Determination of 1,2–Dichloroethane (DCE) Contaminated in Packaged Food by Gas Chromatography with Electron Capture Detector

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Master of Science Thesis in Analytical Chemistry

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ชื่อวิทยานิพนธ์	วิเคราะห์สารปนเปื้อน 1,2 ไดคลอโรอีเทน ในอาหารที่บรรจุในบรรจุภัณฑ์ โดยเครื่องแก๊สโครมาโตกราฟฟีด้วยตัวตรวจวัดชนิดตัวตรวจจับอิเล็กตรอน
ผู้เขียน	นางสาวกิ่งแก้ว กาญจนรัตน์
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บทคัดย่อ

วิเคราะห์เชิงคุณภาพและปริมาณสาร 1,2-ไดคลอโรอีเทน (ดีซีอี) ปนเปื้อนในตัวอย่างอาหารบรรจุภัณฑ์ ด้วยเทคนิคแก๊สโครมาโทกราฟฟี โดยใช้ตัวตรวจวัดชนิดอิเลคตรอนแคปเจอร์ คอลัมน์แก้วขนาดเส้นผ่าศูนย์กลางภายใน 2 มิลลิเมตร ยาว 1.8 เมตร ภายในบรรจุด้วยสเตชันนารีเฟสคือ 0.1%SP-1000 เคลือบบนตัวซัพพอร์ทชนิด Carbopack C ขนาด 80/100 mesh ร่วมกับเทคนิคเฮดสเปซ ในงานวิทยานิพนธ์นี้ได้ศึกษาการวิเคราะห์สารดีซีอี โดยใช้เทคนิคเฮดสเปซ โดยเปรียบเทียบระบบเฮดสเปซ 2 ระบบคือ ระบบที่ทำใช้ในห้องปฏิบัติการและระบบอัตโนมัติสำเร็จรูป จากการศึกษาพบว่า สภาวะที่เหมาะสมสำหรับการวิเคราะห์สารดีซีอีเป็นดังนี้ อัตราการไหลของแก๊สพา (ไนโตรเจน) 20 มิลลิลิตรต่อนาที อุณหภูมิคอลัมน์ 90 องซาเซลเซียส อุณหภูมิหัวฉีด 170 องซาเซลเซียส อุณหภูมิตัวตรวจวัด 280 องซาเซลเซียส อุณหภูมิที่ทำให้ตัวอย่างเข้าสู่สมดุล 80 องซาเซลเซียส โดยใช้เวลา 10 นาที และ อัตราส่วนเฟส 0.8 จากสภาวะเหมาะสมสามารถตรวจวัดสารดีซีอีได้ต่ำถึง 0.92 ไมโครกรัมต่อลิตร สำหรับระบบที่ทำใช้ในห้องปฏิบัติการและ 0.72 ไมโครกรัมต่อลิตร สำหรับระบบอัตโนมัติสำเร็จรูป โดยให้ช่วงการตอบสนองเชิงเส้นตั้งแต่ 1 - 750 ไมโครกรัมต่อลิตร ด้วยค่าสหสัมพันธ์ของเส้นตรง (R^2) มากกว่า 0.99 และให้ค่าความแม่นยำโดยมีค่าความเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า 4%

ผลการวิเคราะห์ตัวอย่างอาหารบรรจุภัณฑ์ 37 ตัวอย่างจากซุปเปอร์มาร์เก็ตในอำเภอหาดใหญ่ จังหวัดสงขลา ด้วยสภาวะที่เหมาะสมดังกล่าว พบว่ามีการปนเปื้อนของสารดีซีอีในตัวอย่างเครื่องแกง 7 ตัวอย่างซึ่งเป็นผลิตภัณฑ์ตราเดียวกันแต่เป็นเครื่องแกงต่างชนิด โดยตรวจพบอยู่ในช่วง 0.79 นาโนกรัมต่อกรัมถึง 29.42 นาโนกรัมต่อกรัม และตัวอย่างบางชนิดมีการปนเปื้อนสาร

ดีซีอีในปริมาณต่ำกว่าขีดจำกัดการตรวจวัดของวิธี ดังนั้นเพื่อยืนยันการปนเปื้อนสารดีซีอีในตัวอย่างดังกล่าว โดยนำวิธี standard addition มาใช้ร่วมด้วย ได้ค่าการปนเปื้อนของสารดีซีอีอยู่ในช่วงตั้งแต่ตรวจไม่พบ ถึง 0.76 นาโนกรัมต่อกรัม

