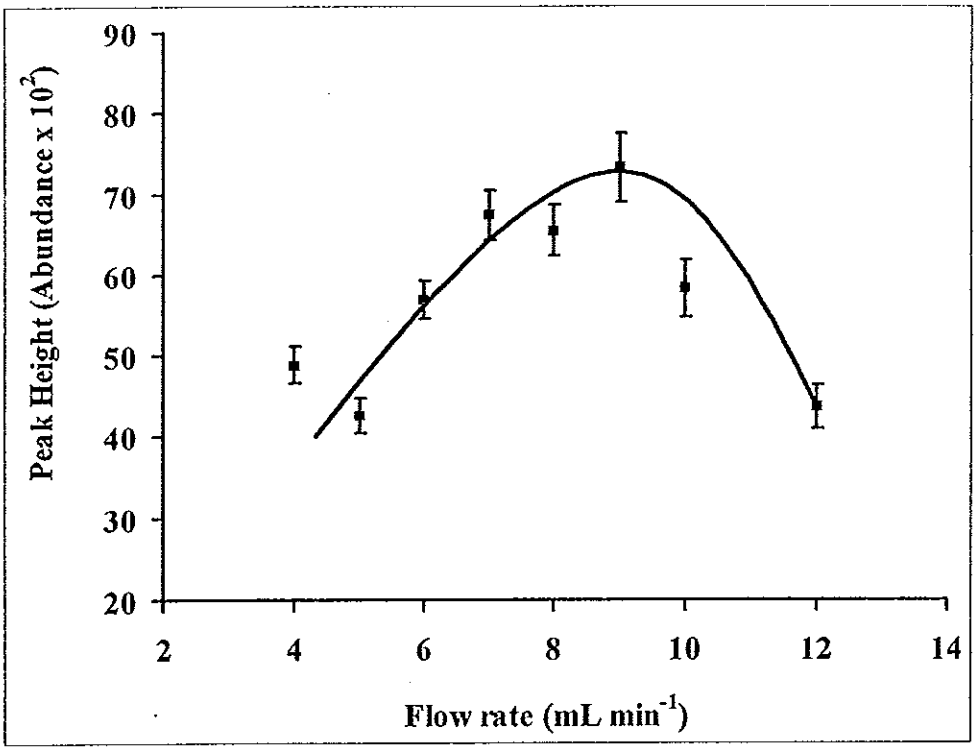


Figure 5.8 Effect of stripping gas flow rate to peak response of 4,4'-DDT (1.0 mg L^{-1}) by fixing sample flow rate at 6 mL min^{-1}

5.5.2.2. Desorption temperature

In a continuous flow-through extraction system, permeation of SVOCs at room temperature may take a relative long time to reach a steady state, and any measurement during the transition period will provide erroneous results. Typically, relatively high sample flow rate was used to increase the extraction efficiency and to minimize the extraction time. However, in a case where the analyte has a large partition coefficient, like SVOCs, it is difficult to monitor with conventional MIMS at the standard temperature (25°C). An improvement of the extraction efficiency could be achieved by rising the extraction temperature, as this is the temperature is a parameter controlling the physicochemical properties such as extraction rate, efficiency and selectivity. The high temperature can enhance the solubility of less-polar compounds dramatically in the extraction phase (Kuosmanen *et al.*, 2003; Miller and Hawthorne, 1998).

A paper box with a 60 watt lamp was used as a thermal desorption unit to enhance the diffusion of 4,4'-DDT in water and desorb analyte from the membrane into the GC-MS. Desorption temperatures from 30 to 60°C with an increment of 10°C were investigated and the results are shown in Figure 5.9. It can be seen that the response of 4,4'-DDT increased with desorption temperature. Over 60 °C the membrane started to deform and bubbles were formed in the system. Therefore, the desorption temperature of 60 °C was chosen for used in this work in order to enhance diffusivity.

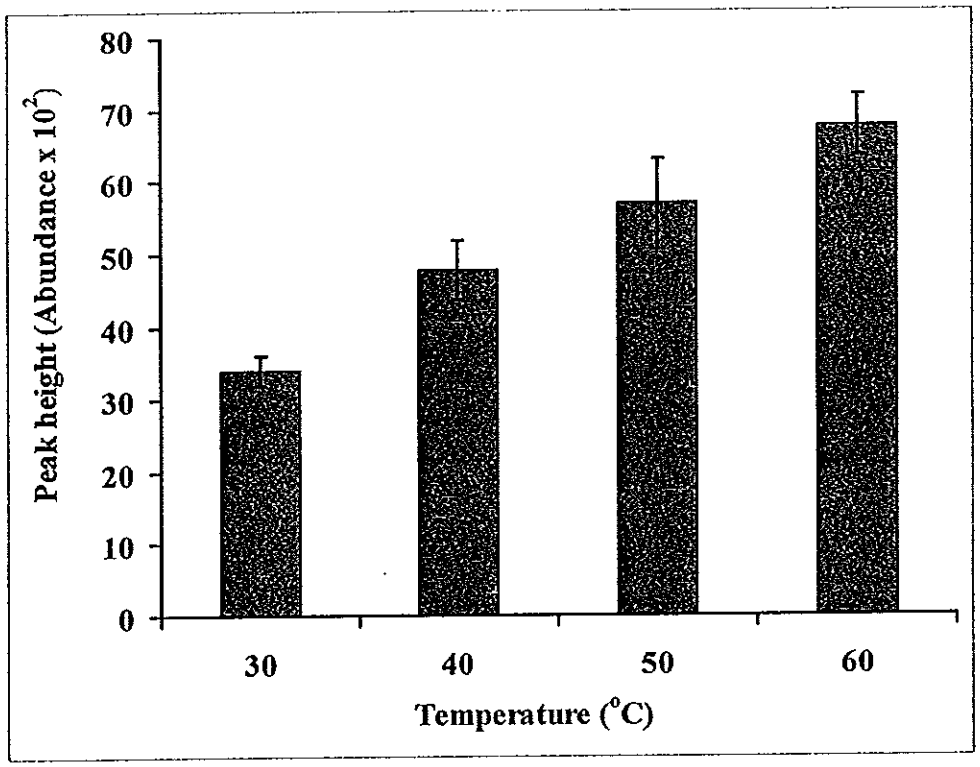


Figure 5.9 Effect of desorption temperature to peak response of 4,4'-DDT

In order to ensure that the temperature inside the thermal desorption unit was relatively uniform. Five positions in the thermal desorption unit were tested at optimum desorption temperature (60°C). The temperatures were measured every 5 minutes for 20 minutes and the results are shown in Table 5.1.

Table 5.1 Temperatures from five positions in the thermal desorption unit, measured every 5 minute.

Position	Temperature (°C)
P1	59, 57, 57, 59
P2	60, 58, 59, 61
P3	60, 61, 61, 59
P4	59, 59, 57, 59
P5	60, 58, 57, 59

One-way ANOVA Analysis of variance (Miller and Miller, 2000), was used as the statistical test to determine whether there is any significant difference between the means of the five positions. Testing parameters were calculated using the following equations.

Between-sample variation

$$\sigma_0^2 = n\sum_i (\bar{x}_i - \bar{x})^2 / (h-1) \qquad (5.2)$$

Within-sample variation

$$\sigma_0^2 = \sum_i \sum_j (x_{ij} - \bar{x}_i)^2 / h(n-1) \qquad (5.3)$$

Total variation

$$\sigma_0^2 = \sum_i \sum_j (x_{ij} - \bar{x})^2 / (hn-1) \qquad (5.4)$$

Where σ_0^2 is a sum of square

n is number of measurement per position (or sample); $n = 4$

h is number of measured position (or sample); $h = 5$

n-1 is degree of freedom of between-sample

$h(n$-1) is degree of freedom of within-sample

The values obtained from equation 5.2-5.4 were then used to calculate the mean square (= (sum of square) / (degree of freedom)) and the F value (= (between-sample mean square) / (within-sample mean square)). This F value was then compared to the critical value, $F_{h-1,hn-1}$ (Miller and Miller, 2000). The values obtained are as follows.

ANOVA

Source of variation	Sum of Square	Degree of freedom	Mean Square	F	$F_{4,15}$ crit
Between-sample	13.20	(5-1) = 4	13.20/4 = 3.30	3.30/78.60 = 0.042	3.056
Within-sample	1179.00	5(4-1) = 15	1179.00/15 = 78.60		
Total	32.95	(5x4)-1 = 19			

The F value, calculated from experiment data in Table 5.1, was less than a critical value F4,15 with $P < 0.05$ (see F table in Appendix A) indicating that there is no difference between positions (Miller and Miller, 2000).

In order to find out how much the heat helps to enhance the diffusion of analyte through the membrane, we considered the following.

In this experimental set up, the pervaporation process can be described by Fick's equations of diffusion (Equation 5.5 and 5.6) (Srinivasan *et al.*, 1997).

$$I_m(x,t) = -AD\left\{\frac{\partial C_m(x,t)}{\partial x}\right\} \qquad (5.5)$$

$$\left\{\frac{\partial C_m(x,t)}{\partial t}\right\} = D\left\{\frac{\partial^2 C_m(x,t)}{\partial x^2}\right\} \qquad (5.6)$$

where $I_m(x,t)$ is the rate of analyte flow inside the membrane (mol s^{-1}), $C(x,t)$ is the concentration inside the membrane (mol cm^{-3}), D is the diffusivity (cm^2 s^{-1}), A is the membrane surface area (cm^2), x is the depth in the membrane (cm), and t is the time (s).

Equation 5.5 helps one to understand the analyte flow in the membrane, while Equation 5.6 reveals how the concentration within the membrane changes with time. At steady state, $\partial C_m(x,t)/\partial x$ through the membrane is constant. Therefore,

$$I_{ss} = AD\left\{\frac{C_s - C_v}{L}\right\} \tag{5.7}$$

where C_s is the concentration of the analyte on the feed side, C_v is the concentration of the analyte on the permeate side, and L is the membrane thickness (cm). By rewriting the equation becomes

$$I_{ss} = AD\left\{\frac{C_m}{L}\right\} \tag{5.8}$$

where $C_m = C_s - C_v$

Applying Henry's law ($C_m = S \times P_s$) to express concentrations in the terms of partial pressure, the steady state flow rate during pervaporation, represented by Equation (5.9), is obtained.

$$I_{ss} = ADS\left\{\frac{P_s}{L}\right\} \tag{5.9}$$

Where I_{ss} is the steady state flow through the membrane (mol s^{-1}), S is the solubility constant of the analyte in the membrane (mol $torr^{-1}$ cm^{-3}), and P_s is the vapor pressure of analyte in the sample (torr).

It is evident from Equation 5.9 that larger and thinner membranes are preferable for maximum analyte flow, and the standard technique of separation variables can be applied to Equation 5.6 to obtain the concentrations inside the membrane as a function of time, when there is small change in concentration. The concentration inside the membrane (C_m) can then be substituted into Equation 5.5 and solved for the flow through the membrane.

In this work, the membrane was exposed to a continuous feed of analyte. Therefore, the diffusivity of 4,4'-DDT could be calculated from the analyte

response time ($t_{10\text{-}90\%}$ of peak rise) which is related to membrane thickness (Kotiaho *et al.*, 1991).

The rise time ($t_{10\text{-}90\%}$) can be calculated using Equation 5.7.

$$t_{10-90\%} = 0.237\left(\frac{L^2}{D}\right) \qquad (5.7)$$

where L is the membrane thickness (L = 0.015 cm) and this gave a diffusity value of 7.4×10^{-5} cm^2 s^{-1}. Comparing to the diffusivity of 4,4′-DDT at standard temperature of 5.0×10^{-6} cm^2 s^{-1} (Batson, 1998), this shows that the heating could enhance the diffusion rate of 4,4′-DDT through hollow fiber membrane by 15 times.

5.5.2.3. Loading time

Before injecting the analyte into the GC-MS, the permeated 4,4′-DDT was stripped by helium to be collected in the gas phase in a 2-mL sampling loop during a loading time. Loading time was investigated from 2 to 25 seconds with an increment of 5 s. Figure 5.10 shows that the response increased with time up to 15 seconds and then became constant. This is the time when the permeated 4,4′-DDT was fully collected in the sample loop. Therefore, a loading time of 15 seconds was chosen to be the optimum value.

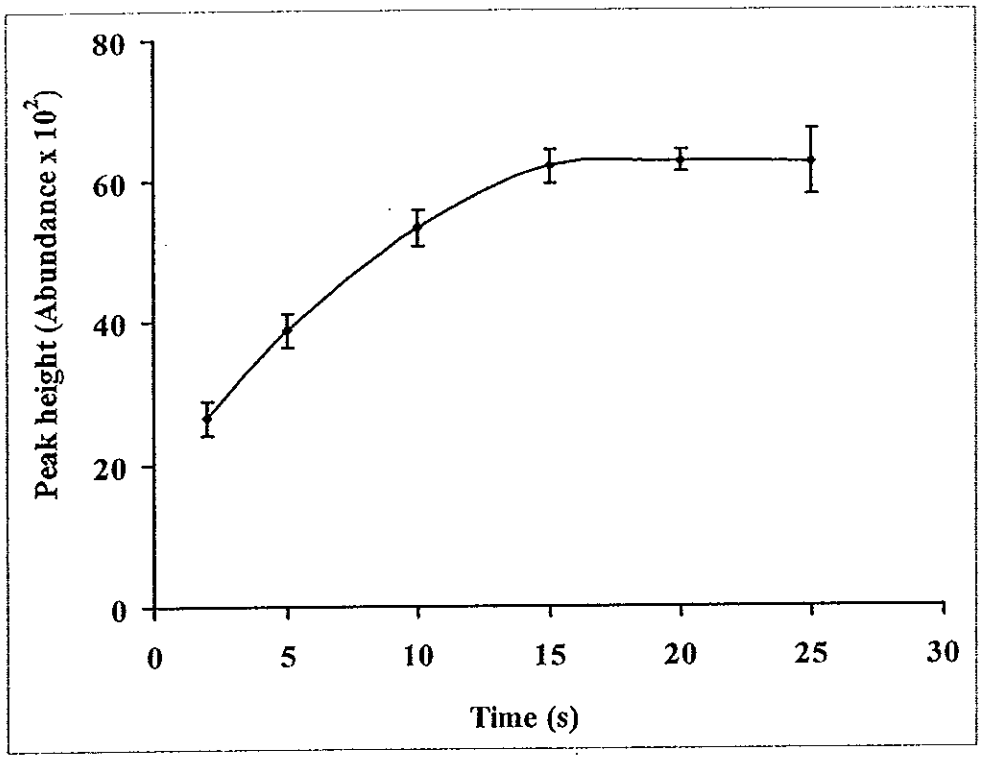


Figure 5.10 Effect of loading time to peak response of 4,4'-DDT

5.5.3 System performance

In order to validate the method, system performances were studied based upon the optimum condition as shown in Table 5.2.

Table 5.2 Summary of conditions for analysis of 4,4'-DDT.

Parameters	Condition value
GC-MS	
Injection mode	Splitless
carrier gas flow rate	0.8 mL min^{-1}
column temperature	Isothermal at 300°C
injector temperature	250 °C
mass detector temperature	230 °C (MS source), 150 °C (MS quadrupole)
selected ion monitoring (SIM)	m/z 235, 165
MI Unit	
Sample flow rate	6 mL min^{-1}
Stripping gas flow rate	9 mL min^{-1}
Desorption temperature	60 °C
Loading time	15 seconds

5.5.3.1. Linear dynamic range (linearity)

The linear dynamic range is defined as a range of concentration or mass over which the detector exhibits an incremental signal response linearly with an incremental change in concentration or mass of solute reaching the detector (Grob, 2004). Spiked ultra pure water in the concentration range 50.0 ug L^{-1} - 5.0 mg L^{-1} was analyzed. Linearity was determined by plotting a calibration curve. The linearity of the response was determined by considering the correlation coefficient.

Figures 5.11 and 5.12 show very good linearity in the range of 0.1-1.0 mg L^{-1} with the correlation coefficient (r) = 0.9969. This result indicated that the MSD gave a linear response over the range being studied.

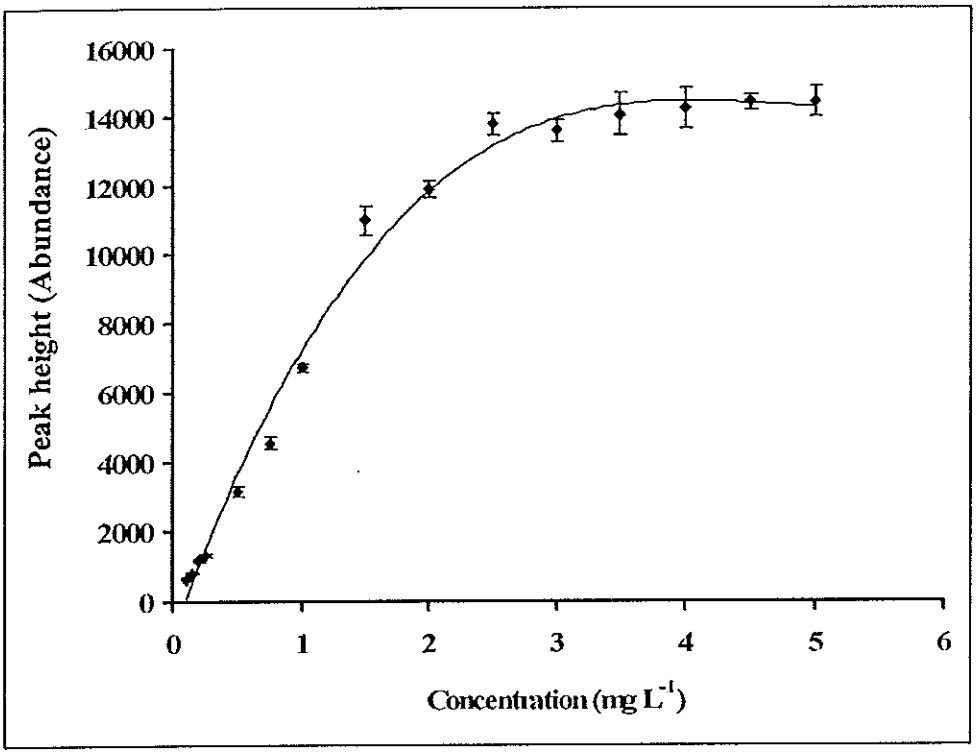


Figure 5.11 Linearity study of 4,4′-DDT at various concentrations

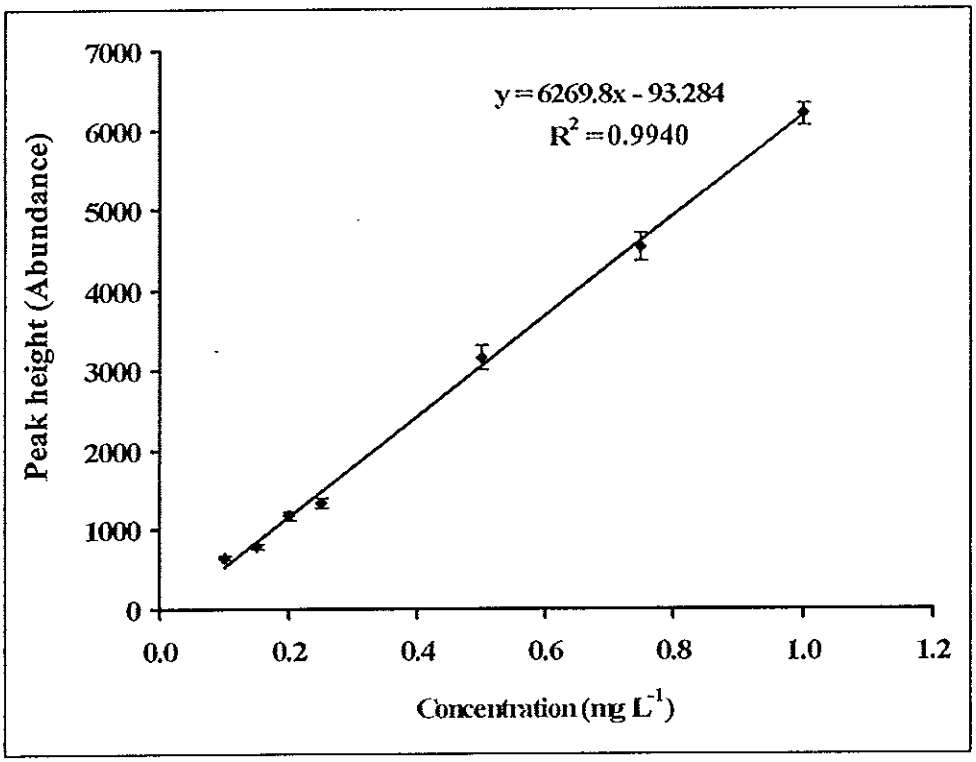


Figure 5.12 Linearity of 4,4′-DDT analysis by membrane inlet coupled to gas chromatography-mass spectrometry

5.5.3.2. Limit of detection (LOD)

The LOD refers to the quantity or concentration of analyte which generates a peak height (or peak area) corresponding to a signal to noise ratio (S/N) of 3 (Grob, 2004). This is the minimum concentration of the substance of interest in the mobile phase for which the detector can give a response that represents a sample peak. Generally, the LOD can be obtained by measuring a signal ratio between analyte peak and noise. But in this work, the lowest concentration or amount of 4,4'-DDT cannot be detected and quantify by SIM data acquisition from the mass selective detector due to matrix effect on the membrane surface which matrix interferences may be caused by contaminants that are co-extracted from the sample. Therefore, IUPAC method (Long and Winefordner, 1983) was used to estimate the LOD by considering the relationship between the standard deviation of blank measurements (Equation 5.10) and analytical sensitivity or slope of the calibration curve (Equation 5.11) as shown in Equation (5.12).

$$S_B = \frac{\sum_{j=1}^{n_B}(x_{Bj-}\bar{x}_B)^2}{(n_B - 1)} \qquad (5.10)$$

Normally, $n_B = 20$ or greater

$$x = mc + i \qquad (5.11)$$

$$C_L = \frac{kS_B}{m} \qquad (5.12)$$

Where C_L is the limit of detection, S_B is the standard deviation of blank measurement and k is equal to 3 as this value allows a confidence level of 99.86% for a measurement based on the error of the blank signal following a normal distribution (Long and Winefordner, 1983).

Table 5.3 shows the results of the 20 blank injection of ultra pure water which was pumped through MI unit and analyzed by GC-MS. Using the IUPAC method, the detection limit under optimum conditions was obtained at 90 μg L^{-1} corresponding to the instrument detection limit of 85 μg L^{-1} (with S/N=3) which was obtained from analysis without the MI unit. It indicated that this developed system, MI-GC-MS, gave a low detection limit, much better than a standard MIMS which has

been used to analyze the semivolatile organic compounds, including DDT (Lauritsen and Ketola, 1997), that is , reducing from 1,000 μg L^{-1} to 90 μg L^{-1}.

Table 5.3 The peak response of blank (ultra pure water) at optimum condition.

Injection Number	Peak height (Abundance)
1	3112
2	3002
3	3105
4	3204
5	3086
6	3312
7	3157
8	3116
9	3009
10	3124
11	3175
12	3067
13	3222
14	3129
15	3030
16	3077
17	3179
18	3047
19	3212
20	3102
Average	3123.35
Standard deviation	78.36
% Relative standard deviation	2.51

5.5.3.3 Extraction efficiency

The efficiency of aqueous samples extracted using MI-GC-MS was studied in two ways. First, the sensitivity of the membrane inlet unit was compared between the results obtained from direct injection of standard solutions to the GC-MS and those obtained from spiked ultra pure water passing through the hollow fiber membrane. The slope of the spiked water was less than the standard solution by 25% as shown in Figure 5.13. This indicated that the extraction process is not 100% and the aqueous samples are needed to calibrate the system before real sample analysis.

Then it was tested by comparing the responses between spiked ultra pure water and spiked bottled water sample at various concentrations (0.1-1.0 mg L^{-1}). The results (Table 5.4 and Figure 5.14) indicated that a good extraction efficiency of between 83 and 94% were obtained with %RSD, based on five replicate injections, less than 10%, better than the 15% RSD reported by the EPA method 8270D (U.S. EPA, 1998a). This is the system which could perform well for real samples.