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ABTRACT

The qualitative and quantitative analysis of 1, 2-Dichloroethane (DCE) contaminated in packaged food were carried out by gas chromatography with electron capture detector (GC-ECD) equipped with a 1.8m x 2mm i.d., glass column, 0.1% SP-1000 on Carbopack C, 80/100 mesh and headspace technique. DCE was analyzed by a lab-built thermal system, a conventional technique, and an automated headspace system. The optimum conditions of GC-ECD and headspace technique of both systems were investigated and obtained *i.e.* flow rate of nitrogen carrier gas 20 ml min^{-1}, column temperature 90°C, injector temperature 170°C, detector temperature 280°C, equilibration temperature 80°C, equilibration time 10 minutes and phase ratio 0.8. A the optimum conditions, the system provided the limit of detection of the lab-built thermal bath and the automatic headspace systems at 0.92 μg L^{-1} and 0.72 μg L^{-1} respectively. The linear dynamic range was 1 μg L^{-1} to 750 μg L^{-1} with linear regression (R^2) larger than 0.99 and the relative standard deviation (%RSD) less than 4% of both system.

The thirty-seven packaged food samples were sampling from supermarkets, in Hat Yai, Songkhla and analyzed at the optimum conditions of GC-ECD and headspace analysis. The results showed that DCE was contaminated in seven curry paste samples of the same brand in the range of

0.79 ng g^{-1} to 29.42 ng g^{-1}. The standard addition method was also used to confirm of trace DCE in packaged food that was lower than the limit of detection. The results showed in the range of not detectable to 0.76 ng g^{-1}.

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Determination of 1,2–Dichloroethane (DCE) Contaminated in Packaged Food by Gas Chromatography with Electron Capture Detector is Master of Science Thesis in Analytical Chemistry. It is a basic research which creates a new knowledge to develop country.

This work related to the food safety and environment. The organization that could be used the outcome of this work were:

- Ministry of Public Health
- Ministry of Environment and Natural Resource
- Ministry of Education

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Chapter 1

INTRODUCTION

1.1 Introduction

Packaging makes food more convenient and gives the food greater safety assurance from microorganism, biological, and chemical change such that the packaged food can provide a long shelf life. As a result, packaging becomes an indispensable element in food manufacturing process. In order to the high demand of the food industry, there was a notable growth in the development of food packaging in the past decade. At present more than 30 different plastics are being use as the packaging material. Also, different type of additive, *i.e.* lubricants, stabilizer, anti-blocking agent and plasticizers have been developed to improve the performance either during processing and fabrication or in use of this polymeric packaging materials (Lau and Wong, 2000). The concern about the benefit and safety of food has rapidly increase recently. Most concern are usually focused on the food additive, both those added intentionally to the foods and those ending up in the food from packaging material or processing equipment.

The migration of the main ingredient of packaging material, plasticizers, from food contact into food had raised many concerns in communities. This was attributed to the demonstrated carcinogenic and mutagenic effect in rodents and potential effect in human as revealed by toxicology studies of several commonly used plasticizers. Such incidence indicated that the packaging could itself represent a substance from the packaging material into food. A large number of research regarding the migration of volatile, additive,

and monomer from plastic packaging material into food were conducted as a regulation. Analysis of the migration in the foodstuff can be vary expensive and time consuming because of the low concentration of migrated substances found in the foodstuff and the complexity of food matrix (Lau and Wong, 2000).

Food is a complex non-homogeneous mixture of a wide range of chemical substances that makes it hard to isolate and determine analytes of interest. Even with the appearance of advanced techniques of separation and identification, it is rarely possible to analyse food without manipulation. After sampling, it is necessary to prepare the sample for the analysis of analytes through its dissolution, trace enrichment (Buldini *et al.*, 2002). The highly needs for food analysis arise from health concern, but other reasons for food analysis include process-control or quality-assurance purposes, flavor and palatability issues, food contamination, identification of origin, or mining the food for natural products that can be used for variety of purpose. Different type of environmental contaminants can be contaminated in food through unintention exposure to the food, air, soil, or water. Food may also be contaminated by toxin from various micro-organisms, such as bacteria or natural toxins that already presented in the food or arise from spoilage. Packaging materials (compounds) can also leach into food unintentionally. All type of additives and contaminants are regulated by
government agencies world-wide (Lehotay *et al.*, 2002). A wide variety of additives were developed and used to improve the performance either during processing and fabrication or in used of polymeric packaging materials. Additives are all commonly encountered in various types of polymer materials. Plasticizers, monomers, stabilizers, and/or other chemical agent were also added into packaging materials that might be migrated into food.