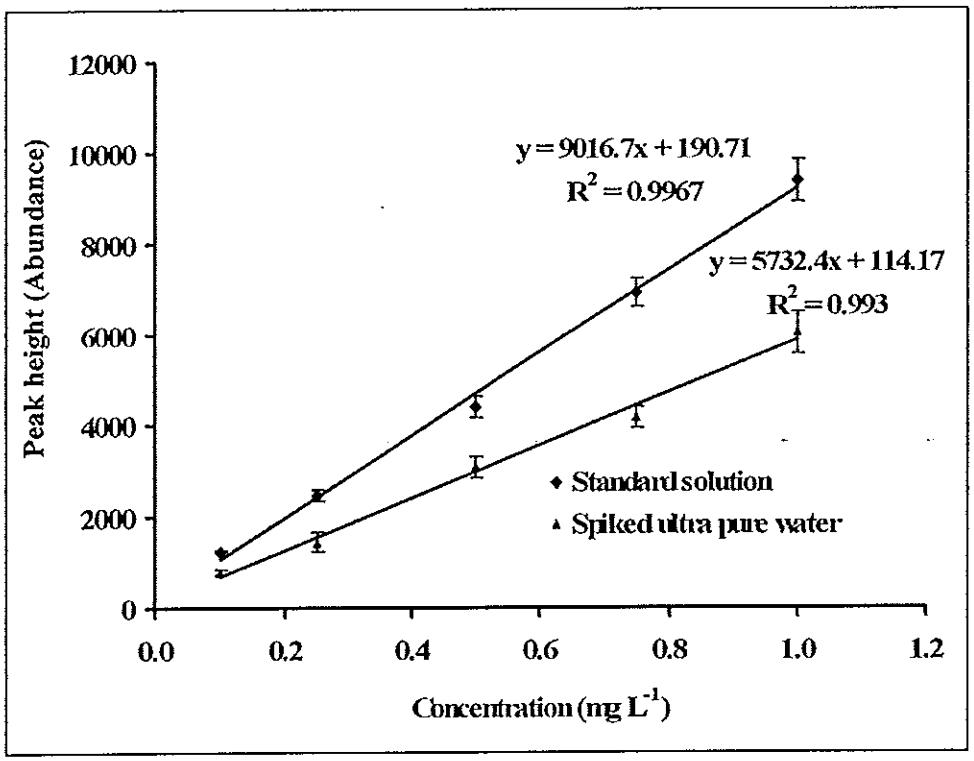


Figure 5.13 Responses of standard solution and spiked ultra pure water at various concentrations

Table 5.4 The extraction efficiency study by comparing response between spiked ultra pure water and spiked water sample at various concentrations

Concentration (mg L^{-1})	Spiked ultra pure water		Spiked water sample		% Extraction
	Peak height (Abundance)	SD	Peak height (Abundance)	SD	
0.10	1086	74.5	910	27.4	84
0.25	2046	165.7	1690	68.6	83
0.50	3985	275.5	3374	277.5	85
0.75	5753	263.3	5428	261.6	94
1.00	8005	562.0	7286	278.1	91

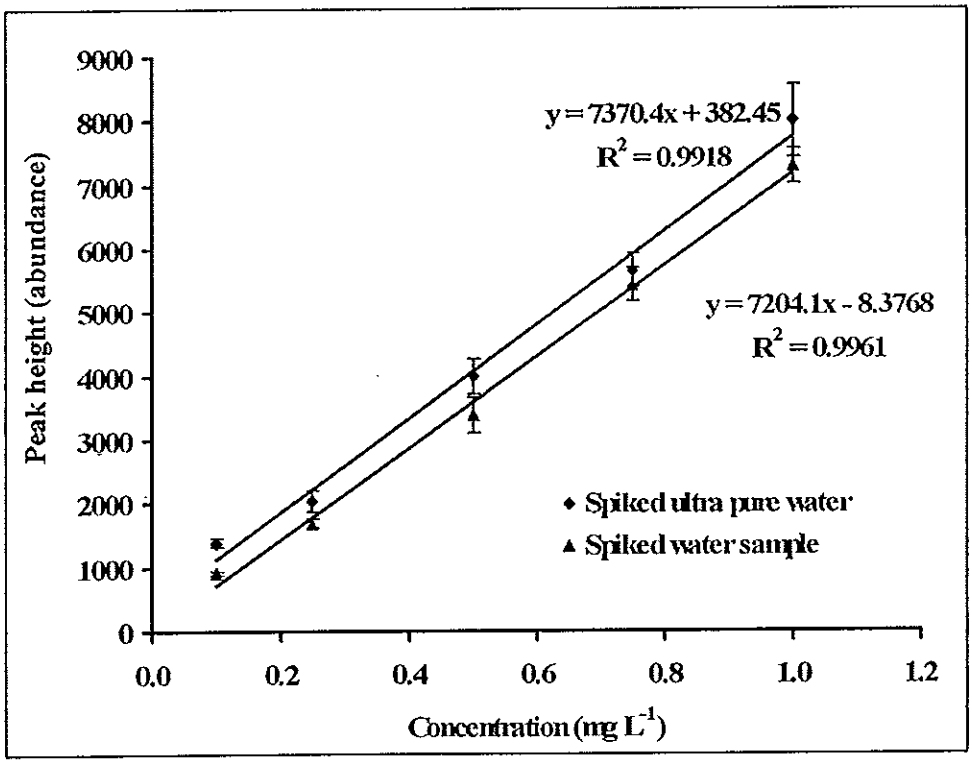


Figure 5.14 Responses of spiked ultra pure water and spiked water sample at various concentrations

5.5.4 Qualitative and quantitative analysis

At optimum conditions, the membrane inlet coupled to gas chromatograph-mass spectrometer system provides a good profile of total ion chromatogram (Figure 5.15) and the qualitative analysis of 4,4′-DDT was determined by comparing the sample mass spectrum with the characteristic ions in a reference mass spectrum. The contaminants were identified based on the criteria that the relative intensities of the characteristic ions agreed within 20% of the relative intensities of these ions in the reference spectrum by referred to EPA8270D (U.S. EPA, 1998a). The analysis was quantified using SIM mode and 4,4′-DDT was monitored at m/z 235 and 165 (Figure 5.16).

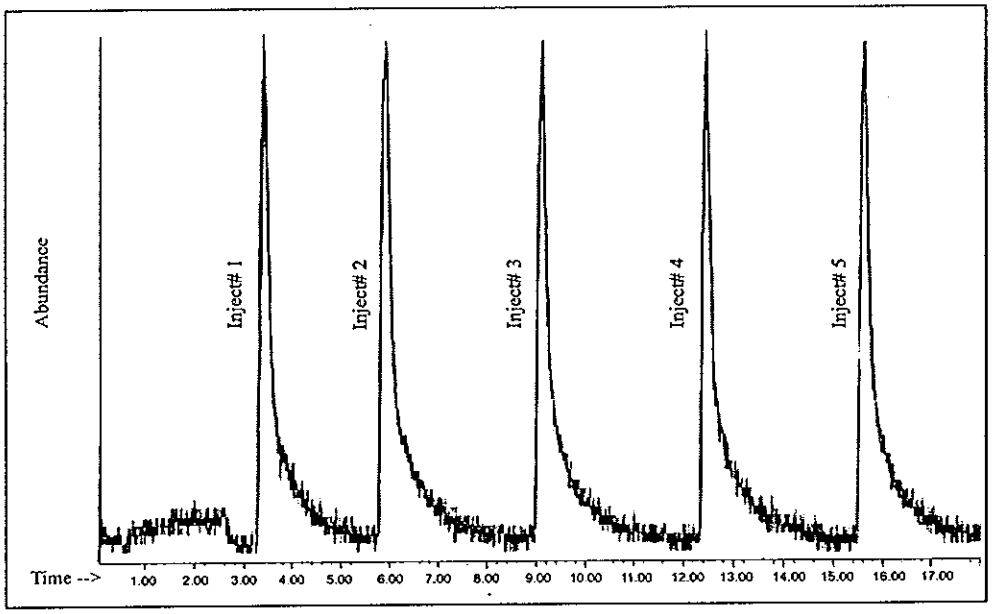


Figure 5.15 Replicated total ion chromatogram of in-line analysis of 4,4′-DDT in water by MI-GC-MS

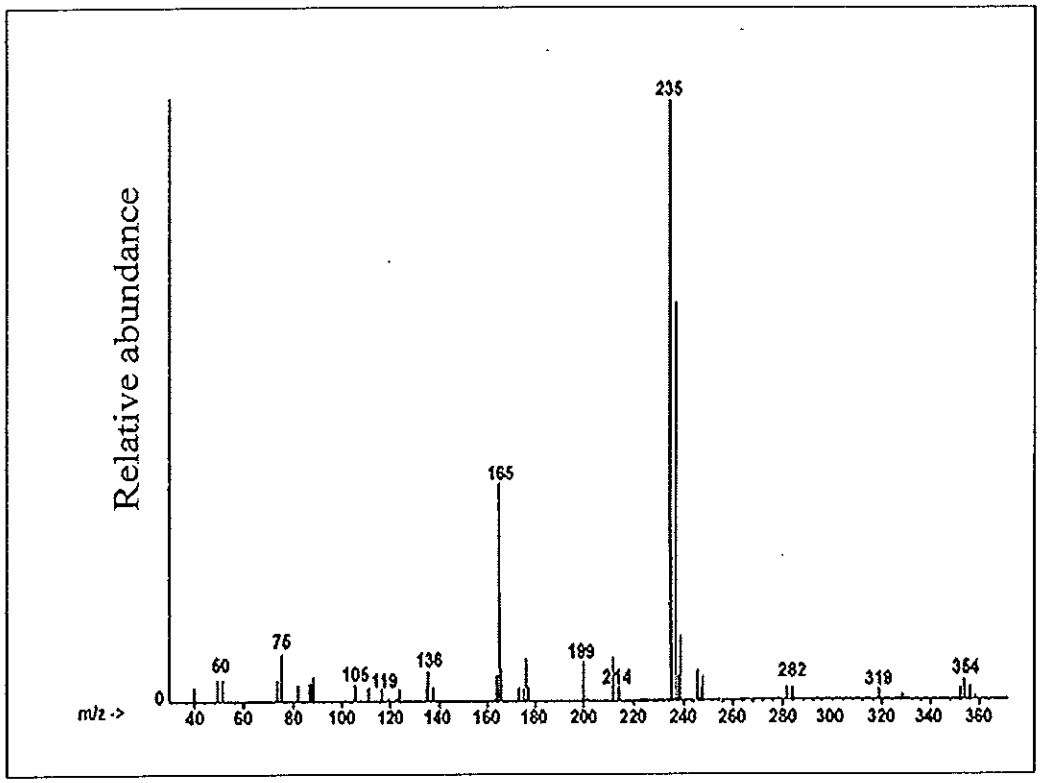


Figure 5.16 Mass spectrum of 4,4′-DDT

5.5.4.1 Real sample analysis

In order to test the capabilities of the technique of using membrane inlet coupled to a gas chromatograph-mass spectrometer, several types of water samples, bottled water (locally produced, unregistered and registered) and tap water, were sampled in Hat Yai City, Thailand, and analyzed. They were collected in clean amber container (Nalgene bottle) and stored at $\leq 4^{o}C$ from the time of collection until being analyzed by the membrane inlet coupled to gas chromatography-mass spectrometry.

Due to the very low concentration of 4,4′-DDT in real sample, the analyte was not detected (its concentration was lower than 90 $\mu g\ L^{-1}$). Therefore, the standard addition method was applied to check the presence of 4,4′-DDT in the samples by spiking five concentrations (0.1, 0.25, 0.50, 0.75 and 1.0 mg L^{-1}) into the water samples as shown in Figure 5.17. The results are shown in Table 5.5.

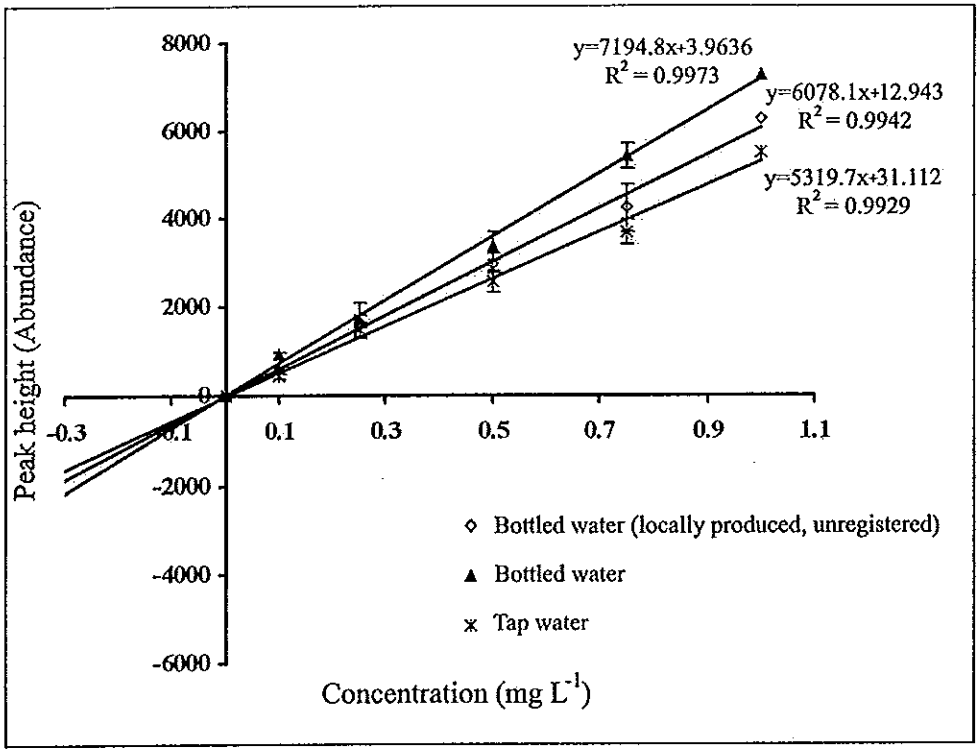


Figure 5.17 Analysis of real samples by membrane inlet coupled to gas chromatography-mass spectrometry using the standard addition method

Table 5.5 Quantity of 4,4′-DDT in real water sampled in Hat Yai City, Songkhla, Thailand

Water sample	Concentration of 4,4′-DDT (μg L^{-1}) (% RSD < 10, n=3)
Bottled water (A) (locally produced, unregistered)	3.0 ± 0.5
Bottled water (B) (Registered)	0.60 ± 0.03
Tap water	7.0 ± 0.5

According to an international guideline, DDT in drinking water must be less than 2 μg L^{-1} (WHO, 2004). From the results (Table 5.5), 4,4′-DDT in bottled water samples (0.60 ± 0.03 μg L^{-1}) was lower than this limit. As for the locally produced, unregistered bottled water, its 4,4′-DDT concentration was higher than the guideline for drinking water. This may be the result of the wide spread of hemorrhagic fever in the country, and to prevent the spreading by mosquitoes, the 4,4′-DDT was sprayed in a very wide area causing it to accumulate in soil and then leach into raw water used to process this bottled water. It could also be lack of regular monitoring by the authority and no quality control of the production process. Consumer should therefore, be careful with their purchase. Tap water also showed high levels of 4,4′-DDT (7.0 ± 0.5 μg L^{-1}). This indicated that the water source is contaminated and any treatment process does not sufficiently remove of 4,4′-DDT. Therefore, regular monitoring is necessary and a warning should be provided to the public that the water is not suitable for drinking.

5.6 Conclusion

The membrane inlet system coupled to a gas chromatograph-mass selective detector (MI-GC-MS) for in-line membrane extraction is an alternative approach of analytical method for semivolatile organic compounds (SVOCs), especially the persistent organic pollutants (POPs), in water. If the concentration of SVOCs is higher than the limit of detection, this system can certainly be used for in-line monitoring. Although standard addition was needed because the concentration of analyte in the sample was very low, this developed system still has many advantages, *i.e.*, it can provide good precision with RSD lower than 10%, the sample can be injected directly without any sample preparations because the extractor unit is set up in-line. Therefore, it does not require any sample handling and preservative step. In some cases where standard addition is required, either when the concentration is lower than the detection limit and/or when the real sample has a matrix effect, it can be performed in-line in a short period of time. Furthermore, this method is less laboring, not require solvent and can be applied to real samples for qualitative and quantitative analysis.

Chapter 6

Capillary microextraction for simultaneous analysis of multi residual semivolatile organic compounds in water

6.1 Introduction

Continuous monitoring of priority hazardous substances in environmental samples is of great importance, especially for residue of some semivolatile organic compounds (SVOCs) in water, *i.e.*, chlorinated hydrocarbons, pesticides and polycyclic aromatic hydrocarbons (PAHs) (ATSDR, 2006), since these trace substances can affect human health (López-Blanco *et al.*, 2002; Olejniczak *et al.*, 2003). In order to monitor and control the quality of water, a reliable and highly sensitive analytical method is required, and either high performance liquid chromatography or capillary gas chromatography is usually employed. The latter is preferred as it offers high resolution and is easy to couple to sensitive and selective detectors (Mol *et al.*, 1995; Pocurull *et al.*, 1995). Since the concentration of SVOCs in water is normally very low, sample preparation techniques are required in order to enrich the analytes so as to meet the instrumentation detection limit (ATSDR, 2006; López-Blanco *et al.*, 2002; Olejniczak *et al.*, 2003).

Several sample preparation techniques aimed at reducing solvent use have recently been reported such as micro liquid-liquid extraction (μLLE) (Zapf *et al.*, 1995), solid phase extraction (SPE) (Ackerman and Hurtubise, 2002; Columé *et al.*, 2001; García-Falcón *et al.*, 2004b; Nogueira *et al.*, 2004; Stajnbaher and Zupancic-Kralj, 2003; Zhang *et al.*, 2004; Zulin *et al.*, 2002), solid phase microextraction (SPME) (Dalvie *et al.*, 2005; Eisert and Pawliszyn, 1997; Zambonin *et al.*, 2004), membrane extraction (ME) (Nardi, 2003b; Zimmerman *et al.*, 2000), in-tube solid phase microextraction (IT-SPME) (Globig and Weickhardt, 2005; Gou *et al.*, 2000; Tan *et al.*, 1999) and stir bar sorptive extraction (SBSE) (Blasco *et al.*, 2002; García-Falcón *et al.*, 2004a; Kolahgar *et al.*, 2002; León *et al.*, 2003; Peñalver *et al.*, 2003;

Popp *et al.*, 2004; Popp *et al.*, 2001). Although the reduction of solvent consumption can be accomplished with the SPE technique, it still requires solvents in the order of milliliters and has limited sample loading volume (Saito *et al.*, 2002).

Lately, the most demanding trend is to detect target analytes at parts-per-million to parts-per-trillion levels in aqueous samples and SPME has become a favorite. It is used to adsorb the analyte and desorb it to the gas chromatographic injector or high performance liquid chromatographic interface. In addition, it requires only a small sample volume and is simple to operate. However, with the small amount of sorbent coated on the SPME fiber (up to 0.5 μL), the sensitivity problem was hard to overcome (Baltussen, 2000). Moreover, SPME has limiting lifetime, is fragile and the fibers and assembly holder are relatively expensive (Kabir *et al.*, 2004). SBSE can overcome these problems and has further extended the detection limits to parts-per-quadrillion due to high capacity of large film thickness of polydimethylsiloxane (PDMS). However, it requires a dedicated hot injector and suffers from unavoidable disadvantages such as high bleed, injection artifacts and carry over (Baltussen *et al.*, 2002; Montero *et al.*, 2004; Nardi, 2003b).

Recently, a few researchers have investigated the use of a chromatographic capillary column as both extractor and pre-concentrator in order to enrich SVOCs in aqueous samples. The sorption of SVOCs onto the thin stationary phase PDMS film of a gas chromatographic capillary column helps to extract and pre-concentrate the analytes. They were then desorbed with either suitable solvent or thermal desorption. The sorption area in the capillary microextractor is much higher than with SPME resulting in high recovery and high sample capacity. Moreover, a thin film thickness can overcome the high bleed and carry over problems of SBSE (Globig and Weickhardt, 2005; Gou *et al.*, 2000; Gou and Pawliszyn, 2000; Mitani *et al.*, 2003; Mullett *et al.*, 2002; Raghani and Schultz, 2003; Tan *et al.*, 1999; Wang *et al.*, 2004).

Many reports employed this technique with a column length of 60 cm up to more than one meter with relatively large film thickness (0.5 - 4.0 nm) (Djozan and Amir-Zehni, 2004; Gou *et al.*, 2000; Nardi, 2003b; Olejniczak *et al.*, 2003; Tan *et al.*, 1999; Wang *et al.*, 2004) by coupling it to high performance liquid chromatography (Gou and Pawliszyn, 2000; Mitani *et al.*, 2003; Mullett *et al.*, 2002;

Raghani and Schultz, 2003; Wang *et al.*, 2004) with fewer reports on its use coupled to gas chromatography (Globig and Weickhardt, 2005; Gou *et al.*, 2000; Tan *et al.*, 1999; Wang *et al.*, 2004). To the best of our knowledge, no one has reported using this technique to simultaneously extract multi residues of different groups of SVOCs. The aim of this work is to investigate the use of a chromatographic capillary column as microextractor coupled to a GC-MS to simultaneously analyze multi residual semivolatile organic compounds in water, *i.e.*, chlorinated hydrocarbons, pesticides and PAHs that are in the toxic priority list of the Agency for Toxic Substances and Disease registry (ATSDR) (ATSDR, 2006).

6.2 Chemicals and materials

6.2.1 Standard chemicals (Certified solutions with purity 99 - 100%, Restek Company., USA):

- Hexachlorobutadiene 1,000 mg L^{-1} in methanol
- Dieldrin 1,000 mg L^{-1} in methanol
- 4,4′- Dichlorodiphenyltrichloroethane; 4,4′-DDT 1,000 mg L^{-1} in methanol
- Benzo[*a*]pyrene 1,000 mg L^{-1} in acetone
- Benzo[*b*]fluoranthene 1,000 mg L^{-1} in acetone
- Dibenzo[*a,h*]anthracene 1,000 mg L^{-1} in dichloromethane

6.2.2 General solvents and chemicals

- Methanol (CH_3OH, GR GRADAE: Merck, USA)
- Acetone (CH_3COCH_3, ACS-ISO-For analysis: Carlo Erba, USA)
- Dichloromethane (CH_2Cl_2, AR Grade: LAB-SCAN, Thailand)
- Ethanol (CH_3CH_2OH, GR Grade: MERCH, USA)
- Acetonitrile (CH_3CN, AR Grade: LAB-SCAN, Thailand)
- HPLC-grade water (Scientific instrument center, Prince of Songkla University, Thailand)

6.2.3 Materials

- Capillary Column: HP-5, 50 and 100 cm (0.32 mm I.D. × 0.25 μm film thickness of 5% diphenyl and 95% dimethylpolysiloxane) (Restek company, USA)
- Fused capillary column , 38 cm × 0.10 mm I.D. (Composite Metal company, UK)
- Teflon tube® sample loop 1 mL, $^{1}/_{16}$ in O.D. (Waters Company, USA)

6.2.4 Samples

Tap water and raw water (source of tap water production) samples were collected from Hat Yai City, Thailand. They were analyzed for the six SVOCs by capillary microextractor coupled to GC-MS at the optimum conditions.

6.3 Instruments and apparatus

6.3.1 Gas chromatograph-mass spectrometry system (GC-MS)

- Gas chromatograph model 6890 Series (Agilent Technologies, USA)
- Capillary Column: HP-5MS, 30 m × 0.25 mm I.D. × 0.25 μm film thickness of 5% diphenyl and 95% dimethylpolysiloxane (Agilent Technologies, USA)
- Helium Carrier Gas: Ultra High Purity, 99.99999% (Southern Liquid Oxygen Ltd., Thailand)
- Mass Selective Detector (MSD) model 5973 (Agilent, Technologies, USA)
- Computer system model KAYAK (Hewlett Packard, USA)

6.3.2 Apparatus

- Syringe 10 µl (Agilent Technologies, USA)
- Interchangeable glass syringe 1 and 5 mL (Vitayasom Co. Ltd., Thailand)
- Vial 2 ml with Silver aluminum cap (Agilent Technologies, USA)
- Amber wide-mouth bottle 250 mL (Nalgene, USA)
- Microliter pipette (Gilson, France)
- 2 units of switching valves Model CW6E and E4C10WE, (Valco instrument company, USA)

6.4 Experiments

6.4.1 Preparation of standard stock solutions

Six certified standard stock solutions; hexachlorobutadiene, dieldrin, 4,4′-DDT, benzo[*a*]pyrene, benzo[*b*]fluoranthene and dibenzo[*a,h*]anthracene (99.99% purity) were prepared in their appropriate solvents; hexachlorobutadiene, dieldrin, and 4,4′-DDT in methanol, benzo[*a*]pyrene, and benzo[*b*]fluoranthene in acetone and dibenzo[*a,h*]anthracene in dicholromethane as indicated on the labels, at a concentration of 100 mg L^{-1}. All solutions were kept in amber glass bottles and stored at 4°C.

6.4.2 Preparation of spiked aqueous solutions

Aqueous solutions used to investigate all parameters of extraction and chromatographic analysis were prepared by spiking 100 mL of HPLC-grade water with various concentrations of each standard stock solution to give the desired concentrations. These solutions were used to optimize parameters for analysis.

6.4.3 Analysis

Figure 6.1 shows the schematic diagram of the capillary microextractor system developed in this work. It consisted of two switching valves (C6WE and E4C10WE). Valve A, a six port valve, was employed for loading and purging the sample, and valve B, a 10 port valve, for extracting and desorbing analytes into GC-MS. A capillary microextractor was a 100 cm (see 6.5.2.4) HP-5

capillary column (95% polydimethylsiloxane, 0.32 mm I.D. × film thickness 0.25 μm) while the eluent loop (3 μL) was a 38 cm blank capillary column (0.10 mm I.D.). An additional blank column (10 cm, 0.05 mm I.D.) was used as a transfer line to transport the desorbed analytes to the GC-MS. It was connected directly to the inlet with 5 cm of its end placed inside the liner. The pressure between inlet and transfer line was controlled by the pneumatic system and the force of gas flow.

To start the analysis, a water sample was loaded into the 1 mL Teflon tube® sample loop ($^{1}/_{16}$ in O.D.) on valve A (Figure 6.1, Step 1). After loading, Valve A was switched (Figure 1, Step 2) to allow the water sample to be transported through the capillary microextractor on valve B, by helium gas flow, when the analytes were adsorbed on the PDMS phase in the column. The capillary microextractor was purged with helium gas to dry the column and to remove any residual water. While step 2 was being carried out, organic solvent was loaded into the eluent loop on valve B. When this was complete, valve B was then switched to allow the eluent to desorb analytes from the capillary microextractor and pass through the transfer line into GC-MS (Figure 6.1, Step 3). Analysis was performed using SIM mode to quantify target analytes. After elution, the capillary microextractor was re-equilibrated with an aliquot of acetonitrile, methanol, HPLC-grade water and then dried with gas purging, respectively, before the next injection.

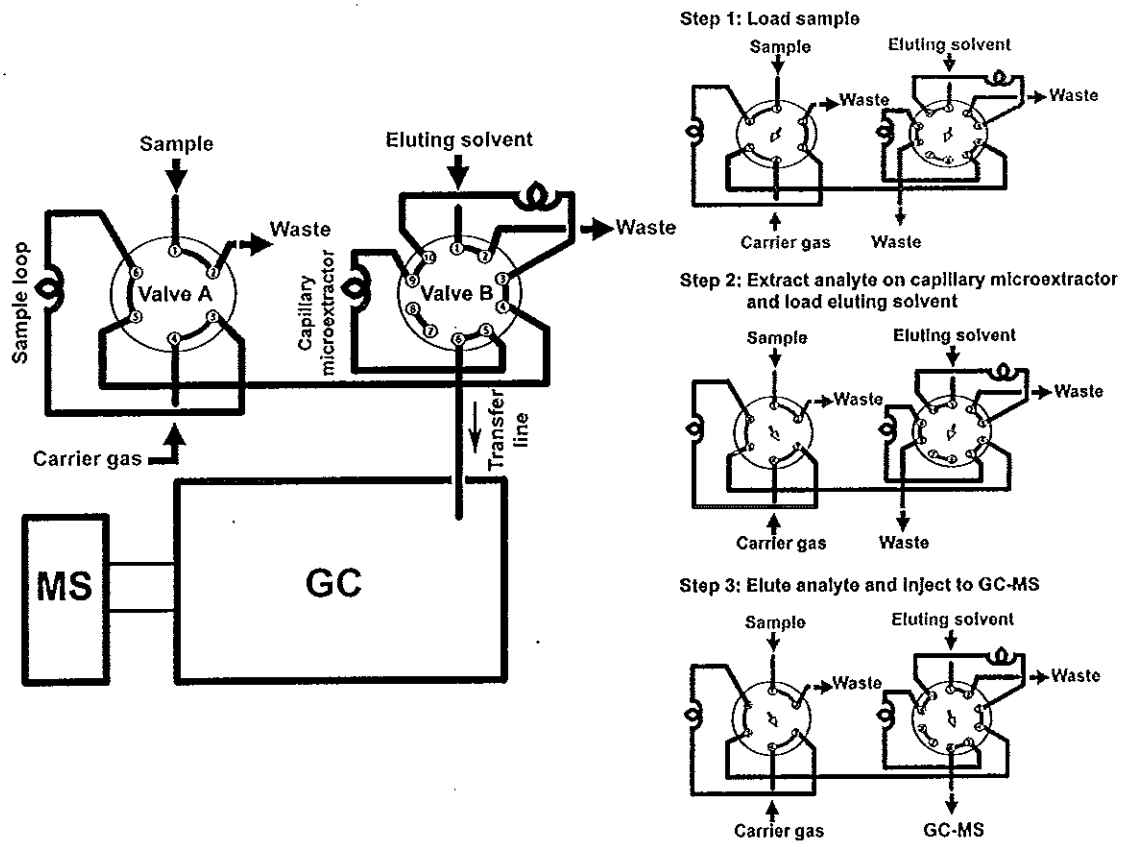


Figure 6.1 Schematic diagram of the capillary microextraction system coupled to gas chromatography-mass spectrometry (CME-GC-MS)

6.5 Results and Discussion

6.5.1 Optimization of the analysis condition of GC-MS

The aim of this study was to investigate the simultaneous analysis of three groups of SVOCs with different properties by using a capillary microextractor coupled to a GC-MS. Parameters affecting the performance of GC-MS were first optimized, *i.e.*, carrier gas flow rate, temperature program, inlet temperature and split ratio. The optimum conditions were chosen by considering two criteria, namely high response and short analysis time.

The analyses were carried out using a gas chromatograph - mass selective detector (Model HP6890- HP5973) with a HP-5MS fused silica capillary column (95% Polydimethylsiloxane, 30 m × 0.25 mm I.D., film thickness 0.25 μm). The split mode was used at a split flow of 1.6 mL min^{-1}. Electron ionization, 70 eV,

with a selected ion monitoring (SIM) mode was used to quantify the analytes with molecular ion at m/z 225(100), 227(63) (hexachlorobutadiene), m/z 79(100), 149(35) (dieldrin), m/z 235(100), 165(66) (4,4'-DDT), m/z 252(100), 250(18) (benzo[*b*]-fluoranthene), m/z 252(100), 250(25) (benzo[*a*]pyrene) and m/z 278(100), 276(16) (dibenzo[*a,h*]anthracene).

6.5.1.1 Carrier gas flow rate

A 1 μL aliquot of six compounds at a concentration of 1.0 mg L^{-1} was analyzed by GC-MS. The optimum carrier gas flow rate was investigated by varying the flow rate of helium carrier gas from 0.3 to 0.9 mL min^{-1} with an increment of 0.1. Column efficiency was determined by the retention time (t_R) and peak width (W) from the chromatogram of six analytes. The number of theoretical plate (N) at each flow rate was calculated using the plate theory equation; $N = 5.54\ (t_R/W½)^2$. The height equivalent to theoretical plate (HETP) was then obtained by substituting N in the equation; HETP = L/N where L was the length of chromatographic column (30 m). A plot between HETP *vs* carrier gas flow rate, the van Deemter plot, provided the optimum carrier gas flow rate, *i.e.*, the best efficiency for separation was at minimum HETP (Grob, 2004). Figure 6.2 shows the relationship between the HETP and carrier gas flow rate. The van Deemter plot of six analytes gives the minimum HETP at 0.7 mL min^{-1}.

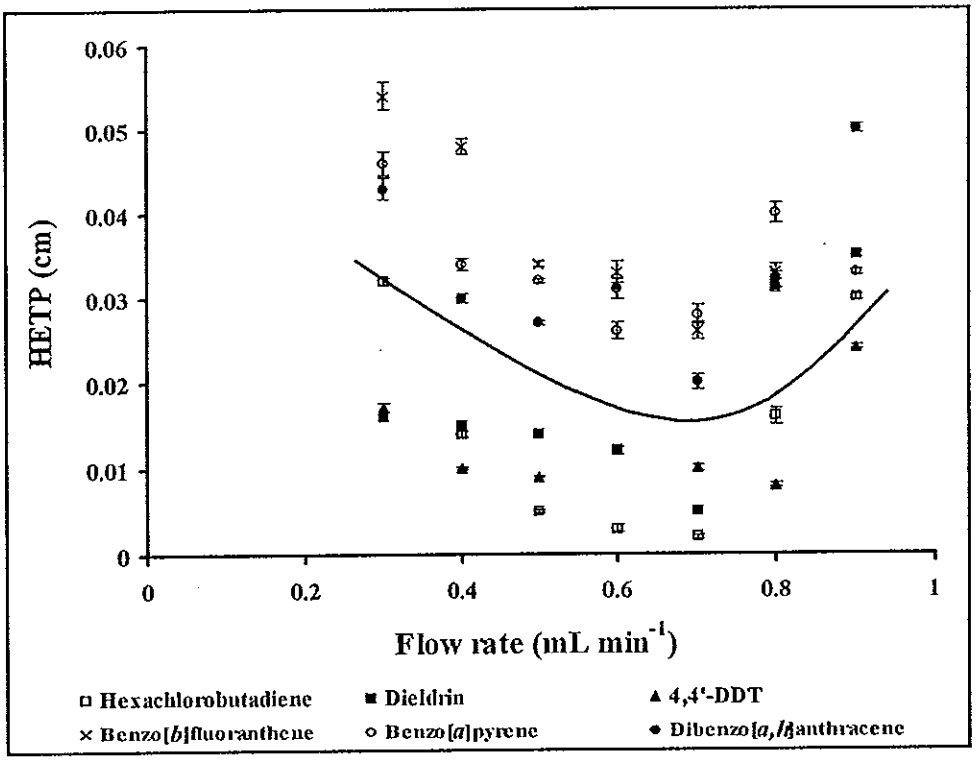


Figure 6.2 van Deemter plot of six compounds

6.5.1.2 Column temperature program

Since the six analytes have different boiling point the analysis time for each analyte would all be different and would take rather a long time to obtain the responses for all analytes. The long analysis time also gave wide and flat peaks which are difficult to evaluate quantitatively (Poole and Schuette, 1985; Scott, 1998). Therefore, the column temperature program was chosen to carry out the analysis of multi residues in order to reduce these problems (Grob, 2004; Scott, 1998). The initial program was started at 50°C then ramped with 20°C min^{-1} to 300°C and held for 5 min. Then the optimum condition for each step was investigated by injecting a 1 µL aliquot of six compounds at a concentration of 1.0 mg L^{-1} into the GC-MS. The MS mass was monitored using SIM mode to obtain the relevant information. Criteria of selection were high response, good resolution and short analysis time.

Step I Initial temperature

The optimum initial temperature was investigated by varying the temperature from 30 to 60°C with an increment of 5°C. The temperature was increased to 300°C with a ramp rate of 20°C min^{-1} and was held at 300°C for 5 minutes. The responses obtained from the different initial temperature were compared. The optimum initial temperature was the one that provided the best abundance of all components. Figure 6.3 shows the responses of all six compounds at various initial temperatures. The response increased when the temperature increased until above 40°C when the response decreased. This was probably due to the weak intermolecular force between analyte and stationary phase at the higher temperature resulting in a low response and poor separation (Jennings, 1987; Poole and Schuette, 1985). From these results, an initial column temperature at 40°C was chosen to be the optimum. At this temperature, the vaporized analytes were trapped at the head of the GC column ("cold trapping") before being swept through the column. This provided a chromatogram with sharp profile and high resolution for the six analytes.

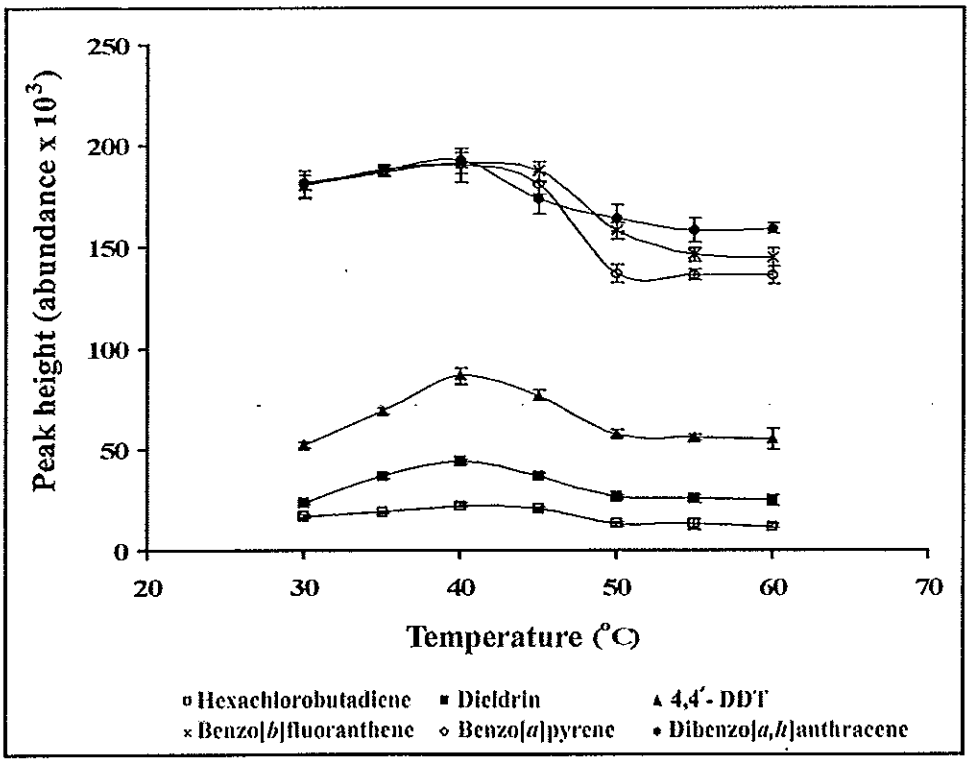


Figure 6.3 Effect of initial column temperature for the analysis of 1.0 mg L^{-1} of six compounds

Step II Hold time at the initial temperature

With the initial temperature set at 40°C (optimum from Step I) the hold time was investigated between 0 and 4 minutes with an increment of 1 minute. The temperature was programmed to 300°C with a ramp rate of 20°C min^{-1} and held at 300°C for 5 minutes. Figure 6.4 shows the responses of the six analytes at various hold times at the optimum initial column temperature. The response increased when the hold time increased because the compounds required equilibration time to partition into the stationary phase (Baltussen, 2000; Grob, 2004; Zweig and Sherma, 1982). The hold time of 3 minutes at 40°C was chosen to be an optimum value because it gave the highest response similar to 4 min, but less analysis time.

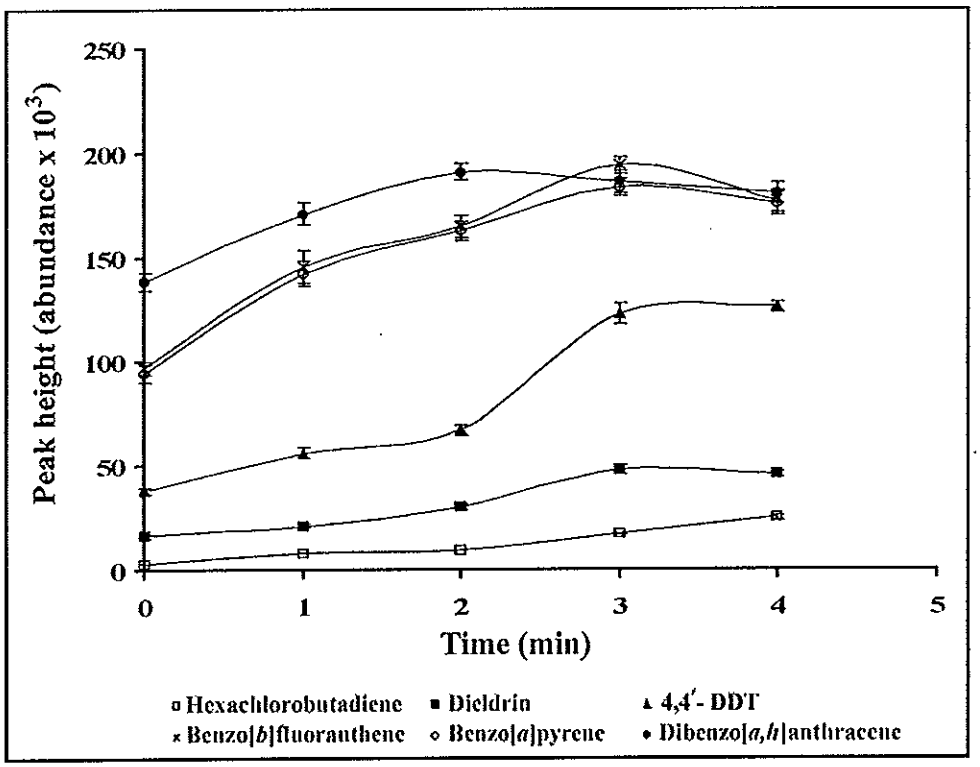


Figure 6.4 Effect of hold time for the analysis of 1.0 mgL^{-1} of six compounds

Step III Ramp rate

The initial temperature was set at 40°C, held for 3 minutes (results from Step I and II respectively) and then programmed to 300°C by varying the ramp rate between 20 and 40°C min^{-1} with an increment of 10°C min^{-1}, with the temperature then held at 300°C for 5 minutes. Figure 6.5 shows the responses of the six analytes at various ramp rates. Their responses increased when the ramp rate was between 20 and 25°C. Then, the response decreased because when the ramp rate was too high the adsorption time to retain analytes on the column was reduced, then resulting in the low response (Debbrecht, 2004; Jennings, 1987; Pettersson, 2004). A ramp rate at 25°C min^{-1} was then chosen as an optimum value as it gave the highest response, best resolution and short analysis time.

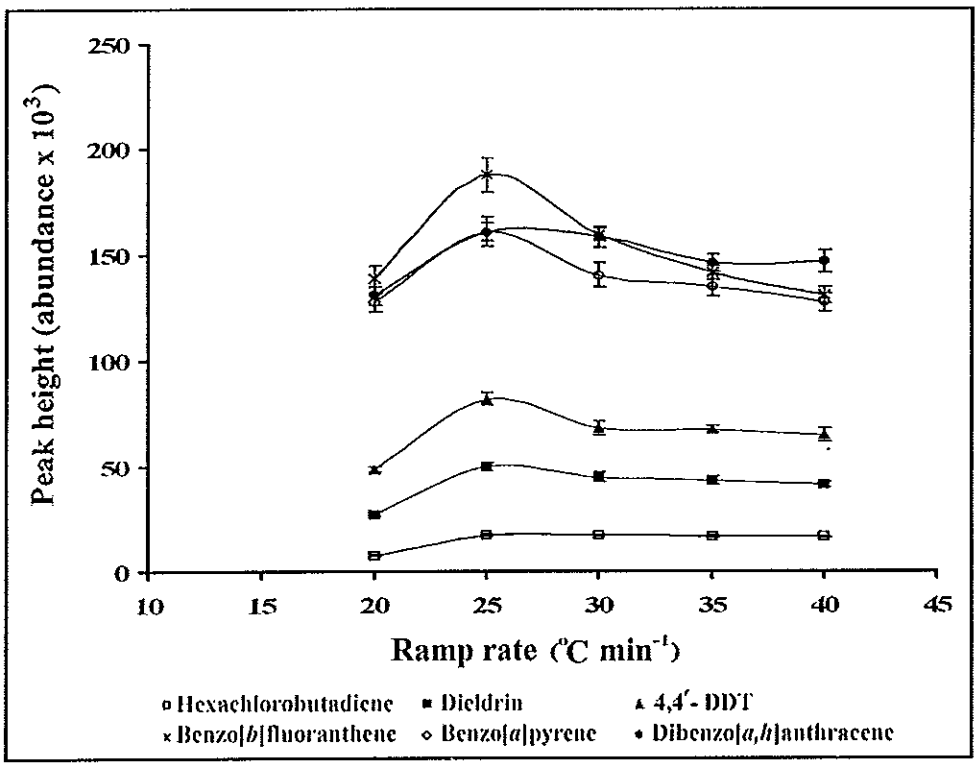


Figure 6.5 Effect of ramp rate for analysis of 1.0 mgL^{-1} of six compounds

Step IV Final temperature

The initial temperature was set at 40°C; held for 3 minutes and then increased to the final temperature with a ramp rate of 25°C min^{-1} (obtained from step I-III). Since all analytes are in the semivolatile organic compound group with relatively high boiling points, they need a high temperature in order to desorb them from the column. The final temperature was investigated between 250 and 300°C. Figure 6.6 shows the responses of the six analytes at various final temperatures. The response increased when temperature increased and the highest response was obtained at 300°C. Higher temperature was not tested because the maximum acceptable temperature of the HP-5MS column is 325°C and in order to ensure the high abundance and to maintain the lifetime of the column, the analysis should not be over this value (Grob, 2004) Therefore, 300°C was used as the optimum final temperature.

Among the six analytes, four of them have boiling point (385-524°C) higher than the optimum final temperature. However, vaporization of these compounds at this temperature is possible since the value of the boiling point is also dependent on the pressure in the system. In this work, the operating pressure is only 2-5 psi due to the narrow inner diameter (0.25 mm I.D.), long column length (30 m) and air vacuum from the MS. When the pressure of the system is lower than the standard pressure (14-15 psi ~ 1 atm) (Atkin, 1982) the vapor pressure of the analytes is higher (referring phase diagram (Atkin, 1982; Oxtoby *et al.*, 2002)) resulting in a lower boiling point. This means that is the analytes can be vaporized and separated by the column. In addition, there are other parameters that may also have some indirect effect on the vaporization of analytes at this temperature, *i.e.*, the surface of the liner (Agilent, 2004), the presence of trace analyte in the large solvent dilution (O'Neill, 1993; Oxtoby *et al.*, 2002; Stoker, 1993), enthalpy and entropy of vaporization of analytes during pressure and temperature changes (Atkin, 1982; Castellan, 1971).