Plasticizers are used in the polymer industry to improve flexibility, workability and general handing properties. They are commonly used in films,

in tubing, in liners or seals for bottle caps or lids. Butyl stearate, acetyltributyl citrate, alkyl sebacates and adipate are types of plasticizers commonly used with low toxicity. However, restriction has been brought up to the use of phthalate plasticizers due to their carnogenic potential and estogenic effect as revealed in some toxicological studies. Recently, it was also reported that phthalates might impair human fertility. Many type of plasticizers such as the phthalates group; di-2-ethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), and adipate group; di-2-ethylhexyl adipate (DEHA) were demonstrated in packaged food (Balafas *et al.*, 1999).

The other additives are added in polymeric packaging *i.e.* anti-oxidants, stabilizers, lubricants, and slip additives. Two types of stabilizers are used in polymeric processing, thermal stabilizers and light stabilizers. Thermal stabilizers are used in a wide range of food-contact plastics heat stabilizer. The other stabilizers, light stabilizer, are used to improve the long term weathering characteristic of plastic, especially poly-olefin such as Chimosorb 944, that is one of commonly used of light stabilizer. Slip additives are added to plastic formulation for useful properties, prevention of film sticking together, and reduction of static charge. Fatty acid amides are slip additives most used in plastic polymeric packaging. Anti-oxidants are also added to stabilize the polymer by preferently degrading themselves from oxidation process. Inganox 1010, Tanuvan P or Irgafos 168 are some types of anti-oxidants (Lau and Wong, 2000).

Benzene and other volatile compounds including 1,2-dichloroethane are used in polymeric packaging materials. 1,2-Dichloroethane is used as a dispersant, wetting agent for clear plastic film and added in PVC cleaning process (EHC 176, 1995). These are the chemical agents in packaging material that can consequently penetrate and migrate into food that comes into contact.

For food safety and health effect of human, 1, 2-dichloroethane that may be contaminated in packaged foods should be evaluated.

1.2 Background

1.2.1 Identity

The empirical formula for 1,2-dichloroethane (ethylene dichloride) is $C_2H_4Cl_2$ and the molecular structure is as in Figure 1.

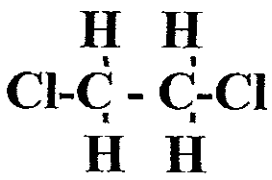

Figure 1 Molecular structure of 1,2-dichloroethane (DCE)

1.2.2 Common name , synonyme and trade name

The common name of 1,2-DCE is 1, 2-Dichloroethane (DCE) and the synonyme of DCE are 1,2-bichloroethane, 1,2-ethylene dichloride, acethylenchloride, alpha, beta-dichloroethane, glycol dichloride, ethylene chloride and sym-dichlorothane. Trade name including Borer sol, Brocide, Destruxo, Dutch liquid, Dutch oil, Gaze Olefiant and Granosan (which also contains carbon tetrachloride).

The Chemical Abstract Service (CAS) registry number for 1,2-Dichloroethane is 107-06-2.

1.2.3 Physical, and Chemical properties

1,2-Dichloroethane is a synthetic chemical that is not found naturally in the environment. It is a clear, colourless, liquid at room temperature and a highly volatile and flammable synthetic chemical which absorbs infrared light at several wavelengths (7, 12 and 13 μm). Other properties of 1, 2-Dichloroethane are presented in Table 1.

Table 1 Physical properties of 1,2-Dichloroethane

Properties	
Physical state	liquid
Colour	colourless
Odour	Sweet, chloroform-like
Relative molecular mass	98.96
Density d^{20}	1.253
Reflective index r^{20}	1.4449
Boiling point	83°C
Melting point	-35°C
Water solubility	8690 mg/litre (20°C)
Vapour pressure	8.5 kPa (20°C)
Log octanol/water partition coefficient	1.76
Log octanol/chloroform partition coefficient	1.28
Henry's law constant	111.5 $Pa.m^3/mol$ (25°C)
Flash point	12-15°C

Source: International Programme on Chemical Safety, 1995

1.2.4 Uses

1,2-Dichloroethane is a primary used in the production of vinyl chloride monomer as well as other chemicals. It is used in solvent in closed system for variously extraction and cleaning purpose in organic synthesis. It is also added to leaded gasoline as a lead scavanger. 1,2-Dichloroethane is also used as a dispersant in plastic and rubber, as a wetting and penetrating agent. It was formerly used in ore flotation and in textile and PVC cleaning (EHC176, 1995; ATSDR-ToxFAQs, 1995).