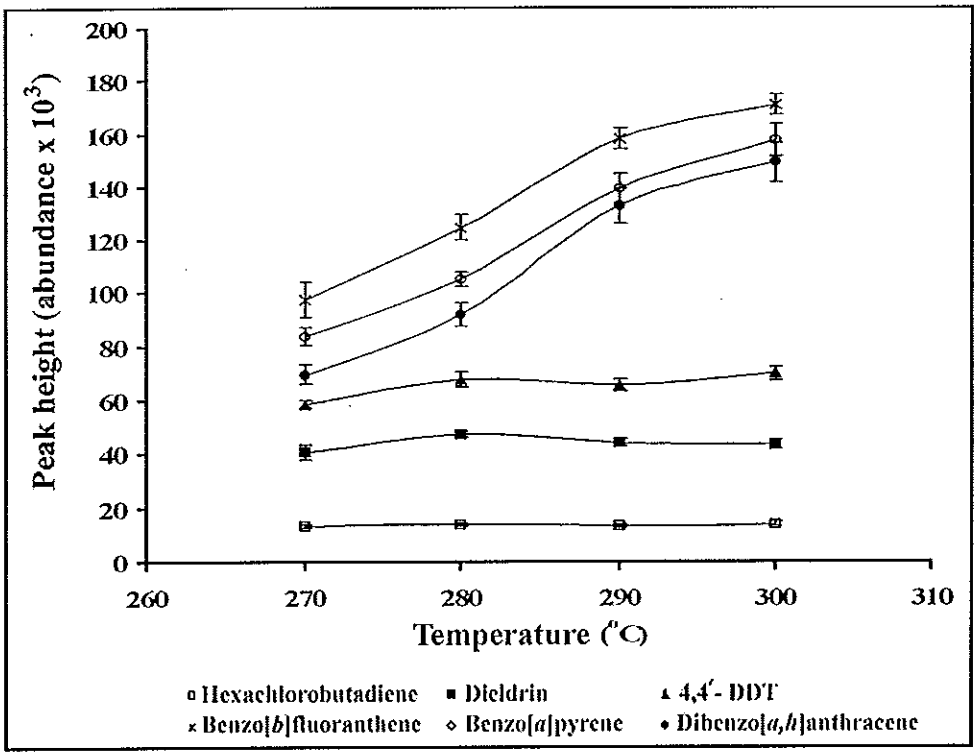


Figure 6.6 Effect of final column temperature for the analysis of 1.0 mgL^{-1} of six compounds

Step V Hold time at the final temperature

The initial temperature was set at 40°C; held for 3 minutes and then increased to the final temperature (300°C) with a ramp rate of 25°C min^{-1} (obtained from step I-IV). The hold time at the final temperature was investigated between 0 and 6 minutes with an increment of 1 minute. Figure 6.7 shows the responses of the six analytes at various hold times at optimum final column temperature. Their responses increased with the hold time and reached a constant value at 5 minutes. The reason of this being the hydrophobic property of the analytes making them well adsorb strongly on the stationary phase of the HP-5ms column even at high temperature, thus, a reasonable period of time was required to desorb them. The hold time for 5 minutes at 300°C was chosen to be an optimum value because it gave the highest response and the analysis time allowed for complete elution of all analytes.

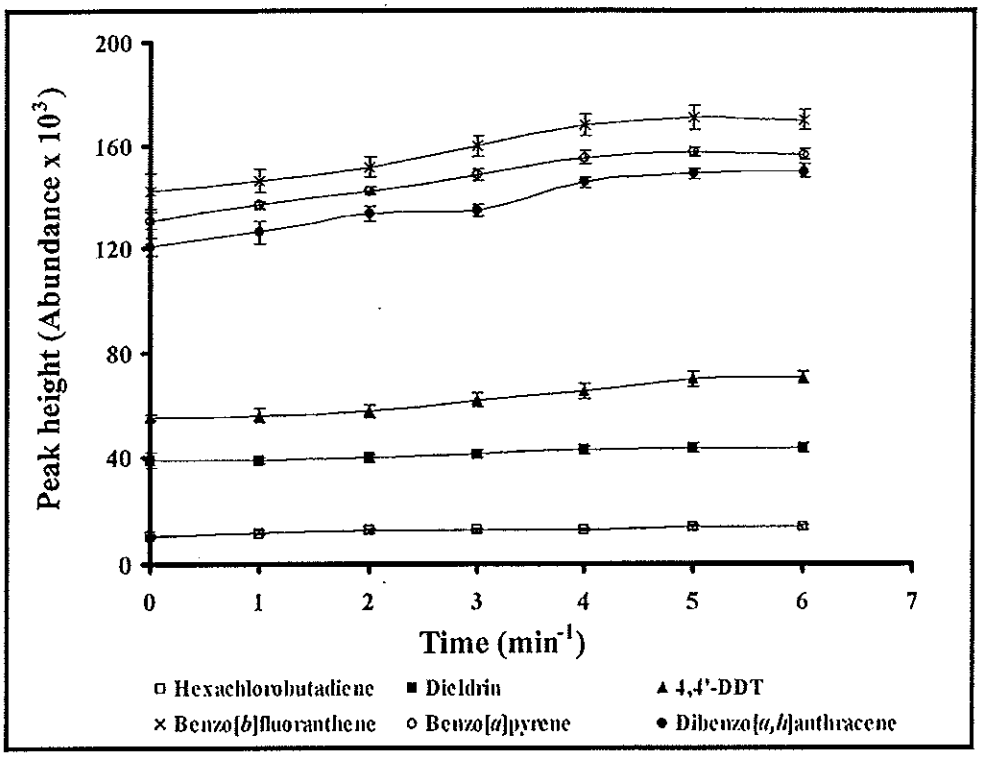


Figure 6.7 Effect of hold time at final temperature for the analysis of 1.0 mgL^{-1} of six compounds

In conclusion, the optimum conditions of the temperature program started with an initial temperature of 40°C which was then held for 3 min before ramping to 300°C at 25°C min^{-1} and then held at the final temperature for 5 min.

6.5.1.3 Injector temperature

The temperature program and carrier gas flow rate were set at the optimum conditions obtained earlier. Other parameters (MS ion source, MS quadrupole and transfer line temperature) were fixed at the default value as recommended by the manufacture, *i.e.*, 230°C for MS ion source, 150°C for quadrupole and 280°C for transfer line) (Hewlett-Packard, 1998). The injector temperature was investigated between 210°C and 250°C.

Since the six analytes have different boiling points, the optimum inlet temperature was chosen as the one that could provide relatively high responses for all analytes and this was found to be 220°C (Figure 6.8).

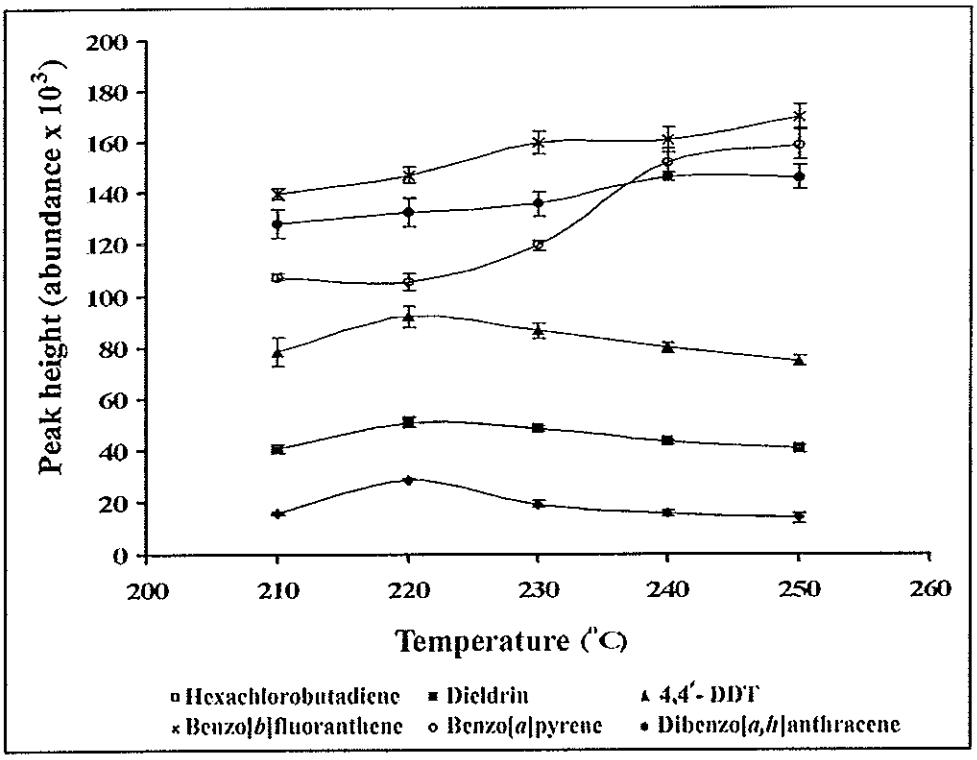


Figure 6.8 Effect of injector temperature for the analysis of 1.0 mg L^{-1} of six compounds

6.5.1.4 Split ratio

The term of "chromatographic system" encompasses all the hardware associated with operation of the separation, including the injector or sample inlet, oven, detector and data system. The function of the injector is to receive a representative sample aliquot and to vaporize it without degradation or loss of sample. One of the most popular sample introductions is the split mode. In this mode, only a small portion of the vaporized analytes will go to the column while the rest is vented out.

In this work the injection was chosen to perform in the split mode due to a relatively large volume of eluting solvent (3 μL) (typical maximum for a split/splitless mode injection is 2 μL) (Grob, 2004). A split mode injection was used instead of the splitless mode in order to reduce the volume loading to the inlet liner caused by its vaporization and expansion. Split ratios from 2:1 to 10:1 were

investigated. Figure 6.9 shows the responses of the six compounds at various split ratios. The split ratio at 2:1 gave the highest response. That is for every 2 units of gas sample which were vented out, 1 unit went into the GC column.

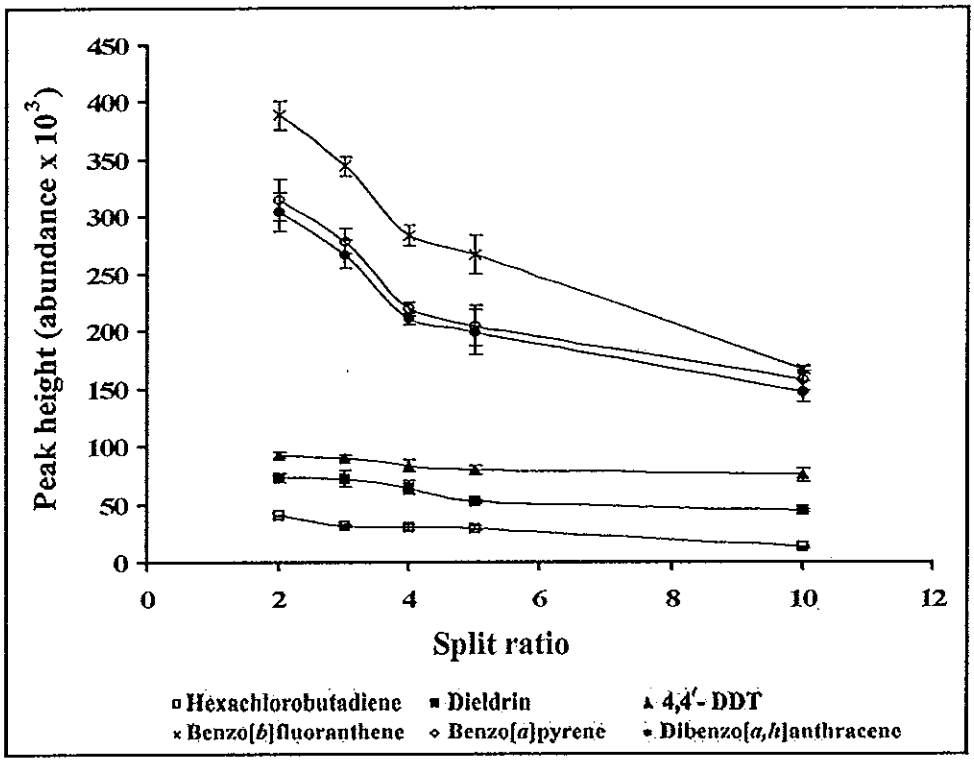


Figure 6.9 Effect of split ratio for the analysis of 1.0 mg L^{-1} of six compounds

6.5.2 Optimization of extraction

Capillary microextraction was performed by liquid desorption instead of thermal desorption. This was to avoid the loss of some polar analytes, *i.e.*, pesticides that have weak interaction with PDMS material when using thermal desorption (Wu *et al.*, 2002). The goal of the method was to obtain high extraction efficiency and low detection limit in a relatively short time. Therefore, the effects of sample flow rate, breakthrough volume, organic modifier, eluting solvent and purging time were investigated. HPLC-grade water spiked with a mixture of the six compounds (hexachlorobutadiene, dieldrin, 4,4′-DDT, benzo[*b*]fluoranthene, benzo-[*a*]pyrene and dibenzo[*a,h*]anthracene) were used to study all extraction parameters.

6.5.2.1 Sample flow rate

Spiked water sample containing 1.0 mgL^{-1} of each compounds (hexachlorobutadiene, dieldrin, 4,4′-DDT, benzo[*b*]fluoranthene, benzo[*a*]pyrene and dibenzo[*a,h*]anthracene) was used to study the effect of sample flow rate (0.8, 1.0, 1.5, 2.0 and 2.5 mL min^{-1}). At the lowest flow rate 0.8 mL min^{-1}, there was no signal due to back pressure from the inlet of the GC-MS to the transfer line, therefore, the eluent could not be transported into GC-MS for separation. A signal could be obtained at flow rates of 1.0 mL min^{-1} and higher (Figure 6.10) however the responses decreased when flow rate increased. This is probably because at higher flow rates, the adsorption time was reduced allowing less analytes to be sorbed into the bulk of the PDMS film (Baltussen *et al.*, 2002). The flow rate of 1.0 mL min^{-1} which provided the highest response was therefore chosen as the optimum.

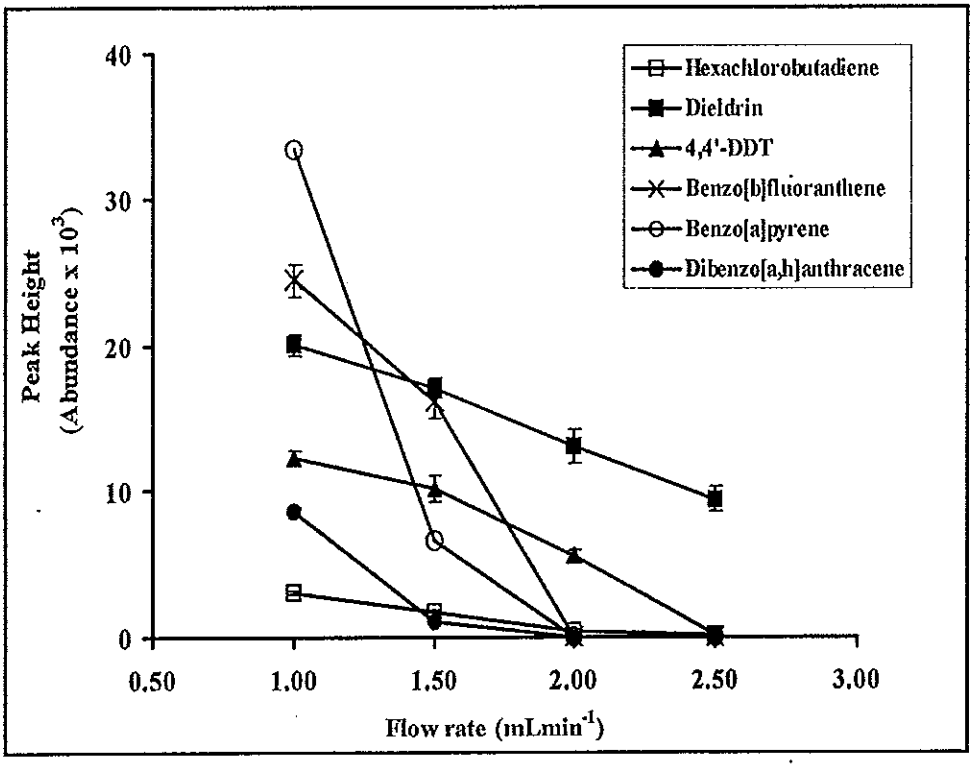


Figure 6.10 Effect of sample flow rate on the extraction of the six analytes in water sample

6.5.2.2 Eluting solvent

Methanol, acetonitrile, acetone and ethyl acetate which were mostly used in liquid desorption, were investigated as potential organic solvents to desorb the analytes from the capillary microextractor. A spiked water sample (hexachlorobutadiene 0.5 mg L^{-1}, dieldrin 0.2 mg L^{-1}, 4,4′-DDT 0.2 mg L^{-1}, benzo[*b*]-fluoranthene 0.15 mg L^{-1}, benzo[*a*]pyrene 0.15 mg L^{-1} and dibenzo[*a,h*]anthracene 0.5 mg L^{-1}) was used to investigate this parameters. The results (Figure 6.11) showed that acetonitrile gave relatively high responses for all analytes. This could be because the polarity of acetonitrile is suitable to desorb both polar and non polar compounds. Moreover, this solvent has been applied widely for SVOCs analysis in liquid chromatography. After elution, contaminants were washed out by a cleaning process described in 6.4.3. No memory effect was found after this re-equilibrated step.

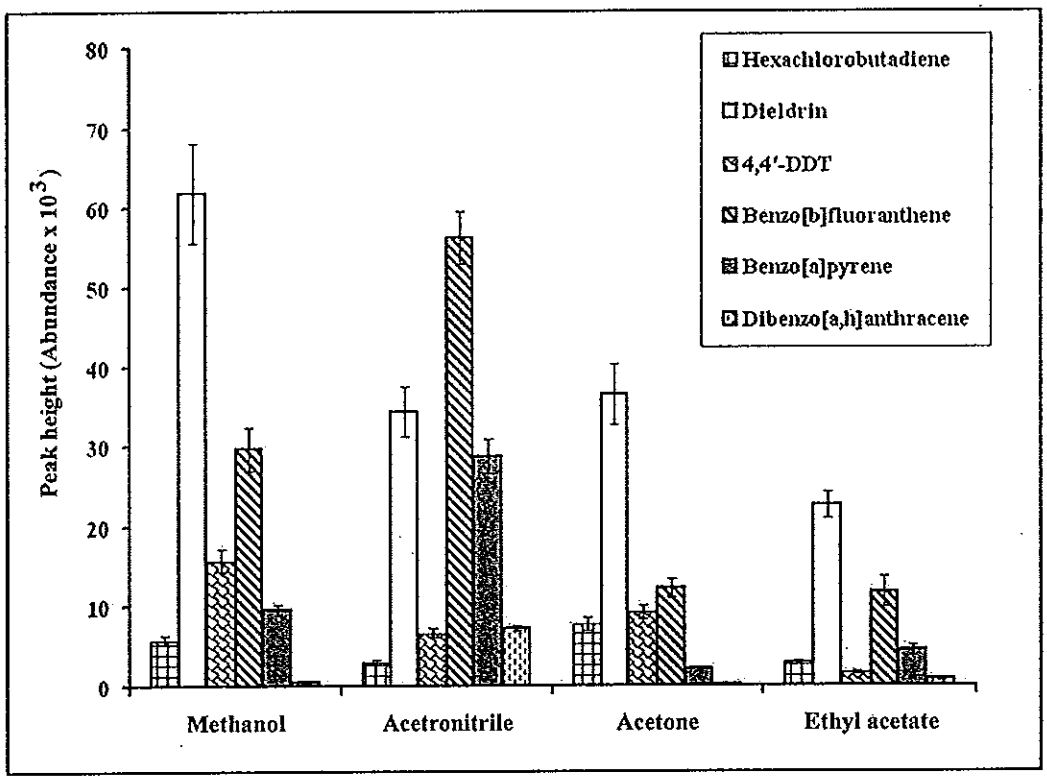


Figure 6.11 Effect of eluting solvents on the desorption of the six analytes in capillary microextraction system

6.5.2.3 Organic modifier

Before detection, SVOCs in the water sample could be lost due to possible adsorption on the sample container wall (polyethylene or glass) and the Teflon® tubing used as the sample loop (U.S. EPA, 1998b). This effect can be reduced by adding a small amount of organic modifier into the water sample in order to increase solute-aqueous phase dispersion interactions (West and Lesellier, 2005) and allow the particulate matter to settle, which will speed the extraction process (U.S. EPA, 1998b). Three organic solvents, in which both water and organic analytes were soluble *i.e.*, methanol, ethanol and acetone (5% v/v) were investigated for a suitable organic modifier for increasing the recovery (Waters, 2000). The organic modifiers were added to the spiked water samples (hexachlorobutadiene 1.0 mg L^{-1}, dieldrin 0.5 mg L^{-1}, 4,4′-DDT 0.5 mg L^{-1}, benzo[*b*]fluoranthene 0.3 mg L^{-1}, benzo[*a*]pyrene 0.3 mg L^{-1} and dibenzo[*a,h*]anthracene 1.0 mg L^{-1}) and then analyzed. The results (Figure 6.12) indicated that ethanol provided the best response for all analytes while methanol gave high responses for polar compounds in the pesticides group but low responses for chlorinated hydrocarbons and PAHs. When acetone was added, the recovery is less than without acetone. This may be due to the increase in solubility of the analytes in acetone-water mixtures, resulting in a low efficiency of adsorption on the extracting phase (Shukla *et al.*, 2006; West and Lesellier, 2005). Therefore, ethanol was chosen as modifier, since it also has lower toxic effects (Cháfer *et al.*, 2005).

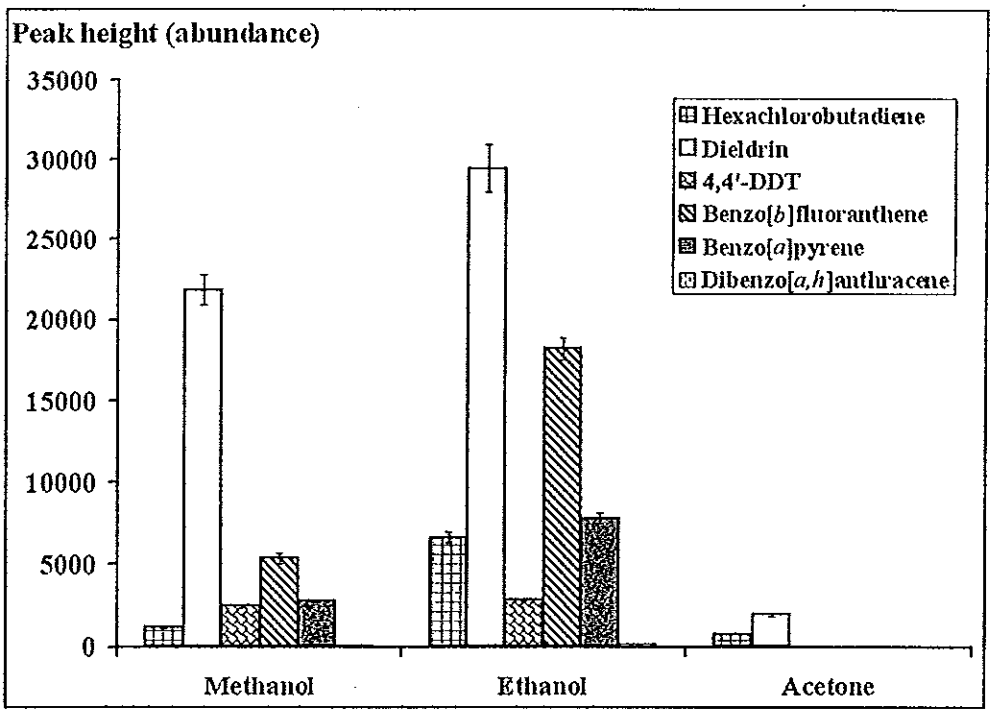


Figure 6.12 Comparison of the organic modifier (5% v/v) for the extraction of the six analytes by capillary microextraction

6.5.2.4 Microextractor column length

Initially a 50 cm length of the capillary microextractor was studied but the limits of detections (LOD) of four of the six analytes were higher than the limits set by the U.S. EPA. Therefore, longer columns (75 and 100 cm) with the same inner diameter (0.32 mm) and film thickness (0.25 μm) were investigated in order to enhance the responses, and hence the limit of detections. Spiked HPLC-grade water at a concentration of 0.5 mg L^{-1} was tested and Figure 6.13 shows that the longer the column the better the response. At 100 cm the extraction efficiency could be enhanced by 39-75% when compared to 50 cm. Therefore, 100 cm column length was used for further study.