1.2.5 Source and Potential exposure

1,2-Dichloroethane (DCE) is a synthetic chemical and is not found in natural source. It is a chlorinated aliphatic hydrocarbon, one of several halogenated solvents. 1,2-Dichloroethane can be released to environment from the production facilities that occurred from a number of sources; vinyl production, monomer production, solvents, process of PVC production, and lead scavanger in gasoline, fumigant and contamination during processing. 1, 2-Dichloroethane is recovered from waste stream of manufacturing facilities in a two-stage distillation operation. In addition, it may release from packaging into food. It has also been in the production of chlorinated solvents such as trichloroethane, tetrachloroethylene, 1,1,1-trichloroethane and vinyl chloride, and in the manufacture of anti-knock fluids containing tetraethylead, although this latter use has declined with phase-out of Lead-petrol. 1,2-Dichloroethane has also been used as fumigant. However, it is no longer registered for use on agricultural product. 1,2-Dichloroethane could be used in the medical and health services, automotive dealers and service stations, machinery, printing and publishing, eating and drink places, chemical and allied products and miscellaneous business service.

The primary routes of human exposure potential to 1,2-dichloroethane are inhalation, and dermal contact. The greatest source of exposure to 1,2-dichloroethane for most of the U.S. population are by inhalation of the contaminated air, since releases of 1,2-dichloroethane to the environment as a result of industrial activity are expected to be primary to the atmosphere. Emission to the atmosphere comprised the largest component of all release of 1,2-dichloroethane to the environment. 1,2-Dichloroethane released to the atmosphere may be transported long distances before being washed out of precipitation. 1,2-Dichloroethane has been detected in not only ambient urban and rural air and indoor samples of residence located near hazardous waste disposal sites but also in surface water, ground water, and

drinking water (ATSDR, 1994-R-043). EPA reported that 1,2-dichloroethane was present at concentration of 1-90 ppb in 53 of 204 surface water samples taken near heavily industrialized area. Drinking water samples from urban and rural located in USA have been report to be contaminated with 1,2-dichloroethane. Concentration in domestic surface waters used as drinking water source have been reported to range from trace amount to 400 μg/L. Exposure of 1,2-dichloroethane though ingestion of contaminated drinking water is expected to be important source for only 4-5% of the population. However, for populations with drinking water supplies containing more than 6 μg/L of the compound, this route is expected to be more important than inhalation. 1,2-Dichloroethane has also been detected in food items and in human breath, urine, and milk (ATSDR, 1994-R043).

1.2.6 Health effect

The effects of 1,2-dichloroethane on humans are similar for ingestion, inhalation, and skin adsorption. Acute exposures result in nausea, vomiting, dizziness, internal bleeding, bluish-purple discoloration of the mucous membranes and skin, rapid but weak pulse, and unconsciousness. There is no definitive evidence that dichloroethane causes cancer in humans (OSHA, 1979).

Acute (short-term) inhalation exposure of humans to 1,2-dichloroethane can induce neurotoxic, nephrotoxic, and hepatotoxic effects, as well as respiratory distress, cardiac arrhythmia, nausea, and vomiting. Similar effects have been reported in animals exposed by inhalation. Clouding of the cornea and eye irritation have been observed in humans and animals.

Chronic (long-term) inhalation exposure to 1, 2-dichloroethane produced effects on the liver and kidneys in animals. Some studies have reported change in the liver and kidney and effects to the immune and central nervous systems (CNS) in animals chronically exposed by ingestion.

Epidemiological studies are not conclusive regarding the carcinogen effect of 1,2-dichloroethane, due to concomitant exposure to other chemicals. Following treatment by gavage (experimentally placing chemical in the stomach), several tumor types were induced in rat and mice. An increased incidence of lung papillomas has been reported in mice after topical application. EPA has classified 1,2-dichloroethane as a Group B2, probable human carcinogen of low carcinogenic hazard (U.S. EPA, 2002).

1.2.7 Analytical methods

Analytical methods are important because they are the keys in studying the migration of packaging components from the package or food-contact material into food. The methods are also required by regulatory who response for ensuring public safety by monitoring food, for harmful levels of contaminant and/or required to evaluate changing residue level as well as calculated dietary intake. The analytical procedures typically involve sample preparation, extraction, clean up and a final determination step. The analytical methods have been used and applied to determine different types of packaging migrations, group of plastic additives and other contaminants migrated into food (Lau and Wong, 2000).

1,2-Dichloroethane is a highly volatile organic compound that is present in trace amount. 1,2-Dichloroethane was contaminated in many media of environment such as soil, air, drinking water, groundwater, and food. Different analytical approaches have been applied, including sampling and sample preparation *i.e.* solvent extraction, distillation/extraction, and vacuum distillation. Most of these methods are time consuming, expensive, and required considerable sample preparation and exposure to high temperature.

In this work, contamination of 1,2-dichloroethane in packaged food was investigating, since the isolation of this compound from food composition

depended on the type of matrix, there is a need for a rapid and simple analytical procedure that could provide the analysis of these volatile composition.

Gas chromatography (GC) is a popular analytical method, for the measurement of chemical contaminants and additives. Typically, Gas chromatography is useful for analyzing non-polar and semi-polar, volatile and semi-volatile chemical without chemical derivertization. Gas chromatography is often used for analysis of sterols, oils, aromatic components and many contaminants, such as industrial pollutants and certain types of drugs in foods (Lehotay *et al.*, 2002). Gas chromatography is also the method of choice for analysis of any volatile component in food. Two of the most used procedures for volatile compounds sampling.

1) Static headspace sampling. The sample is placed in a glass vessel that is sealed with septum and aluminum crimp cap. After heating for a given period of time at moderated temperature (60-80°C) a volume of vapour phase, in equilibrium with the solid or aqueous phase, is injected into Gas chromatograph system (Grob, 1995, Dewulf *et al.*, 2002, Snow and Slack, 2002).

2) Dynamic headspace sampling (purge-and-trap). The sample is placed in the purge vessel. The volatile component is purged off by inert gas flow and trapped on a solid sorbent. Ballistic heating of the trap desorbs the volatile compounds which are then carried to gas chromatograph (Santos *et al.*, 2001, Snow and Slack, 2002,.Sides *et al.*, 2000)

Analytical method for 1,2-dichloroethane in various environmental media is described in Table 2. Gas chromatography coupled with electron capture or flame ionization detection or mass spectrometry, are commonly used for analysis of 1,2-dichloroethane in most media (EHC 176, 1995).

Table 2 Levels of 1,2-dichloroethane in some environmental media.

Media	Location	Year	Concentrations
Ambient air	Canade	1988-1990	0.07-0.28 μg/m^3
Ambient air	Japan	1992	<0.004-3.8 μg/m^3
Indoor air	Canada	1991	<0.1 μg/m^3
Drinking water	Canada	1990	<0.2 μg/littre
Surface water	Japan	1992	0.01-3.4 μg/littre
Food (34 group)	Canada	1992	<5 μg/kg (solids)
			<1 μg/kg (liquids)
Food (19 items)	USA	Not specified	<9-30μg/kg

Source: Concise international chemical assessment document no.1, 1998.

In this work, Gas chromatography coupled with electron capture detector and static headspace sampling method were used for determination of 1,2-dichloroethane contaminated in packaged food.

1.3 Literature reviews

In recent year, more interest in migration of contaminant from container into food are focusing on packaging material that are contact with food. The additives in packaging, especially plasticizers, are most of substances used in polymeric processing. In 1999, Balafas *et al.* reported the determination of phthalate and adipate esters in Australian packaging materials. The amount of phthalates in packaged foods depended on many factors including the concentration of phthalates in packaging material or printing ink, the storage period, the storage temperature, the fat content in the food and the contact area. The 136 packaging material samples were divided into the eight groups *i.e.* dairy packaging, baked goods packaging, beverage packaging, breakfast cereal

packaging, confectionary packaging, pasta packaging, and miscellaneous packaging. All samples of packaging materials were extracted with mixed solvent of chloroform and methanol at a ratio of 2:1 for 6 hours, by Soxhlet apparatus, the condensers were connected to cool water system for ensuring no loss of volatile. The extracts were transferred and analyzed by gas