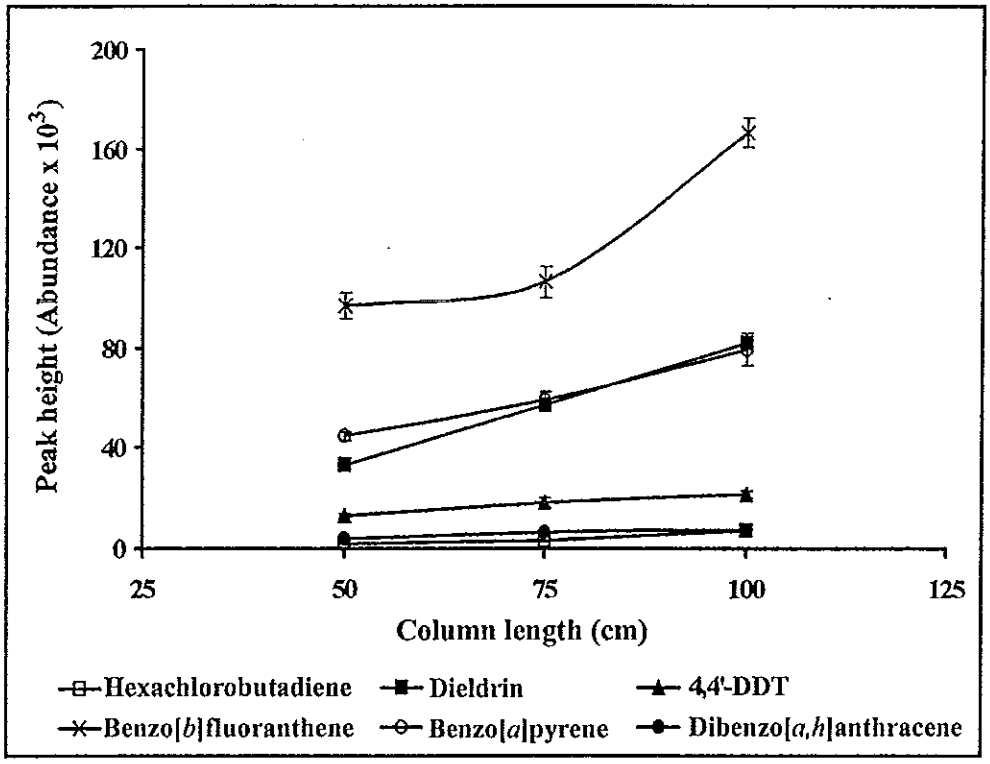


Figure 6.13 Effect of microextractor column length on the extraction of six analytes (0.5 mg L^{-1}) in water sample

6.5.2.5 Purging time

To ensure that there was no carry over from water residue left in the capillary microextractor from the previous sample, purging gas was used to dry and remove the residue. Matrix blank (HPLC-grade water) was passed through the capillary microextractor coupled to the gas chromatography-mass spectrometry system and then purged by helium gas at various purging times of 20, 30, 40, 50 and 60 seconds. By monitoring the mass ion of water using SIM mode, it was found that when the purging time was less than 50 seconds, residual water (m/z 18) was detected and gave a high response that could interfere with the response of analytes. At 50 seconds there was no carry over from residual water found as demonstrated by a clear chromatogram baseline. Therefore, a minimum of 50 seconds purging time was used. To test whether there was any loss of analyte if the column was purged after a spiked water sample containing 0.5 mg L^{-1} of each compound (hexachlorobutadiene, dieldrin, 4,4′-DDT, benzo[*b*]fluoranthene, benzo[*a*]pyrene and dibenzo[*a,h*]anthracene) was

passed through the system, it was purged for 50 and 60 seconds. The results showed relatively no losses of analytes. This was due to the rapid equilibrium in the capillary microextractor during the dynamic sampling process (Guo and Mitra, 1999b; Zhaolun *et al.*, 1988).

6.5.2.6 Sample volume

Since the length of the capillary microextractor and the film thickness of the extracting phase coated on the microextractor were fixed, the sample volume providing maximum adsorption or equilibrium between the two phases was determined in order to obtain high recovery. Water samples from 4 to 8 mL were tested. Since the sample loop can hold only 1 mL of sample, each "loading" through valve A will pass 1 mL of sample to valve B (Figure 6.1). For samples with higher volume, loading was carried out more than once by switching valve A on and off until the desired volume was transported through the capillary microextractor on valve B. A spiked water sample .containing 0.5 mg $L^{-1}$of the six compounds (hexachloro-butadiene, dieldrin, 4,4′-DDT, benzo[*b*]fluoranthene, benzo[*a*]pyrene and dibenzo-[*a,h*]anthracene) was employed for the test. This concentration was chosen following the U.S. EPA method for performance test (U.S. EPA, 2005b; U.S. EPA, 2005a). When the capacity of the capillary microextractor is reached, all the sorptive sites are fully occupied so the increase in concentration or sample volume will no longer lead to an increase in the amount of compound adsorbed. From Figure 6.14, the response increased with sample volume up to 7 mL, and this is the breakthrough volume which is used for further studies.

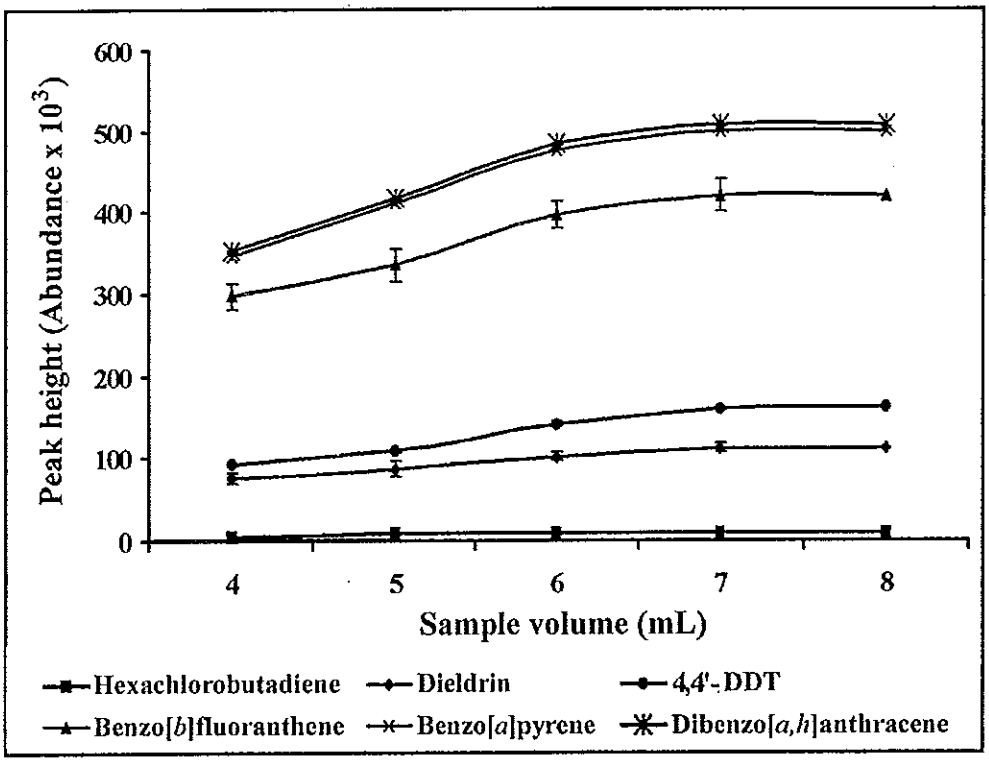


Figure 6.14 Effect of sample volume on the extraction of the six analytes in water sample

The optimum conditions for the analysis of the six compounds of interest using CME-GC-MS system are summarized in Table 6.1. The chromatogram obtained from these optimum conditions gave high resolution and sensitivity as shown in Figure 6.15.

Table 6.1 Summary of optimum conditions for analysis of six compounds.

Parameters	Optimum conditions
GC-MS	
Injection mode	Split 2:1
carrier gas flow rate	0.7 mL min^{-1}
column temperature	40°C (hold 3 min) to 300°C with rate 25°C min^{-1} and hold at 300°C for 5 min
injector temperature	220 °C
mass detector temperature	230 °C (MS ion source), 150 °C (MS quadrupole)
selected ion monitoring (SIM)	Hexachlorobutadiene m/z 225(100), 227(63)
	Dieldin m/z 79(100), 149(35)
	4,4'-DDT m/z 235(100), 165(66)
	Benzo[*b*]fluoranthene m/z 252(100), 250(18)
	Benzo[*a*]pyrene m/z 252(100), 250(25)
	Dibenzo[*a,h*]anthra-cene m/z 278(100), 276(16)
CME	
Sample flow rate	1 mL min^{-1}
Sample volume	7 mL
Organic modifier	Ethanol (5% v/v)
Eluting solvent	Acetonitrile
Purging time	50 seconds

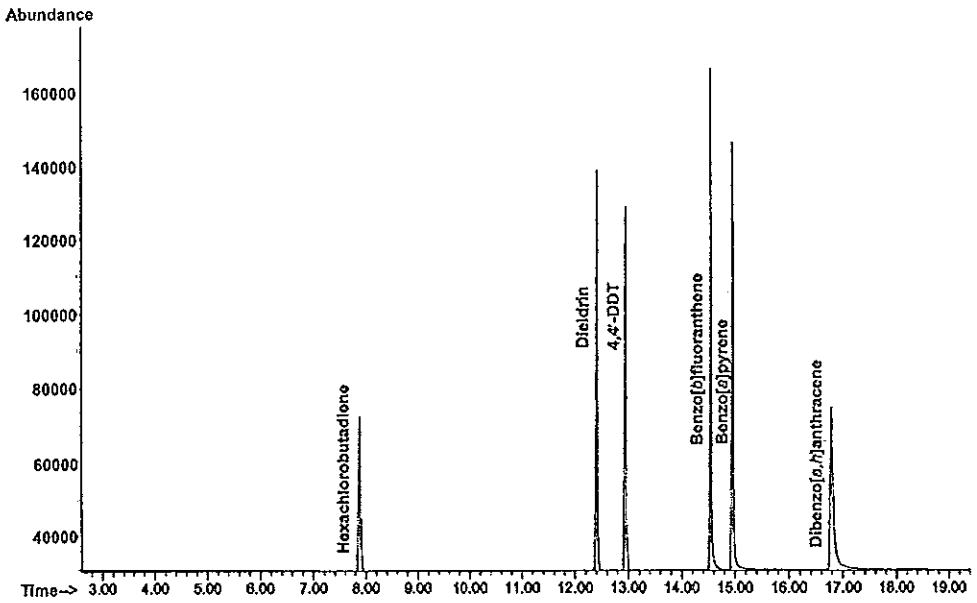


Figure 6.15 Total ion chromatogram of six hazardous substances in water (2.0 mg L^{-1} hexachlorobutadiene, 1.0 mg L^{-1} dieldrin, 1.0 mg L^{-1} 4,4′-DDT, 1.0 mg L^{-1} benzo[*b*]fluoranthene, 1.0 mg L^{-1} benzo[*a*]pyrene and 2.0 mg L^{-1} dibenzo[*a,h*]anthracene) enriched by capillary microextraction coupled to gas chromatography-mass spectrometry

6.5.3 System performance

6.5.3.1 Linear dynamic range (linearity)

HPLC-grade water was spiked in order to obtain the concentration in the range 10.0 ng L^{-1} - 5.0 mg L^{-1}. A 7 mL of each spiked aqueous solution was injected into the CME-GC-MS system under optimum conditions obtained from 6.5.1-6.5.2. Linearity was determined from a calibration curve at the correlation coefficients greater than 0.99 as shown in Table 6.2.

6.5.3.2 Limit of detection (LOD)

Spiked HPLC-grade water in the concentration range 0.01 – 1.0 μg L^{-1} was injected into the CME-GC-MS system at the optimum conditions. The lowest concentration or amount of analytes that the mass selective detector can detect was taken from the total ion chromatogram using the SIM data acquisition. The Signal to

Noise ratio (S/N) was calculated automatically by the Chemstation Operating Software.

The detection limits of the six compounds were in the range of 10 ng L^{-1} to 1.0 μg L^{-1}, obtained by considering the signal to noise ratio equal to 3 (S/N = 3), the relative standard deviation (RSD), based on five replicate injections was less than 10% (Table 6.2).

Table 6.2 Linear range, correlation coefficient and limit of detection obtained by capillary microextraction coupled to gas chromatography-mass spectrometry (%RSD less than 10, n=5) and the limit set by U.S.EPA

Compounds	Linear equation Y : Peak height (Abundance) x : Concentration (mg L^{-1})	Linear range (μg L^{-1})	Correlation coefficient (r)	LOD (μg L^{-1}) (S/N=3)	Limits set by U.S. EPA (μg L^{-1}) (U.S. EPA, 2005a)
Hexachlorobutadiene	Y=32087x+30.3	0.01 - 1000	0.9982	0.01	0.05
Dieldrin	Y=242930x+534.8	0.02 - 1000	0.9972	0.02	0.03
4,4'-DDT	Y=74044x+2945.7	1.00 - 2000	0.9998	1.00	2.00
Benzo[*b*]fluoranthene	Y=456492x+6545.5	0.05 - 3000	0.9959	0.05	0.10
Benzo[*a*]pyrene	Y=116651x+5445.7	0.01 - 2000	0.9998	0.01	0.01
Dibenzo[*a,h*]anthracene	Y=17327x+22.4	0.10 - 1000	0.9998	0.10	0.10*

* There is no implemented maximum contaminant level, however, U.S. EPA recommended that it should not exceed 0.10 μg L^{-1}(García-Falcón *et al.*, 2004a; OJEC, 1998).

6.5.3.3 Recovery

The term of recovery is at present used to indicate the yield of an analyte in a preconcentration or extraction stage in an analytical method. It is the ratio of observed value obtained from an analytical process via a calibration graph divided by the reference value. The reference value may be that of a certified reference material or the increased amount of analyte observed from the addition of a known amount of analyte to the test sample (Burns *et al.*, 2002). However, since the extraction process of the capillary microextractor involved the partitioning of analytes between water matrix and extraction phase contained inside the capillary, both the water matrix and extracting phase are competing for the analytes due to the different K_D (the partition coefficient) (Wang *et al.*, 2004) of the analytes in the sample. Therefore, recovery is also dependent on water matrix and this would influence the extraction efficiency. Because of this, relative recovery was used instead of recovery. This was carried out by comparing the responses obtained from spiked HPLC-grade water and spiked real water samples containing 0.5 mg L^{-1} of hexachlorobutadiene, dieldrin, 4,4′-DDT, benzo[*b*]fluoranthene, benzo[*a*]pyrene and dibenzo[*a,h*]-anthracene. They were passed through the capillary microextractor and analyzed by GC-MS at optimum conditions. All component peaks were quantified by SIM mode at specific mass ion. The obtained recoveries were higher than 80% (Table 6.3) and these were acceptable (70-130%) (U.S. EPA, 2003a). The results indicated that this method is effective for multi residues analysis.

Table 6.3 Relative recoveries obtained by comparing the response between HPLC-grade water and spiked sample under the same concentration at 0.5 mg L^{-1} (n = 5).

Compounds	Recovery (%)	
	Tap water	Raw water
Hexachlorobutadiene	101 ± 5	99 ± 6
Dieldrin	112 ± 3	109 ± 5
4,4'-DDT	94 ± 7	95 ± 6
Benzo[*b*]fluoranthene	92 ± 4	90 ± 3
Benzo[*a*]pyrene	90 ± 5	93 ± 7
Dibenzo[*a,h*]anthracene	89 ± 5	88 ± 4

6.5.4 Qualitative and quantitative analysis

6.5.4.1 Real sample analysis

The capillary microextractor coupled to the gas chromatography-mass spectrometry system was validated with real samples, *i.e.*, raw water and tap water collected in Hat Yai City, Thailand. Each water sample was introduced directly into the CME-GC-MS system. Qualitative analysis was evaluated using the retention time and the molecular ion while for quantitative analysis, the amount of analytes was determined from the calibration curve. When the responses were lower than the detection limits, the standard addition method was employed by spiking the real sample with various concentrations of stock standard solution and passing through CME-GC-MS. The standard addition method was also used to control the effect of the matrix in each sample.

Figure 6.16 and 6.17 show the results of tap and raw water analysis after using the standard addition method, *i.e.*, spiking sample with concentration of a stock standard solution in the range of 0.001-1.0 mg L^{-1}. All analysis provided a linearity with R^2 greater than 0.99. Some of these compounds gave minus intercept

values which means that quantitative information could not be obtained or these compounds were not detectable (Harris, 1995; Mendham *et al.*, 2000).

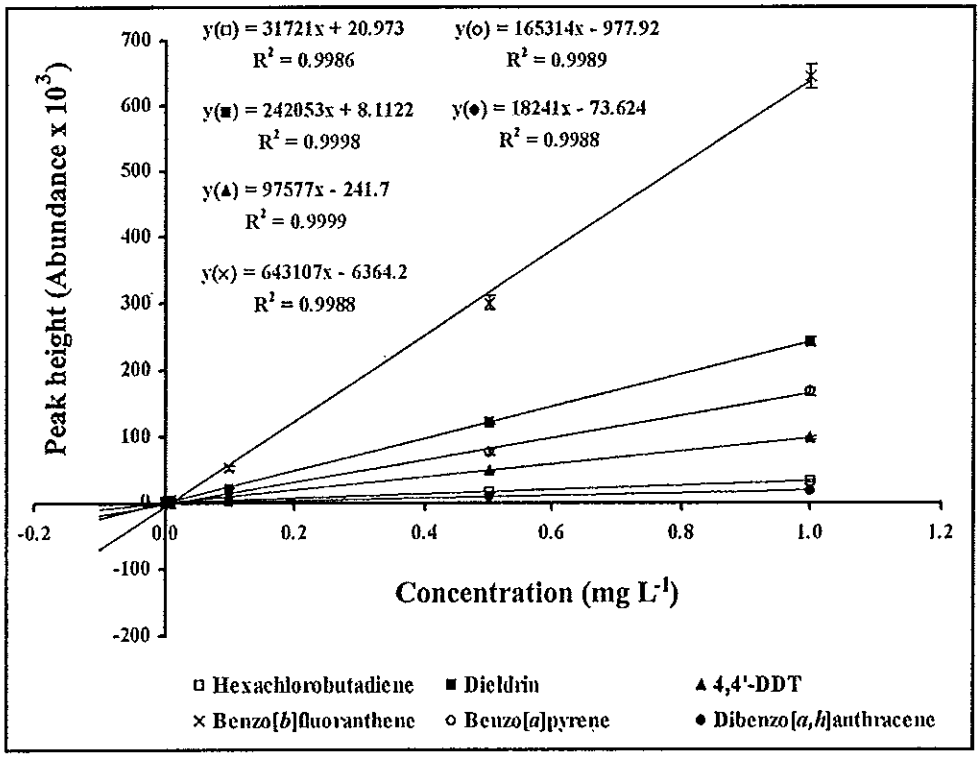


Figure 6.16 Standard addition of analysis of tap water sample

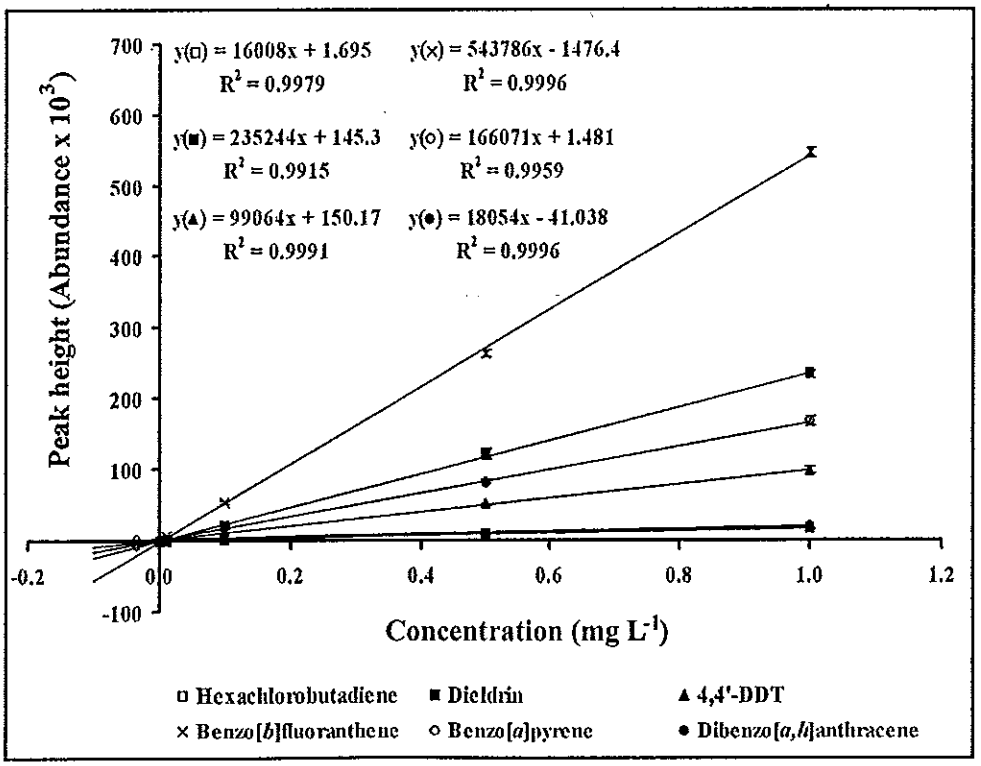


Figure 6.17 Standard addition of analysis of raw water sample

Table 6.4 Concentration of semivolatile organic compounds in water sample (tap water and raw water) from Hat Yai City, Songkhla, Thailand analyzed by capillary microextraction coupled to gas chromatography-mass spectrometry

Compounds	Tap water ($\mu g\ L^{-1}$) (%RSD)	Raw water ($\mu g\ L^{-1}$) (%RSD)	Limit set by the Metropolitan Waterworks Authority, Thailand (Water quality analysis devision, 2004)
Hexachlorobutadiene	0.66 (6)	0.11 (7)	-
Dieldrin	0.03 (8)	0.62 (6)	0.03
4,4′-DDT	ND	1.50 (5)	2.00
Benzo[*b*]fluoranthene	ND	ND	-
Benzo[*a*]pyrene	ND	0.01 (8)	0.02
Dibenzo[*a,h*]anthracene	ND	ND	-

ND: Not detectable (determined by standard addition method)

(-): No implementation

The results in Table 6.4 indicated that these water samples were contaminated with most of the priority hazardous substances. The presence of 4,4′-DDT are lower than the legal limits of drinking water set by the Metropolitan Waterworks Authority, Thailand, which are based on the World Health Organization guideline 1993 addendum 1998 (Water quality analysis division, 2004). This could be a result of an attempt to control the mosquitoes with 4,4′-DDT to avert the wide spread of hemorrhagic fever and malaria (Ome and Kegley, 2004) in the country.

Therefore, insecticides were sprayed in a very wide area causing these compounds to be accumulated in soil and then leached into raw water. As for benzo[*a*]pyrene, it could be the result of leakage from an old water pipe line. Combustion products (U.S. EPA, 2006a) may also be a possible cause since benzo[*a*]pyrene adsorbed onto smoke particles can be transferred into water by rainfall (Kolahgar *et al.*, 2002) which finally, has an impact on water quality. For hexachlorobutadiene and dieldrin, they were detected with concentrations higher than the legal limits. The higher concentration of hexachlorobutadiene in tap water was probably caused by the pretreatment of raw water. In the process of tap water production the water was pretreated with chlorine and this could lead to chlorination of hydrocarbon such as tri- and tetrachloroethene, and tetrachloromethane resulting in the formation of hexachlorobutadiene (ATSDR, 1994). This compound could also come from the leachate of rubber manufacturing where hexachlorobutadiene is generally used as an intermediate product in rubber production (Howard, 1989). In the case of dieldrin it has been used in place of 4,4′-DDT and has been proved to be a highly effective insecticide (IPCS, 1989). It is now being used and this may be the reason why it was detected at relatively high concentration in both real samples. For benzo[*b*]fluoranthene and dibenzo[*a,h*]anthracene, their concentrations were not detectable for all samples. Although the U.S. EPA has not implemented the maximum contaminant level (MCL) of dibenzo[*a,h*]anthracene for drinking water, they have recommended that this compound be included in water quality criteria of surface water used as a drinking water source for the protection of aquatic life and human health. Therefore, regular monitoring is necessary. The finding in this study suggested that it would be necessary to monitor these compounds and a warning should be provided to the public that this tap water is not suitable for drinking.