chromatograph (GC) detected with mass selective detector that operated in the multiple ion detection (MID) mode. The HP-5MS capillary column was used and the GC column was programmed as initial temperature 70°C for 1 min, heated at 20°C/min to 280°C for 10 min, with the injector temperature of 250°C. The concentration of plasticizers was calculated by calibration curve. A total of packaging materials presented six phthalate esters *i. e.* di-2-ethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), dioctyl phthalate (DOP), diethyl phthalate (DEP), and dimethyl phthalate (DMP) and one adipate ester, di-2-ethylhexyl adipate (DEHA). The concentration of DEHP and DBP was found higher than limitation of packaging in food. The highest concentrations of plasticizers were detected in printed polyethylene materials that plasticizers migrated from printed inks or contacted with other components during production and transportation.

Apart from plastics, paper is one of the most important packaging materials. Paper and cardboard have been used both as packaging for a wide range of food. As public interest in conservation of natural resource increases there are more use of recycled paper and board. Many contaminants in recycle fibers were detected including PCDD, PCDF, aldehydes, alkanes, ketones, phthalates, hydrocarbons and trace elements. In 2002, Triantafyllou *et al.* developed an analytical method for rapid testing at this migration. The concentrations and migration were studied by model method. The paper strips was used for mixture contaminants model. Their sorptions were measured fiber material and evaluated the migration potential of these compounds by studying the migration behavior of them onto Tenex. The selected 12 model compounds

(surrogates) *i.e.* σ-xylene, acetophenone, benzoic acid, dodecane, naphthalene, vanillin, diphenyl oxide, 2,3,4-tirchloroanisole (2,3,4-TCA), benzophenone, diisopropylnaphthalene (DIPN), dibutyl phthalate (DBP) and methyl stearate were mixed. Two types of paper were used. The papers were immerged in the mixture until saturated then dried and extracted with ethanol to evaluate the concentration and migration. For migration behavior, the papers that were saturated with standard were placed the vials, lied in horizontally, and cover with Tenex powder. The vials were heated at a range of temperature and time and analyzed with gas chromatograph equipped with mass spectrometry and gas chromatograph combined with flame ionization detector. The solvent extraction was used to determine the migration of contaminants in recycle paperboard. The large amount of difference sorption of contaminants of both paper types was observed under the same sorption conditions. For less polar compounds low sorption levels were detected and for more polar high sorption levels into paper matrix were found. For migration behavior, the amount of migration was depended on many factors, such as the nature of the test sample, the chemical nature of the test sample, and temperature conditions.

In addition to plasticisers, anti-oxidants are also used in polymeric processing. For example, Butylated hydroxytoluene (BHT) is used in plastics. BHT can appear in food from its use as additives in food and beverages and/or from its use as additive in plastic containers that migrates to its content. Potential increases of daily ingestion of BHT is from bottled water due to its migration from the plastic package into the water. Preconcentration and clean up method that use solid phase microextraction (SPME) was used in the analysis for VOCs in water and air samples. In 2002, Tombesi *et al.* applied the SPME combined with gas chromatography-mass spectrometry to determine the antioxidant in bottled drinking water. The SPME fiber was 100 μm polydimethylsiloxane (PDMS) film in a manual holder. The fiber was exposed to 15 ml of the sample and stirred with magnetic bar 2000 rpm. The extraction

was performed at room temperature (20-25°C). The limit of detection (LOD) was 4.2 μg L^{-1} (3 times the standard deviation of the intercept divided by the slope).

The analyses of volatile organic compounds (VOCs) are important because they can be responsible for health hazard or affect atmosphere. Because of the low concentration level of VOCs such as benzene, toluene, xylene and 1,2-dichloroethane in environmental samples, a preconcentration step is necessary for analysis and detection and a number of techniques have been used to determine them. VOCs are most commonly analysed with dynamic headspace or purge-and-trap (PT) technique and static headspace (SH) technique (Roos and Brinkman, 1998). The advantages of static headspace are rapid, solvent-free, and simple (Snow and Slack, 2002). The purge-and-trap (PT) technique is less matrix dependent and provided a low limit of detection (LOD) 10 to 100 times lower than the static headspace technique (Huybrechts *et al.*, 2000).