6.6 Conclusion

The results from this work showed that CME-GC-MS has advantages, *i.e.*, simultaneous analysis of multi residual semivolatile organic compounds (SVOCs) in water; especially the priority toxic substances, did not need sample preparation and requires small sample for analysis. The chromatograms showed a stable and smooth baseline because the capillary microextractor has a thinner and stronger film of

bonded phase that eliminates practical problems, *i.e.*, ghost peaks or several hours to reach extraction equilibrium which normally occurred with the SPME technique (Wang *et al.*, 2004). The developed system provided good recovery, higher than 80%, good precision with the RSD lower than 10% and also achieved detection at very low concentration (ng L^{-1}). The main advantage is that the sample can be directly injected without any sample preparation because the extractor unit is set up in-line. Therefore, it does not require any sample handling and it can be directly applied to both qualitative and quantitative analysis.

Chapter 7

Conclusions

The development and evaluation of the performance of two sample preparation techniques, namely membrane inlet and capillary microextractor, has been described in this thesis. The extraction of each technique was based on the selective sorption of analyte onto a polydimenthylsiloxane (PDMS) phase coated onto the surface of a hollow fiber membrane or on the inside wall of capillary microextractor.

The first investigation was in-line analysis of SVOCs in water based upon membrane inlet system. A hollow fiber membrane was constructed as a membrane inlet unit coupled in-line to a gas chromatograph-mass spectrometer. Laboratory-built heating box was installed into the system to enhance the permeation of analyte through the membrane. Spiked water containing 4,4′-DDT was used as a case study to evaluate the development of this sample preparation system. Excellent results were obtained, *i.e.*, low limits of detection (90 μg L^{-1}) compared to the standard method (1000 μg L^{-1}) (Lauritsen and Ketola, 1997), higher diffusion rate of extraction, 15 times higher than at standard temperature and pressure (STP) (Batson, 1998). Furthermore, this system could selectively extract organic compounds while prohibiting the passage of the water matrix through the membrane. This proposed system is less time consuming and less laborious.

The second development was the use of a capillary microextractor (CME) coupled to a gas chromatograph-mass spectrometer for the simultaneous determination of trace priority hazardous substances in water. A chromatographic column (HP-5, 100 cm × 0.32 mm I.D. × 0.25 μm film thickness) was used as a microextractor. Three groups of toxic semivolatile organic compounds (SVOCs), *i.e.*, chlorinated hydrocarbons, pesticides and polycyclic aromatic hydrocarbons (PAHs) were chosen as target analytes. The proposed system showed excellent linearity over a

wide concentration range (0.01 ng L^{-1} to 1.0 mg L^{-1}) with correlation coefficients (r) greater than 0.99 and low limits of detection at ng L^{-1} level. High recovery (more than 80%) was obtained with a relative standard deviation less than 10%. The method was successfully applied for the simultaneous analyses of multi residual SVOCs in water samples.

Table 7.1 summarrizes the performance of both developed techniques. They provide advantages over other methods, *i.e.*, low detection limits and a wide linear range. Moreover both techniques could also be expressed as the green and economic methods due to less solvent use (for CME-GC-MS)/or being solventless (for ME-GC-MS) and also less labor intensive since samples could be directly injected without any sample preparation or sample handlings. And lastly, both methods can be directly applied for qualitative and quantitative analysis of water samples contaminated with hazardous substance at trace level.

Table 7.1 Summary the performance of membrane inlet-gas chromatography-mass spectrometry and capillary microextraction-gas chromatography-mass spectrometry techniques in this work

Analytes	Developed techniques	LOD ($\mu g\ L^{-1}$)	LDR ($\mu g\ L^{-1}$)	Solvent volume	Analysis time
4,4'-DDT	MI-GC-MS	90	100 - 1000	0	3 min
*Multi residual SVOCs	CME-GC-MS	0.01 – 1.0	0.01 - 3000	3 µL	18 min

* Multi residual SVOCs: Hexachlorobutadiene, Dieldrin, 4,4'-DDT, Benzo[*b*]fluoranthene, Bezo[*a*]pyrene and Dibenzo[*a,h*]anthracene

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Appendix A

Appendix A

Critical values of F for a one-tailed test ($P = 0.05$)

V_2	V_1												
	1	2	3	4	5	6	7	8	9	10	12	15	20
1	161.4	199.5	215.7	224.6	230.2	234.0	236.8	238.9	240.5	241.9	243.9	245.9	248.0
2	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.41	19.43	19.45
3	10.13	9.552	9.277	9.117	9.013	8.941	8.887	8.845	8.812	8.786	8.745	8.703	8.660
4	7.709	6.944	6.591	6.388	6.256	6.163	6.094	6.041	5.999	5.964	5.912	5.858	5.803
5	6.608	5.786	5.409	5.192	5.050	4.950	4.876	4.818	4.772	4.735	4.678	4.619	4.558
6	5.987	5.143	4.757	4.534	4.387	4.284	4.207	4.147	4.099	4.060	4.000	3.938	3.874
7	5.591	4.737	4.347	4.120	3.972	3.866	3.787	3.726	3.677	3.637	3.575	3.511	3.445
8	5.318	4.459	4.066	3.838	3.687	3.581	3.500	3.438	3.388	3.347	3.284	3.218	3.150
9	5.117	4.256	3.863	3.633	3.482	3.374	3.293	3.230	3.179	3.137	3.073	3.006	2.936
10	4.965	4.103	3.708	3.478	3.326	3.217	3.135	3.072	3.020	2.978	2.913	2.845	2.774
11	4.844	3.982	3.587	3.357	3.204	3.095	3.012	2.948	2.896	2.854	2.788	2.719	2.646
12	4.747	3.885	3.490	3.259	3.106	2.996	2.913	2.849	2.796	2.753	2.687	2.617	2.544
13	4.667	3.806	3.411	3.179	3.025	2.915	2.832	2.767	2.714	2.671	2.604	2.533	2.459
14	4.600	3.739	3.344	3.112	2.958	2.848	2.764	2.699	2.646	2.602	2.534	2.463	2.388
15	4.543	3.682	3.287	3.056	2.901	2.790	2.707	2.641	2.588	2.544	2.475	2.403	2.328
16	4.494	3.634	3.239	3.007	2.852	2.741	2.657	2.591	2.538	2.494	2.425	2.352	2.276
17	4.451	3.592	3.197	2.965	2.810	2.699	2.614	2.548	2.494	2.450	2.381	2.308	2.230
18	4.414	3.555	3.160	2.928	2.773	2.661	2.577	2.510	2.456	2.412	2.342	2.269	2.191
19	4.381	3.522	3.127	2.895	2.740	2.628	2.544	2.477	2.423	2.378	2.308	2.234	2.155
20	4.351	3.493	3.098	2.866	2.711	2.599	2.514	2.447	2.393	2.348	2.278	2.203	2.124

V_1 = number if degrees of freedom of the numerator and V_2 = number of degrees of the freedom of the denominator

Appendix B

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Development of an In-Line System for the Analysis of 4,4′-DDT in Water

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An in-line system for trace persistent organic pollutants (POPs) in water was developed by using a laboratory-made hollow fiber membrane (HFM) unit connected with a high-resolution gas chromatograph-mass spectrometer (HRGC-MS). The semivolatile organic compound, 4,4′-Dichlorodiphenyl trichloroethane (4,4′-DDT), was chosen as a representative of a persistent organic compound. The synthetic water contaminated with 4,4′-DDT was passed through the HFM unit, the extraction occurred by the analyte pervaporated and permeated, then stripped into HRGC-MS. Several factors were investigated for the high extraction efficiency. The best performance was obtained at sample and stripping gas flow rates of 6 and 9 $mLmin^{-1}$, respectively, and desorption temperature of 60°C. At this temperature, the diffusion rate was enhanced by 15 times over 25°C. A wide linear dynamic range was obtained, i.e., 0.10–1.0 mgL^{-1}, with a limit of detection (LOD) of 90 μgL^{-1}. The extraction efficiency of 4,4′-DDT in real water samples was in the range of 83–94%. Real water samples were analyzed and 0.6 μgL^{-1} of 4,4′-DDT was found in unregistered bottled water and 7.0 μgL^{-1} in tap water.

Key Words: 4,4′-DDT; POPs; Water; Pervaporation; In-line.

INTRODUCTION

Persistent Organic Pollutants (POPs) are toxic chemicals, persist in the environment and bioaccumulate if they move up through the food chain. POPs have been linked to adverse effects on human health and animals, such as cancer, damage to the nervous system, reproductive disorders, and disruption

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of the immune system.[1] Some POPs are semivolatile organic compounds and their contamination of water plays a major role in environmental problems. Semivolatile organic compounds (SVOCs) originate from many sources, i.e., electroplating/metal shops, fire-fighting training areas, pesticide mixing areas, wood preservative operations and landfills. The last is the most likely source for releasing these pollutants into the water.[2]

Typically, the analysis of SVOCs in aqueous samples is performed by a combination of various extractions and analytical methods, i.e., gas chromatography (GC) or liquid chromatography (LC), since these techniques are well-known for their good performance for separation.[3–5] The common extraction method employed with liquid samples is liquid-liquid extraction (LLE), which has been the main method for enrichment of organic pollutants from aqueous solutions. Although it is still being widely used, this method requires a large amount of toxic organic solvents, and is time consuming and labor intensive.[6] The microextraction, i.e., solid phase extraction (SPE), solid phase microextraction (SPME) has been reported to replace the LLE[7–8] since these methods are based on the small amount of solvent used for extracting analytes from moderate amount of samples. SPME is particularly suitable for direct injection. Although the microextraction techniques can provide good selective extraction and clean up, there are many disadvantages: easily clogging for SPE if the sample has a large amount of suspension particles; the fragile assembly used in SPME needs more skill of operation. Moreover, the adsorption capacity was limited by the length of coated fiber.

An alternative approach that is currently used for the analysis of trace organic compounds in aqueous matrix is to use a membrane inlet unit coupled to analytical instruments, especially, a mass spectrometer. This membrane-inlet mass spectrometer (MIMS) has been used to determine volatile organic compounds (VOCs). Its sensitivity is high and detection limits at low or sub-ppb concentrations can be obtained for many compounds.[9–11] However, the analysis of SVOCs is much less.[12–14] This is because at normal conditions SVOCs do not evaporate from membrane surface into mass spectrometer and also do not dissolve very well in the membrane that is normally used.[12,15–17] To facilitate SVOCs analysis in aqueous samples a new versatile and advantageous design of a membrane inlet system is developed in this work by coupling it to a high-resolution gas chromatograph-mass selective detector and using a simple thermal desorption unit to enhance the extraction efficiency.

Current research on SVOCs focuses on pesticides that are considered to be endocrine disruptors.[18] Among the SVOCs, 4,4′-Dichlorodiphenyl trichloroethane (4,4′-DDT) has been listed in the top 20 hazardous substances in 2003,[19] is very persistent in the environment, can accumulate in the food chain and is probably a human carcinogen. Although it has been banned since the 1970s, it is still needed in countries where mosquito-borne malaria is a

larger health problem than DDT's potential toxicity, particularly in Asia and the Pacific region[20], including Thailand. Therefore, it was chosen to be a representative of SVOCs for preliminary study in this work.

MATERIALS AND METHODS

Standard and Reagents

4,4'-DDT standard was purchased from Restek Company (USA) and methanol (Analytical grade) from Merck (USA). Ultra pure water was obtained from a Milli-Q system (H_2O, Synthesis in laboratory by Maxima, ELGA, UK). A 100 mgL^{-1} standard stock solution was prepared in methanol. Water samples used for investigating all parameters of extraction and chromatographic analysis were prepared by spiking various concentrations of standard stock solution in ultra pure water.

Membrane Inlet System

A schematic picture of the experimental set-up for the in-line MIMS-measurement of 4,4'-DDT residue in water is presented in Figure 1. The membrane inlet (MI) system consists of a laboratory-built membrane inlet with a desorption unit coupled to a high-resolution gas chromatograph-mass spectrometer. The membrane inlet unit was constructed from a 15 cm Teflon

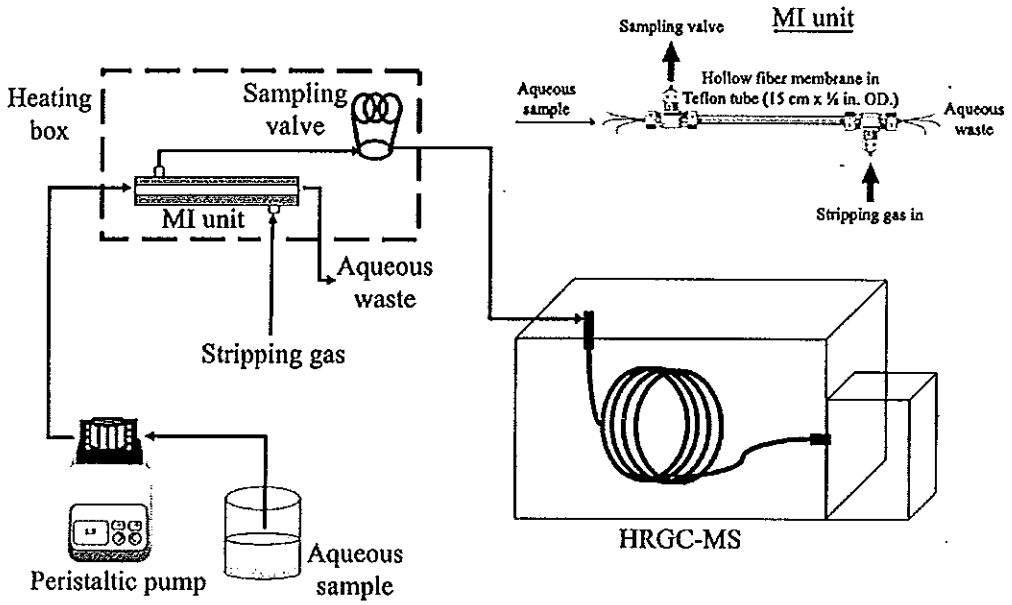

Figure 1: The experiment set-up for in-line measurement of 4,4'-DDT residue in water by MI-HRGC-MS (membrane inlet-high resolution gas chromatography-mass spectrometry).

tube (0.10 in I.D, 1/8 in O.D.) and two Swagelok® T-union (1/8 in). A hollow fiber membrane, micro-porous polypropylene support coated with 1-μm thick film of polydimethylsiloxane, was obtained from Applied Membrane Technology Inc., USA. Three strands of 15 cm (0.290 mm O.D., 0.240 mm I.D.) hollow fiber membrane were inserted inside the Teflon tube. Each end of the tube was connected to one port of the Swagelok® T-union. The side ports of the T-union were used as the inlet and outlet of the sample that flowed through the hollow fiber lumen. The vertical ports were for the flow of the stripping gas (Helium gas, UHP grade, Thai Industrial Gases Public Company Limited, Thailand). Epoxy was used to glue the end of the hollow fiber to the T-union. This epoxy acted as a seal, preventing intermixing of the sample and stripping gas. The system was set up in a flow-through mode as stripping gas flows outside the membrane surface while the sample solution flows inside the lumen of the membrane. The aqueous sample was delivered through an MI unit using a peristaltic pump while stripping gas outside the fiber was flowed in the opposite direction.

A thermostated laboratory-built heating box (13 × 10 × 21 cm^3) was used as a desorption unit. It was designed to cover the MI unit and a ten-port injection valve (Fig. 1) so the pervaporation process occurred while the temperature remained constant until the permeated analyte was direct introduced to the HRGC-MS.

High-Resolution Gas Chromatography-Mass Spectrometry (HRGC-MS)

Sample analysis was carried out by using an Agilent (Agilent Technologies, USA) HP6890 Gas chromatograph—HP5973 Mass selective detector equipped with a HP-5MS fused silica capillary column (95% Polydimethylsiloxane, 30 m × 0.25 mm I.D., film thickness 0.25 μm, Agilent Technologies, USA). The HRGC-MS was operated at 70 eV electron ionization and a selected ion monitoring (SIM) mode for qualitative and quantitative analysis. Helium gas (UHP grade) was used as a carrier gas at a flow rate of 0.8 $mLmin^{-1}$. Gas sample, 2 mL, from the sampling valve was injected into HRGC-MS in splitless mode. The injection temperature was set at 250°C and interface temperature at 280°C. The column temperature was isothermal at 300°C for in-line analysis and the gas sample was analyzed by SIM acquisition mode. Molecular ion at m/z 235 and 165 were chosen as the quantify ion of 4,4′-DDT.

Extraction by Membrane Inlet System

The different parameters that can affect the extraction process, i.e., the sample flow rate, stripping gas flow rate, desorption temperature and loading time, were optimized based on the justification of two criteria: high sensitivity and short analysis time. All data were obtained under SIM acquisition mode.

Extraction performance was determined by comparing the response between the ultra pure water and bottled water samples, which were spiked with various concentrations of 4,4′-DDT (0.1–1.0 mgL^{-1}).

Real Water Sample Analysis

Several types of water samples of bottled water (locally produced, unregistered and registered) and tap water, were collected in Hat Yai city, Thailand, and then analyzed for 4,4′-DDT by MI-HRGC-MS. If it was undetectable (lower than the detection limit), the standard addition method was applied for the analysis to confirm the absence of 4,4′-DDT.

RESULTS AND DISCUSSION

Flow Rate Effects

The pervaporation rate of analyte can be limited by poor mixing at the sample membrane interface which can cause the formation of a layer of analyte depletion, resulting in low response and longer response time.[21] This effect is commonly reported when using linear flow on flat membranes.[22] Therefore, the turbulent or counter current flow is preferred and the latter was applied in this work. The effect of the sample and stripping gas flow rates on the response of 4,4′-DDT in water were investigated. Sample flow rate was optimized at a fixed stripping gas flow rate (4 $mLmin^{-1}$). The results (Fig. 2) showed that the

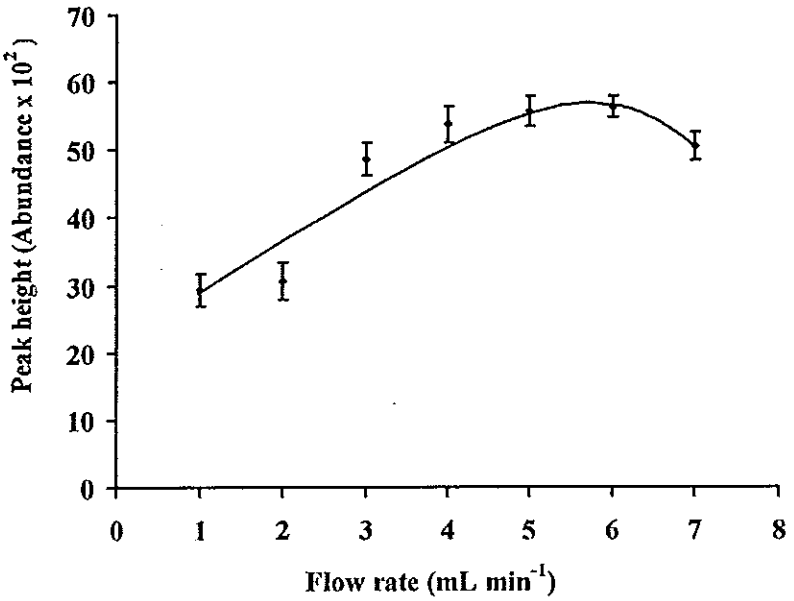

Figure 2: Effect of sample flow rate at a stripping gas flow rate of 4 $mlmin^{-1}$.

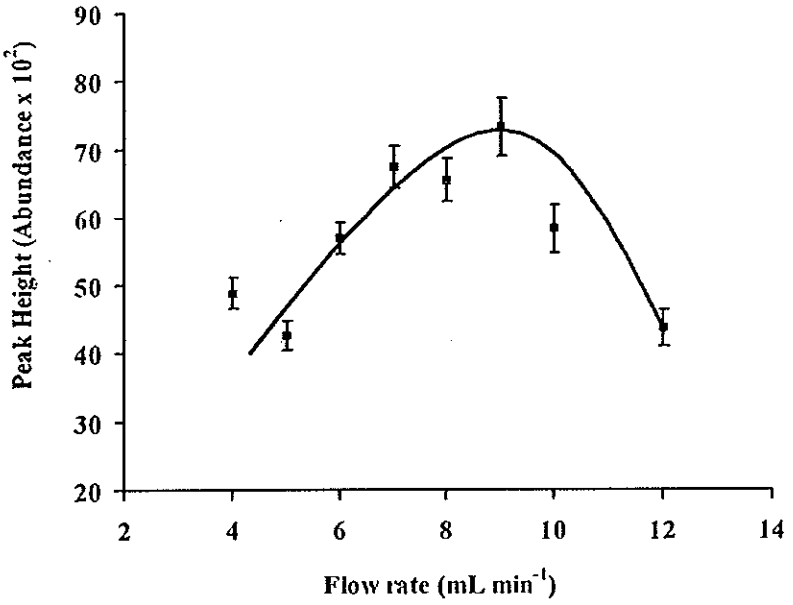

Figure 3: Effect of stripping gas flow rate at optimized sample flow rate of 6 mlmin-1.

response increased with sample flow rate. This was because a high sample flow rate could minimize a layer of depletion on the membrane surface by allowing a larger amount of analyte to contact the membrane per unit time.[23] However, when the permeation of analyte reached a steady state, at flow rate higher than 6 $mLmin^{-1}$, a stable response was obtained. Therefore, the optimum value of the sample flow rate was 6 $mLmin^{-1}$. This optimum sample flow rate corresponded well to a typical flow rate used in MIMS, i.e., below 10 $mLmin^{-1}$.[21–22]

Stripping gas flow rate was then optimized at optimum sample flow rate. The result in Figure 3 indicated that when the sample flow rate is fixed the extraction can be improved by increasing the stripping gas flow rate. The response increased with stripping gas flow rate until 9 $mLmin^{-1}$ then started to decrease due to high dilution from a larger amount of stripping gas with a high flow rate.

Desorption Effect

Typically, a relatively high sample flow rate was used to increase the extraction efficiency and to minimize the extraction time. However, in a case where the analyte has a large partition coefficient, like SVOCs, it is difficult to monitor with conventional MIMS at a standard temperature (25°C). An improvement of the extraction efficiency could be achieved by raising extraction temperature, which resulted in the increase of analyte in water.[23–24]

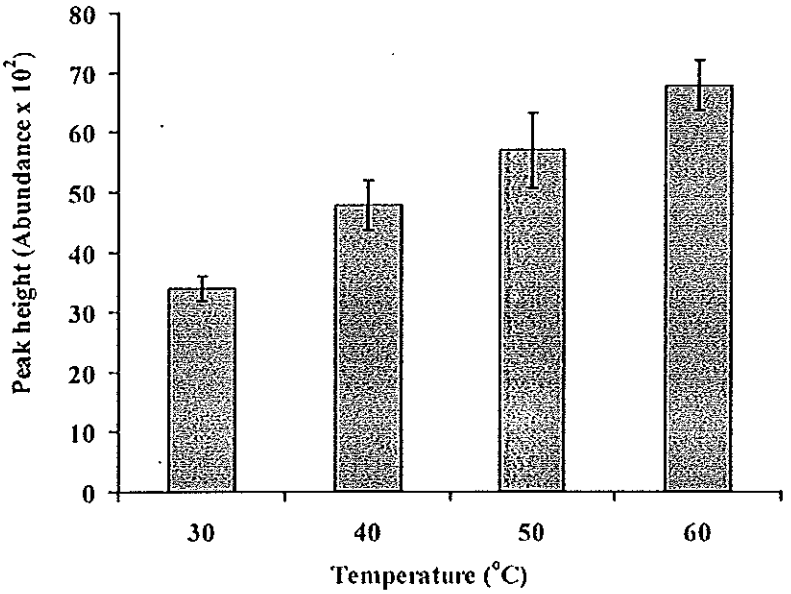

Figure 4: Effect of desorption temperature. The experiment was done with 1.0 mgL^{-1} 4,4'-DDT, injection volume 2 ml, sample and stripping gas flow rate 6 and 9 $mLmin^{-1}$, respectively.

A laboratory-built heating box was designed and set up as a thermal desorption unit to enhance diffusion of 4,4'-DDT in water and desorption of an analyte into HRGC-MS. Five different positions in the heating box were first calibrated and a statistical test indicated that there was no significant difference. The effect of the desorption temperature was then investigated.

The trend for the responses of 4,4'-DDT with increasing desorption temperature is shown in Figure 4. Over 60°C the membrane started to deform and bubbles were formed in the system. Therefore, the desorption temperature at 60°C was chosen to be used in this work to enhance diffusivity.

In this experimental set up, the analyte diffusivity could be calculated from the analyte response time ($t_{10\text{-}90\%}$ of peak rise) which is related to membrane thickness.[25] Using chromatographic results at 60°C, $t_{10\text{-}90\%}$ of the peak was used to calculate diffusivity (D) of 4,4'-DDT from the relationship $t_{10-90\%} = 0.237(\frac{l^2}{D})$ where l is the membrane thickness (l = 0.015 cm) and this gave a value of 7.4×10^{-5} cm^2s^{-1}. Comparing to the diffusivity of 4,4'-DDT at standard temperature of 5.0×10^{-6} cm^2s^{-1}[26], the heating could enhance the diffusion rate of 4,4'-DDT through a hollow fiber membrane by 15 times.

Loading Time

Before injection of the permeated 4,4'-DDT into HRGC-MS, the loading time to collect the gas phase of the analyte in a 2-mL sampling loop was optimized. This effect on extraction efficiency was evaluated from peak height

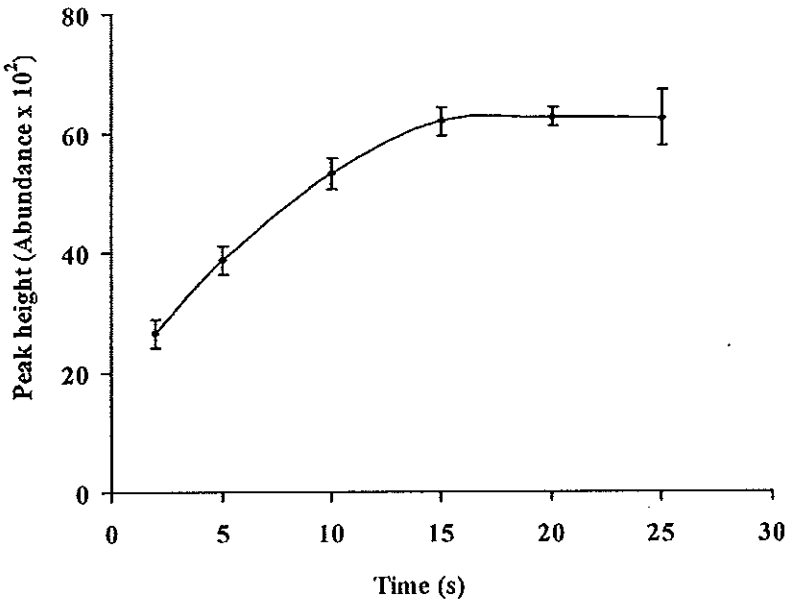

Figure 5: Effect of loading time. The experiment was done with 1.0 mgL^{-1} 4,4′-DDT, injection volume 2 ml, sample and stripping gas flow rate 6 and 9 $mL.min^{-1}$, respectively.

obtained from various loading times. Figure 5 shows a suitable loading time of 15 seconds, which gave the highest response.

System Performance

Figure 6 shows the replicated total ion chromatogram of successive in-line injection of 4,4′-DDT at concentration 0.5 mgL^{-1}. At optimum conditions, the method detection limit was obtained at 90 μgL^{-1} by IUPAC method;[27] which corresponded to the instrument detection limit (85 μgL^{-1} with S/N = 3). The relative standard deviation (RSD), based on five replicate injections was less than 10%, better than the 15% RSD reported in EPA method 8270D.[28] This developed system, MI-HRGC-MS, gave a much better detection limit than a standard MIMS which has been used to analyze the SVOCs include DDT[14], that is, reducing from 1,000 μgL^{-1} to 90 μgL^{-1}. Very good linearity was obtained in the range of 0.1–1.0 mgL^{-1} with $R^2 = 0.9940$.

Extraction Efficiency

The efficiency of extracting aqueous samples by MI-HRGC-MS was studied in two ways. First, the sensitivity of the membrane inlet unit was compared between the results obtained from direct injection of standard solution to the HRGC-MS and those obtained from spiked ultra pure water passing through the hollow fiber membrane. The slope of the spiked water was less than the standard solution by 25%. This indicated that the extraction

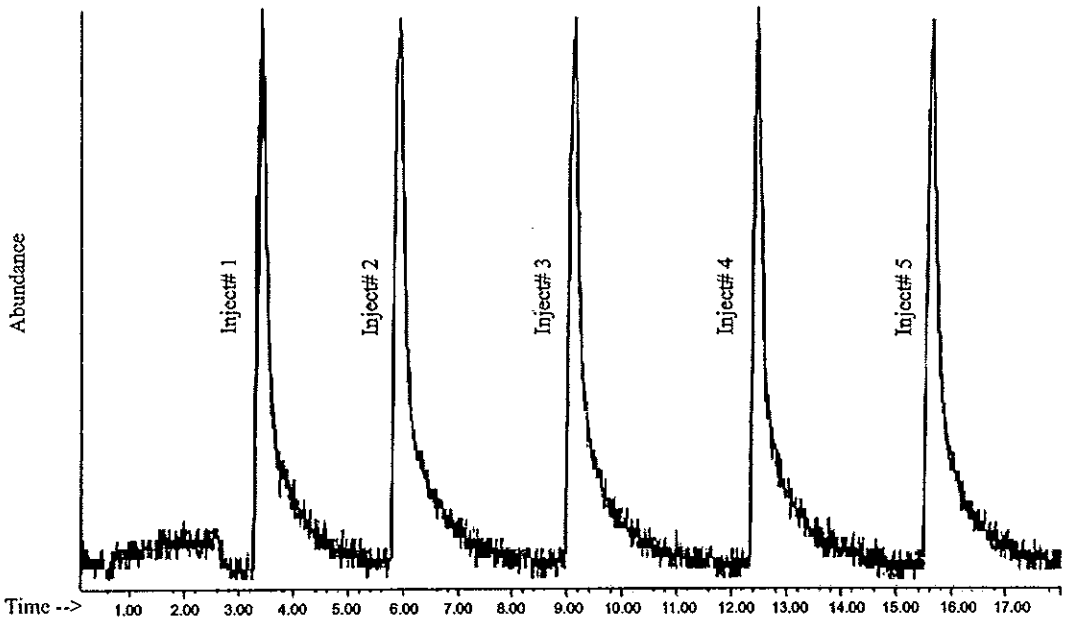

Figure 6: Replicated total ion chromatogram of in-line analysis of 4,4'-DDT in water by MI-HRGC-MS (membrane inlet-high resolution gas chromatography-mass spectrometry).

process is not 100% and the aqueous samples are needed to calibrate the system before real sample analysis. Then it was tested by comparing the responses between spiked ultra pure water and spiked bottled water samples at various concentrations (0.1–1.0 mgL^{-1}). The results (Fig. 7) indicated that good extraction efficiency between 83 and 94% were obtained with%RSD lower than 10. That is, the system could perform well for real samples.

Real Water Sample Analysis

To test the capabilities of the MI-HRGC-MS technique with real samples, some bottled and tap waters were sampled and analyzed. Due to the very low concentration of 4,4'-DDT in real samples, the analyte was not detected (its concentration was lower than 90 μgL^{-1}). Therefore, the standard addition method was applied to check the presence of 4,4'-DDT in the samples by spiking five concentrations (0.1, 0.25, 0.50, 0.75 and 1.0 mgL^{-1}) into water samples.

According to an international guideline, DDT in drinking water must be less than 2 μgL^{-1}.[29] From the results, 4,4'-DDT in drinking water samples (0.60 ± 0.03 μgL^{-1}) was lower than the limit. As for the locally produced, unregistered bottled water, its 4,4'-DDT concentration was higher than the guideline of drinking water. This may be the result of widespread hemorrhagic fever in the country, and to prevent the spreading by mosquitoes, the 4,4'-DDT was sprayed in a very wide area, causing it to accumulate in soil and then

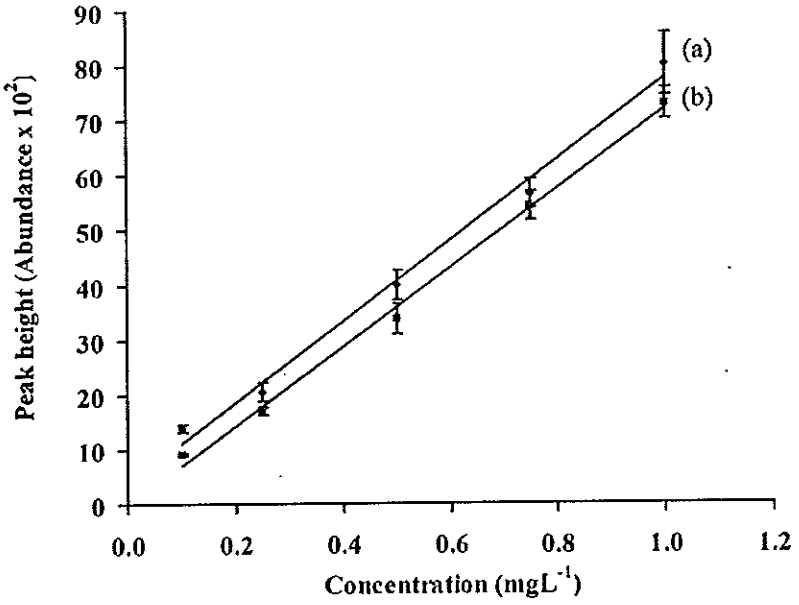

Figure 7: Extraction efficiency by comparing response between a (top line) and b (bottom line). (a) response from spiked ultra pure water and (b) response from spiked bottled water sample.

leach into raw water. Also, it could be the lack of regular monitoring by the authorities and no quality control of the production process. Consumer should therefore, be careful with their purchase. For tap water, it also showed high level of 4,4′-DDT (7.0 ± 0.5 μgL^{-1}). This indicated that the water source is contaminated and the treatment process does not sufficiently remove 4,4′-DDT. Therefore, regular monitoring is necessary and a warning should be provided for the unsuitability for drinking.

CONCLUSIONS

The membrane inlet system coupling with a high resolution gas chromatograph-mass selective detector (MI-HRGC-MS) for in-line membrane extraction is an alternative approach of the analytical method for SVOCs, especially the persistent organic pollutants (POPs), in water. If the concentration of SVOCs is higher than the limit of detection, this system can certainly be used for in-line monitoring. Although standard addition was needed because the concentration of analyte in the sample was very low, this developed system still has many advantages, i.e., it can provide good precision with RSD lower than 10%, the sample can be injected directly without any sample preparations because the extractor unit is set up in-line. Therefore, it does not require any sample handling and preservative step. In some cases where standard addition is required, either when the concentration is lower

than the detection limit and/or when the real sample has a matrix effect, it can be done in-line in a short period of time. Furthermore, this method requires less labor. There is no need of solvent and it can be applied to the real sample for qualitative and quantitative analysis.

ACKNOWLEDGEMENTS

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Capillary microextraction for simultaneous analysis of multi-residual semivolatile organic compounds in water

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Capillary microextractor (CME) in combination with a gas chromatograph-mass spectrometer (GC-MS) was employed for the determination of trace priority hazardous substances in water. Three groups of semivolatile organic compounds (SVOCs), *i.e.*, chlorinated hydrocarbons, pesticides and polycyclic aromatic hydrocarbons (PAHs), were simultaneously determined. SVOCs were extracted from 7 mL of water samples on a 100 cm commercial gas chromatographic column (0.32 mm id × film thickness 0.25 μm, HP-5 capillary column) and eluted with only 3 μL of acetonitrile. The extractant was analyzed by GC-MS in the selected ion monitoring mode. The method showed good linearity over the concentration range 10 ng L^{-1} to 3.0 mg L^{-1} with correlation coefficients (r) greater than 0.99 and low limits of detection ranged from 10 ng L^{-1} to 1.0 mg L^{-1}. High recovery (more than 80%) was obtained with relative standard deviation less than 10%. The method was successfully applied for trace level analyses of SVOCs in water samples.

1. Introduction

Continuous monitoring of residue of some semivolatile organic compounds (SVOCs) in water, *i.e.*, chlorinated hydrocarbons, pesticides and polycyclic aromatic hydro-carbons (PAHs)[1] is of great importance since these trace substances can affect human health.[2,3] A reliable and highly sensitive analytical method is required and high performance liquid chromatography or capillary gas chromatography is usually employed. The latter is preferred as it offers high resolution and is easy to couple with sensitive and selective detectors.[4,5] Since the concentrations of SVOCs in water are normally very low,[1-3] sample preparation techniques are still needed in order to enrich the analytes to meet the instrumentation detection limit.

Several sample preparation techniques have recently been reported such as micro liquid–liquid extraction (μLLE),[6] solid phase extraction (SPE),[7-13] solid phase microextraction (SPME),[14-16] membrane extraction (ME),[17,18] in-tube solid phase micro extraction (IT-SPME)[19-21] and stir bar sorptive extraction (SBSE).[22-28] Although the reduction of solvent usage can be accomplished with SPE technique, it still requires organic solvent volumes of some millilitres and has a limiting sample loading volume.[29] Lately, the most demanding trend is to detect target analytes at part-per-million to part-per-trillion levels in aqueous samples and SPME has been favored. It is used to sorb and then desorb the analyte to the gas chromatographic injector or high performance liquid chromatographic interface. In addition, it requires only a small sample volume, thus, it is a simple and solvent free technique. However, with the low amount of sorbent coated on the SPME fiber (up to 0.5 μL), the sensitivity problem was hard to overcome.[30] Moreover, SPME has limiting lifetime, fragility, and the fibers and assembly holder are relatively expensive.[31] SBSE can further extend detection limits to part-per-quadrillion due to the high capacity of the high film thickness of polydimethylsiloxane (PDMS) but it requires a dedicated hot injector and suffers from unavoidable disadvantages such as high bleed, injection artifacts and carry over.[17,32,33]

Recently, a few researchers have investigated the use of a chromatographic capillary column as both extractor and preconcentrator to enrich SVOCs in aqueous samples. The sorption of the SVOCs on the thin stationary phase PDMS film of a gas chromatographic capillary column helps to extract and preconcentrate the analytes. The analytes were then desorbed with either suitable solvent or thermal desorption. The sorption area in the capillary microextractor is much higher than SPME, resulting in high recovery and high sample capacity. Moreover, a thin film thickness can reduce high bleed and carry over better than SBSE.[19-21,34-38] Many publications have employed this technique with a column length of typically 60 cm and up to more than one metre, or particularly with relatively large film thickness (0.5–4.0 nm)[3,17,20,21,38,39] by coupling it to high performance liquid chromatography,[34-38] with fewer reports on its use coupled to gas chromatography.[19-21,38] To the best of our knowledge, no one has reported using this technique to simultaneously

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extract multi-residues of different groups of SVOCs. The aim of this work is to investigate the use of a chromatographic capillary column as a microextractor coupled with a GC-MS to simultaneously analyze multi-residual semivolatile organic compounds in water, *i.e.*, chlorinated hydrocarbons, pesticides and PAHs that are in the toxic priority list of the Agency for Toxic Substances and Disease Registry (ATSDR).[1]

2. Experimental

2.1 Chemicals and reagents

Six certified standard solutions; hexachlorobutadiene, dieldrin, and 4,4′-DDT in methanol, benzo[*a*]pyrene, and benzo[*b*]fluoranthene in acetone and dibenzo[*a*,*h*]anthracene in dicholromethane (99.99% purity) were purchased from Restek Company (USA). Organic solvents, *i.e.*, methanol, ethanol, acetone, acetonitrile, ethyl acetate and dichloro-methane (analytical grade) were purchased from Merck (USA). HPLC-grade water was purchased from Scientific Instrument Center (Prince of Songkla University, Thailand). A 100 mg L^{-1} of each standard stock solutions was prepared in the appropriate solvent indicated on the label. The solution was kept in an amber glass bottle and stored at 4 °C. Water samples used to investigate all parameters of extraction and chromatographic analysis were prepared by spiking HPLC-grade water with various concentrations of each standard stock solutions.

2.2 Analytical instrumentation and operating conditions

Sample analyses were carried out by a gas chromatograph-mass selective detector (Model HP6890-HP5973, Agilent Technologies, USA) with a HP-5MS fused silica capillary column (95% Polydimethylsiloxane, 30 m × 0.25 mm id, film thickness 0.25 μm, Agilent Technologies, USA). GC-MS parameters were first investigated and the optimum conditions were used for the remaining experiments (see 3.1 and 3.3). The sample introduction was carried out in 2 mm id split inlet liner (Agilent Technologies, USA). Split mode was used (see 3.1) at a split flow of 1.6 mL min^{-1}. Electron ionization, 70 eV, with a selected ion monitoring (SIM) mode was used to quantify the analytes with molecular ion at *m/z* 225(100), 227(63) (hexachlorobutadiene), *m/z* 79(100), 149(35) (dieldrin), *m/z* 235(100), 165(66) (4,4′-DDT), *m/z* 252(100), 250(18) (benzo[*b*]fluoranthene), *m/z* 252(100), 250(25) (benzo[*a*]pyrene) and *m/z* 278(100), 276(16) (dibenzo[*a*,*h*]anthracene).

2.3 Extraction

Fig. 1 shows the schematic diagram of the capillary microextractor system developed in this work. It consisted of two switching valves (C6WE and E4C10WE, Valco Instruments Co. Inc., USA). Valve A, a six port valve, was employed for loading and purging the sample, and valve B, a 10 port valve, for extracting and desorbing analytes into GC-MS. A capillary microextractor was a 100 cm HP-5 capillary column (95% polydimethyl siloxane, 0.32 mm id × film thickness 0.25 μm, Agilent Technologies, USA) while the eluent loop (3 μL) was a 38 cm blank capillary column (0.10 mm id, Composite Company, UK). An additional blank column (10 cm, 0.05 mm id) was used as a transfer line to transport the desorbed analytes to GC-MS. The transfer line was connected directly to the inlet with 5 cm of its end placed inside the liner. The pressure between inlet and transfer line was controlled by the pneumatic system and the force of gas flow.

To start the analysis, water sample was loaded into the 1 mL Teflon tube® sample loop (1/16 in od, Waters Company, USA) on valve A (Fig. 1, step 1). After loading, Valve A was switched (Fig. 1, step 2) to allow the water sample to be transported through the capillary microextractor on valve B by helium gas flow (ultra high purity grade, Thai Industrial Gas, Thailand) where the analytes were sorbed on the PDMS phase in the column. The capillary microextractor was purged with helium gas (see 3.2.5) to dry the column and to remove any residual water. While step 2 was being carried out, organic solvent was loaded into the eluent loop on valve B. When step 2 was complete, valve B was then switched to allow the eluent to desorb analytes from the capillary microextractor and passed through the transfer line into GC-MS (Fig. 1, step 3). Analysis was performed using SIM mode to quantify target analytes. After elution, the capillary microextractor was re-equilibrated with an aliquot of acetonitrile, methanol, or HPLC-grade water then dried with gas purging, respectively, before the next injection.

2.4 Method qualification

To prove the analytical method to be acceptable for its intended use, the method needs to be validated. Spiked HPLC-grade water, with a wide range of analytes concentrations (10 ng L^{-1} to 5 mg L^{-1}), was analyzed by CME-GC-MS at optimum conditions to demonstrate linearity, limit of detection and precision. Real sample analysis for the six SVOCs was performed using tap water and raw water (source of tap water production) collected from Hat Yai City, Thailand.

3. Results and discussion

3.1 Optimum conditions for chromatographic separation

The aim of this study was to investigate simultaneous analysis of three different properties groups of SVOCs by using a capillary micro extractor coupled to a GC-MS. Parameters affecting the performance of GC-MS were first optimized, *i.e.*, carrier gas flow rate, temperature program, inlet temperature and split ratio. The optimum conditions were chosen by considering two criteria, high response and short analysis time. For the carrier gas flow rate, 0.7 mL min^{-1} provided the best resolution and high efficiency. When studying the inlet temperature, since the six analytes have different boiling points, the optimum inlet temperature was chosen as the one that could provide relatively high response for all analytes and this was found to be 220 °C.

In the case of the injection mode, due to a relatively large volume of eluting solvent (3 μL) (typical maximum for a split/splitless mode injection is 2 μL),[40] split mode injection was used instead of the splitless mode to reduce volume loading to the inlet liner caused by its vaporization and expansion. Various split ratios were investigated and a split ratio of 2 : 1 provided the highest response.

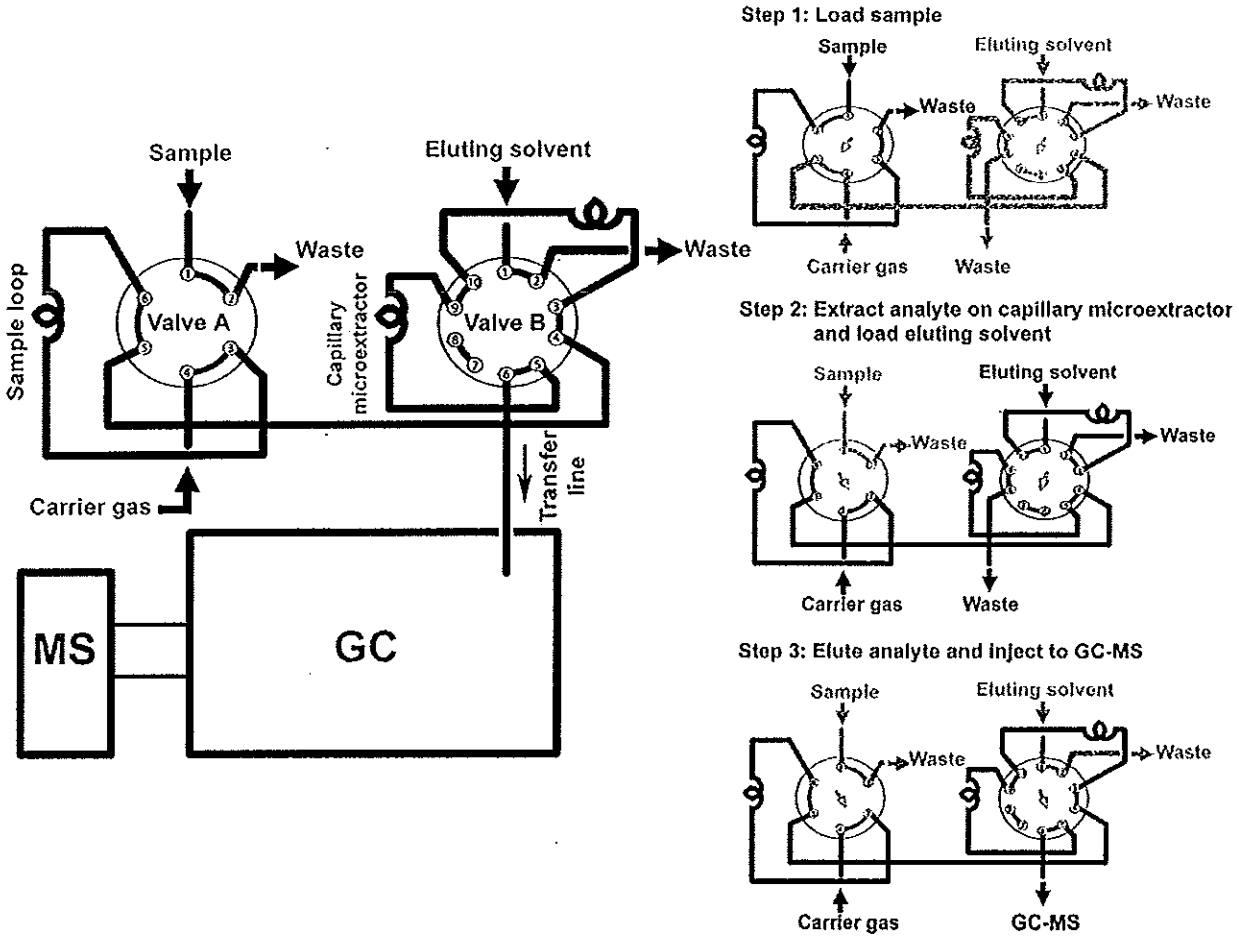

Fig. 1 Schematic diagram of the capillary microextraction system coupled to a gas chromatograph-mass spectrometer (CME-GC-MS).

To obtain a good resolution of the six components, the column temperature program was studied and was found to be: initial temperature 40 °C, held for 3 min then increased to 300 °C at a ramp rate of 25 °C min^{-1}. At the initial temperature of 40 °C, the analytes were trapped at the head of the GC column ("cold trapping") before being swept through the column during the increase in temperature. This provided a chromatogram with a sharp profile and high resolution for the six analytes. The obtained optimum conditions provided good separation for the three groups of SVOCs, *i.e.*, chlorinated hydrocarbon, pesticides and PAHs.

3.2 Optimization of extraction conditions

Capillary microextraction was performed by solvent desorption instead of thermal desorption to avoid the loss of some polar analytes, *i.e.*, pesticides that have weak interaction with PDMS material when using thermal desorption.[41] The goal of the method was to obtain high extraction efficiency and a low detection limit in a relatively short time. Therefore, the effects of sample flow rate, breakthrough volume, organic modifier, eluting solvent and purging time were investigated. Water sample spiked with a mixture of the six compounds (hexachlorobutadiene, dieldrin, 4,4′-DDT, benzo[*b*]fluoranthene, benzo[*a*]pyrene and dibenzo[*a*,*h*]anthracene) was used to study all extraction parameters.

3.2.1 Sample flow rate. Spiked water sample which containing 1.0 mg L^{-1} of each compounds was used to study the effect of sample flow rate (0.8, 1.0, 1.5, 2.0 and 2.5 mL min^{-1}). Helium gas was used to drive the sample through the microextractor column and measured in terms of flow rate. At the lowest flow rate, 0.8 mL min^{-1}, there was no signal due to back pressure from the inlet of GC-MS to the transfer line, therefore, the eluent could not be transported into GC-MS for separation. Signals could be obtained at flow rates of 1.0 mL min^{-1} and higher (Fig. 2) where the responses decreased when flow rate increased. This is probably because at a higher flow rate, the adsorption time was reduced, allowing less analyte to be sorbed into the bulk of PDMS film.[32] The optimum flow rate of 1.0 mL min^{-1}, which provides the highest response, was then chosen.

3.2.2 Eluting solvent. Methanol, acetonitrile, acetone and ethyl acetate which have different polarities and are mostly

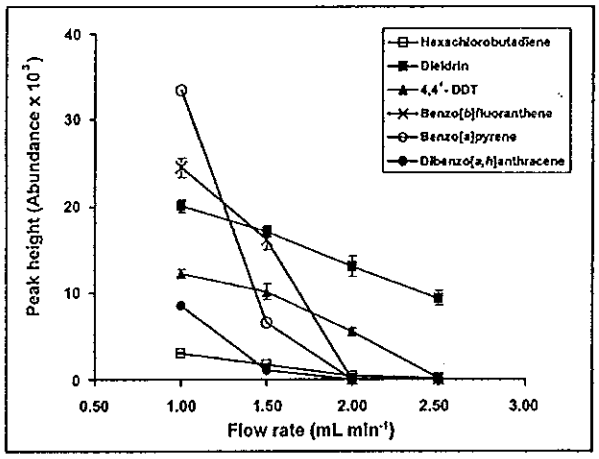

Fig. 2 Effect of sample flow rate on the extraction of the six analytes in water sample.

used in liquid desorption were investigated as potential organic solvents to desorb the analytes from the capillary microextractor. A spiked water sample (hexachlorobutadiene 0.5 mg L^{-1}, dieldrin 0.2 mg L^{-1}, 4,4′-DDT 0.2 mg L^{-1}, benzo[*b*]fluoranthene 0.15 mg L^{-1}, benzo[*a*]pyrene 0.15 mg L^{-1} and dibenzo[*a*,*h*]anthracene 0.5 mg L^{-1}) was used to investigate this parameter. The results (Fig. 3) showed that acetonitrile gave relatively good responses for all analytes. This could be because acetonitrile has suitable polarity (polarity index: methanol > acetonitrile > acetone > ethyl acetate)[42] to desorb both polar and non-polar compounds and this has been applied widely for SVOC analysis in liquid chromatography. After elution, contaminants were washed out by the cleaning process described in section 2.3. No memory effect was found after this re-equilibrated step.

3.2.3 Organic modifier. Before detection, SVOCs in a water sample could be lost due to possible adsorption on the sample container wall (polyethylene or glass) and the Teflon® tubing used as a sample loop.[43] This effect can be reduced by

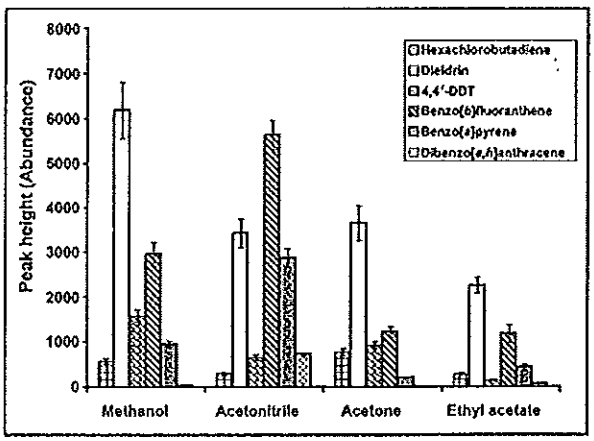

Fig. 3 Effect of eluting solvents on the desorption of the six analytes in capillary microextraction system.

adding a small amount of organic modifier into the water sample to increase solute–aqueous phase dispersion interactions[44] and allow the particulate matter to settle, which will speed up the extraction process.[45] Three organic solvents which are soluble in both water and organic analytes, *i.e.*, methanol, ethanol and acetone (5% v/v), were investigated for a suitable organic modifier that would increase the recovery.[43] The organic modifiers were added to the spiked water samples (hexachlorobutadiene 1.0 mg L^{-1}, dieldrin 0.5 mg L^{-1}, 4,4′-DDT 0.5 mg L^{-1}, benzo[*b*]fluoranthene 0.3 mg L^{-1}, benzo[*a*]-pyrene 0.3 mg L^{-1} and dibenzo[*a*,*h*]anthracene 1.0 mg L^{-1}) and then analyzed. The results (Fig. 4) indicated that ethanol provided the best response for all analytes while methanol gave high responses for polar compounds in the pesticide group but low responses for chlorinated hydrocarbon and PAHs. When acetone was added, the recovery is less than when there was no acetone. This may due to the increase in solubility of the analytes in acetone–water mixture, resulting in a low efficiency for adsorption on extracting phase.[44,46] Therefore, ethanol was chosen as modifier; it also has lower toxic effects.[47]

3.2.4 Microextractor column length. Initially a 50 cm length of the capillary micro extractor was studied. However, the limit of detections of four of the six analytes were higher than the limits set by the United States Environmental Protection Agency (U.S. EPA). Therefore, longer columns (75 and 100 cm) with the same inner diameter (0.32 mm) and film thickness (0.25 μm) were investigated to enhance the responses, and hence the limit for detections. Spiked HPLC-grade water at concentration of 0.5 mg L^{-1} was tested and Fig. 5 shows that the longer the column the better the response. At 100 cm the extraction efficiency could be enhanced by 39–75% when compared to 50 cm. Therefore 100 cm column length was used for further study.

3.2.5 Purging time. To ensure that there would be no carry over from water residue left in the capillary microextractor from the previous sample, purging gas was used to dry and remove the residue. HPLC-grade water (blank) was passed through the system and then purged by helium gas at purging times of 20, 30, 40, 50 and 60 s. By monitoring mass ion of water using SIM mode, it was found that residual water (*m*/*z* 18) could be detected at a purging time less than 50 s with relatively high response that could interfere with the response of analytes. At 50 s there was no carry over from residual water and this was demonstrated by a clear chromatogram baseline. Therefore, a purging time of 50 s was used. To test whether there would be any loss of analyte if the column was purged, after a spiked water sample contained 0.5 mg L^{-1} of each compound (hexachlorobutadiene, dieldrin, 4,4′-DDT, benzo[*b*]fluoranthene, benzo[*a*]pyrene and dibenzo[*a*,*h*]anthracene) was passed through the system, it was purged for 50 and 60 s. No relative losses of analytes were observed. This was due to the rapid equilibrium in the capillary micro-extractor during the dynamic sampling process.[48,49]

3.2.6 Sample volume. Since the length of the capillary microextractor and the film thickness of the extracting phase coated on the microextractor were fixed, therefore, sample

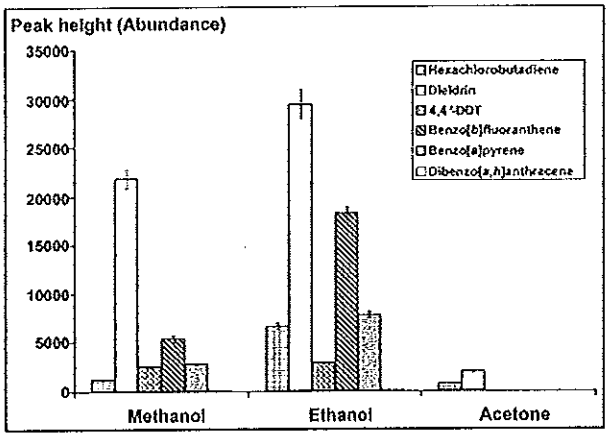

Fig. 4 Comparison of the organic modifier (5% v/v) for the extraction of the six analytes by capillary microextraction.

volume providing maximum adsorption or equilibrium between two phases was determined to provide the highest recovery. Water samples from 4 to 8 mL were tested. Since the sample loop can only hold 1 mL of sample, each "loading" through valve A will pass 1 mL of sample to valve B (Fig. 1). For a sample with higher volume, loading was done more than once by switching valve A on and off until the desired volume was transported through the capillary microextractor on valve B. A spiked water sample that contained 0.5 mg L^{-1} of each compound (hexachlorobutadiene, dieldrin, 4,4′-DDT, benzo [*b*]fluoranthene, benzo[*a*]pyrene and dibenzo[*a,h*]anthracene) was employed for the test. This concentration was chosen following the U.S. EPA method for performance testing.[50] When the capacity of capillary microextractor is reached, all the sorptive sites are fully occupied so the increase in concentration or sample volume will no longer lead to an increase in the amount of compounds sorbed. From Fig. 6, the response increased with sample volume up to 7 mL, and this is the breakthrough volume used for further studies.

3.3 Recovery

Recovery is normally determined by comparing the response of analyte in the sample to the standard calibration curve. However, since the extraction process of capillary microextractor involved the partitioning of analytes between water matrix and extraction phase contained inside the capillary, both the water matrix and extracting phase are competing for the analytes due to the different K_D (the partition coefficient)[38] of the analytes in the sample. Therefore, recovery is also dependent on the water matrix and this would influence the extraction efficiency. Because of this, relative recovery was used instead of recovery. This was done by comparing the responses obtained from spiked HPLC-grade water and spiked real water samples containing 0.5 mg L^{-1} of hexachlorobutadiene, dieldrin, 4,4′-DDT, benzo[*b*]fluoranthene, benzo[*a*]pyrene and dibenzo[*a,h*]anthracene. They were passed through the capillary micro extractor and analyzed by GC-MS at optimum conditions. All component peaks were quantified by SIM mode at the specific mass of the ion. The obtained

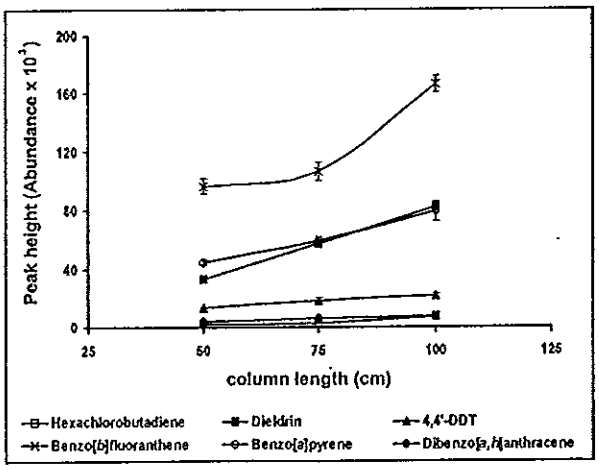

Fig. 5 Effect of microextractor column length on the extraction of the six analytes (0.5 mg L^{-1}) in water sample.

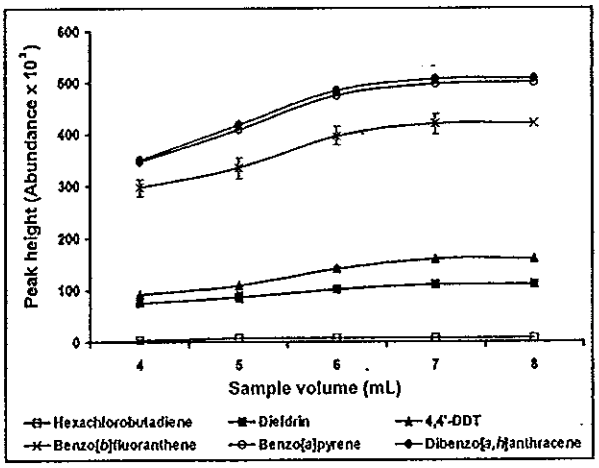

Fig. 6 Effect of sample volume on the extraction of the six analytes in water sample.

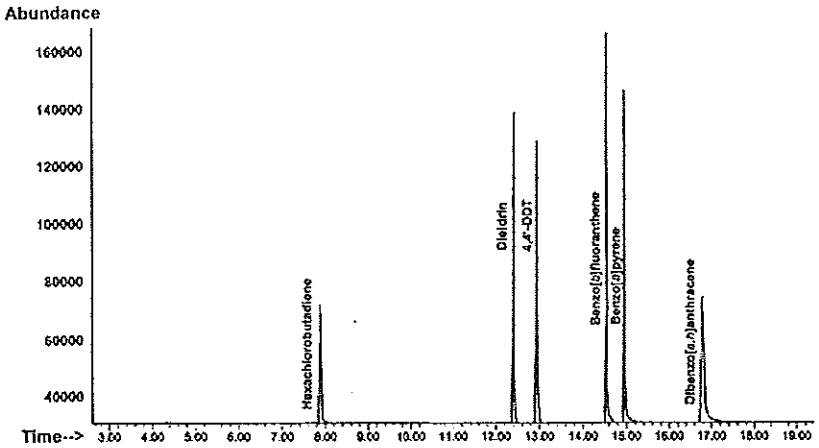

Fig. 7 Total ion chromatogram of six hazardous substances in water (2.0 mg L^{-1} hexachlorobutadiene, 1.0 mg L^{-1} dieldrin, 1.0 mg L^{-1} 4,4′-DDT, 1.0 mg L^{-1} benzo[*b*]fluoranthene, 1.0 mg L^{-1} benzo[*a*]pyrene and 2.0 mg L^{-1} dibenzo[*a*,*h*]anthracene) enriched by CME-GC-MS.

recoveries were higher than 80% (Table 1) and these were acceptable (70–130%).[51] The results indicated that this method is effective for multi-residues analysis.

3.4 System performance

Six compounds were analyzed by capillary microextraction coupled to gas chromatography-mass spectrometry and identified based on their retention time and mass spectrum. Fig. 7 shows the total ion chromatogram of the six components, hexachlorobutadiene 2.0 mg L^{-1}, dieldrin 1.0 mg L^{-1}, 4,4′-DDT 1.0 mg L^{-1}, benzo[*b*]fluoranthene 0.5 mg L^{-1}, benzo[*a*]-pyrene 0.5 mg L^{-1} and dibenzo[*a*,*h*]anthracene 1.0 mg L^{-1}.

These compounds were well adsorbed by PDMS (hydrophobic phase) as well as having high partition coefficient, but their responses were not equal. This is because this separation was directed at the simultaneous analysis of six compounds, therefore, the response of some compounds have been compromised. For instance, the first and last peaks had relatively low responses than the others. The reason being, hexachlorobutadiene, the first peak has the lowest partition coefficient,[52–55] therefore, it was the least adsorbed on the PDMS phase of the microextractor resulting in a low response. For the last peak, dibenzo[*a*,*h*] anthracene, although it has a high partition coefficient and was better retained on the PDMS phase, the optimal eluting solvent could only desorb a small amount of this compound, thus, the lowest response.

Table 1 Relative recoveries obtained by comparing the response between HPLC-grade water and spiked sample under the same concentration at 0.5 mg L^{-1} (n = 5)

	Recovery (%)	
Compounds	Tap water	Raw water
Hexachlorobutadiene	101 ± 5	99 ± 6
Dieldrin	112 ± 3	109 ± 5
4,4′-DDT	94 ± 7	95 ± 6
Benzo[*b*]fluoranthene	92 ± 4	90 ± 3
Benzo[*a*]pyrene	90 ± 5	93 ± 7
Dibenzo[*a*,*h*]anthracene	89 ± 5	88 ± 4

Quantitative analyses of all six analytes were carried out by generating a series of calibration curves at concentrations ranging from 10 ng L^{-1} to 3.0 mg L^{-1}. Linearity of each component was established and the correlation coefficients were greater than 0.99 (Table 2). The detection limits of the six compounds were in the range of 10 ng L^{-1} to 1.0 μg L^{-1} obtained by considering the signal to noise ratio equal to 3 (S/N = 3). These limits of detection were all lower than or equal to the legal limits for drinking water set by the U.S. EPA, therefore, this system can be used to determined these compounds in water. The relative standard deviation (RSD), based on five replicate injections was less than 10%.

3.5 Real sample analysis

The capillary microextraction coupled to gas chromatography-mass spectrometry system was validated with real samples, *i.e.*, raw water and tap water collected in Hat Yai City, Thailand. Each collected water sample was introduced directly into the analysis system. Qualitative analysis was evaluated using the retention time and the molecular ion, while for quantitative analysis the amount of analyte was determined

Table 2 Linear range, correlation coefficient and limit of detection obtained by capillary microextraction coupled to gas chromatography-mass spectrometry (%RSD less than 10, n = 5) and the limit set by U.S. EPA

Compounds	Linear range/ μg L^{-1}	Correlation coefficient (r)	Limit of detection/ μg L^{-1} (S/N = 3)	Limits set by U.S. EPA[56]/ μg L^{-1}
Hexachlorobutadiene	0.01–1000	0.9982	0.01	0.05
Dieldrin	0.02–1000	0.9972	0.02	0.03
4,4′-DDT	1.00–2000	0.9998	1.00	2.00
Benzo[*b*]fluoranthene	0.05–3000	0.9959	0.05	0.10
Benzo[*a*]pyrene	0.01–2000	0.9998	0.01	0.01
Dibenzo[*a*,*h*]anthracene	0.10–1000	0.9998	0.10	0.10[a]

[a] There is no implemented maximum contaminant level, however, U.S. EPA recommended that it should not exceed 0.10 μg L^{-1}.[57,58]

Table 3 Concentration of semivolatile organic compounds in water sample (tap water and raw water) from Hat Yai City, Songkhla, Thailand analyzed by capillary microextraction coupled to gas chromatography-mass spectrometry

Compounds	Tap water/ μg L^{-1} (%RSD)	Raw water/ μg L^{-1} (%RSD)	Limit set by the Metropolitan Waterworks Authority, Thailand[59]
Hexachlorobutadiene	0.66 (6)	0.11 (7)	—
Dieldrin	0.03 (8)	0.62 (6)	0.03
4,4′-DDT	ND	1.50 (5)	2.00
Benzo[*b*]fluoranthene	ND	ND	—
Benzo[*a*]pyrene	ND	0.01 (8)	0.02
Dibenzo[*a*,*h*]anthracene	ND	ND	—

[a] ND: not detectable (determined by standard addition method); (—): no implementation.

from the calibration curve. When the responses were lower than the detection limits, the standard addition method was employed by spiking the real sample with various concentrations of stock standard solution and passed through CME-GC-MS. The standard addition method was also used to control the effect from matrix in each sample.

The results in Table 3 indicate that these water samples were contaminated with most of the priority hazardous substances. The presence of 4,4′-DDT, although lower than the legal limits of drinking water set by the Metropolitan Waterworks Authority, Thailand (which based on World Health Organization guideline 1993, addendum 1998),[59] could be a result of an attempt to control the mosquitoes with 4,4′-DDT to avert the wide spread of hemorrhagic fever and malaria[60] in the country. Therefore, insecticides were sprayed in a very wide area causing these compounds to be accumulated in soil and then leached into raw water. As for benzo[*a*]pyrene, it could be the result of leakage from an old water pipe line. Combustion products[55] may also be a possible cause since benzo[*a*]pyrene sorbed onto smoke particles can be transferred into water by rainfall[24] which finally, has an impact on water quality. For hexachlorobutadiene and dieldrin, they were detected with concentrations higher than the legal limits. The higher concentration of hexachlorobutadiene in tap water was probably caused by the pretreatment of raw water. In the process of tap water production the water was pretreated with chlorine and this could lead to chlorination of hydrocarbon such as tri- and tetrachloroethene, and tetrachloromethane resulting in the formation of hexachlorobutadiene.[52] This compound could also come from the leachate of rubber manufacturing where hexachlorobutadiene is generally used as an intermediate product in rubber production.[61] In the case of dieldrin, it has been used in place of 4,4′-DDT and has been proved to be a highly effective insecticide.[62] It is in current use and this may be the reason why it was detected at relatively high concentration in both real samples. For benzo[*b*]fluoranthene and dibenzo[*a*,*h*]anthracene, their concentrations were not detectable for all samples. Although the U.S. EPA has not implemented the maximum contaminant level (MCL) of dibenzo[*a*,*h*]anthracene for drinking water, they have recommended this compound to be included in water quality criteria of surface water used as a drinking water source, for the protection of aquatic life and human health. Therefore, regular monitoring is necessary. The finding in this study suggested that it would be necessary to monitor these compounds and a warning should be provided to the public that this tap water is not suitable for drinking.

Conclusions

The results from this work show that CME-GC-MS can be used for simultaneous analysis of multi-residual semivolatile organic compounds (SVOCs) in water, especially the priority toxic substances. The chromatograms showed a stable and smooth baseline because the capillary microextractor has a thinner and stronger film of bonded phase that eliminates practical problems, *i.e.*, ghost peaks or several hours to reach extraction equilibrium which normally affect the SPME technique.[38] The developed system provided good recovery, higher than 80%, good precision with RSD lower than 10% and also achieved detection at very low concentration (ng L^{-1}). The main advantage is that the sample can be directly injected without any sample preparations because the extractor unit is set up in-line. Therefore, it does not require any sample handling and it can directly be applied for qualitative and quantitative analysis.

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List of Publications and Proceedings

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1. Pensiri Srichana, Pimpimon Penchumruth, Proespichaya Kanatharana* and Panote Thavarungkul, 2004. Trace analysis of hormone disruption contaminants in water near wastewater treatment pond and landfill, *Malaysian Journal of Science* **23**: 79-84.
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Presentations

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1. Pensiri Srichana and Proespichaya Kanatharana. "Trace Analysis of PCBs Residue in Water Sample by Gas Chromatography – Mass Spectrometry", The First PERCH Annual Scientific Conference (PERCH Conference I), 12-15 May 2002, Garden Sea View Resort, Pattaya, Chonburi, Thailand.
2. Pensiri Peeraprasompong, Panote Thavarungkul and Proespichaya Kanatharana. "On-line Hollow Fiber Membrane Inlet Mass Spectrometry for Semivolatile Organic Compounds (SVOCs) Analysis", RGJ Series XXX: Biosensors and Biotechnology for the development of Sounthern Thailand, 13 Aug 2004, Faculty of Agro-Industry, Prince of Songkla University, Songkhla, Thailand.
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Poster Presentations

1. Pensiri Peeraprasompong, Panote Thavarungkul and Proespichaya Kanatharana. "Hollow Fiber Membrane Inlet Mass Spectrometry (HFMIMS) to Analyze Semivolatile Organic Compounds (SVOCs)", The Third PERCH Annual Scientific Congress (PERCH Congress III), 9-12 May 2004, Jomtien Palm Beach Resort Hotel, Pattaya, Chonburi, Thailand.
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