For dynamic headspace technique, VOCs are forced out of the matrix by heating under a flow or purging with an inert gas. The analytes are trapped for analysis using cyogenic traps, or sorbent traps (Kuo *et al.*, 1997 and Triantafyllpu *et al.*, 2002). The trapped are then desorbed at room temperature or by heating rapidly into an analysis system. The common techniques of analysis is gas chromatography with flame ionization detector (GC-FID) (Sech *et al.*, 1994 and Tudini *et al.*, 2002), gas chromatography with electron capture detector (GC-ECD) (Wolska *et al.*, 1998), or gas chromatography with mass spectrometry (GC-MS) (Kuo *et al.*, 1997 and Peres *et al.*, 2000).

In 2001, Sukphung *et.al.* determined the volatile amines *i.e.* dimethylamine and trimethylamine in prawn and fishes by HS-GC-ECD. The gas solid chromatography (GSC) and gas liquid chromatography (GLC) techniques were used in this work. Chromosorb 103 was used as gas-solid stationary phase, while the gas-liquid phase was 4% Carbowax

20M/0.8%/KOH on Carbopack B. This method could detect trimethylamine and dimethylamine level by using a silicar gel as sorbent for analyte adsorption in headspace system.

In 1997, Kuo *et al.* reported VOCs determination in drinking water, focused on Taiwan three major metropolitan areas, Taipei, Taichung and Kaohsiung. The US EPA method 524.2 was used for determining 171 tap water samples, and 68 boiling water samples. Purge-and-Trap was used as sampling technique. Helium was used as purging gas and the analytes were trapped on sorbent, Tenex /Silica gel and Charcoal. VOCs, chlorinated aliphatic and aromatic compounds and aromatic hydrocarbons were analyzed by gas chromatography with mass spectrometry (GC-MS). The capillary column was DB-5 (30m x 0.25mm x 0.25μm thickness). GC programmed temperature conditions were set at initial temperature 35°C for 3 min, increased at ramp rate 4°C/min, and the final was 100°C. Drinking water showed high concentration of trihalomethanes (THMs) where Chloroform was the highest. For treatment method, it was found that 61-82% of all THMs removed by boiling, but not 1,2-dichloroethane and toluene.

Trace THM in drinking water was also analyzed by HS-GC as reported by Kanatharana *et al.* (2003). In this work, a packed column with 0.1% SP-1000 on Carbopack C 80/100 mesh was used for determining THM in water samples in Hat Yai city, Thailand. The limit of detection was obtained at a very low level of 0.3 ppb and the linearity was in the range of 1 to 100 ppb. The residue of THM in tap water was higher than bottled drinking water.

In 1998, Roos and Brinkman developed method to determine VOCs in marine biota. This method simultaneously detected 11 volatiles by GC-MS, *i.e.* Chloroform, tetrachloromethane, 1,1-dichloroethane, 1,2-dichloroethane, trihalomethane and tetrachlorothene and the volatile aromatics *i.e.* benzene, toluene, ethylbenzene and the xylene (BTEX). VOCs in fish samples placed in a vial were forced out by purging at 70°C and trapped on multisorbent tube

which was Vocarb 4000 occurred in sorbents 8-5 cm Carbopack C, 10 cm Carbopack B, 6 cm Carboxen 1000 and 1 cm Carboxen 1001. After purging, the trapped was backflushed while rapidly heated to 250°C. The desorbed analytes were then trapped in a cyogenic focuser at –120°C that was connected to the analytical column, RTX-502.2. The analytes were transferred into the column by rapid heating from –120°C to 200°C in 0.75 min. The method allowed low detection limit at concentration level at 0.005 ng/g of 1,1-dichloroethane, 1,2-dichloroethane and tetrachloroethane and 0.12 ng/g of Chloroform. These were lower than several methods that were determined only halogenated volatile organic compounds. Benzene Toluene and Xylene (BTX) in water were also reported by Kanatharana *et al.*(2003). A lab built purge and trap, and static headspace (HS) together with solid-phase micro-extraction techniques (SPME) were used for sampling and pre-concentration methods where Tenex TA was used as trapping sorbent. The developed method showed high selectivity with a low level (ppb) limit of detection and a wide range of linearity at 2-1000 ppm.

In another report, Hino *et al.*(1998) detected trace amount of very volatile organic compounds (VVOCs) using a large amount of headspace gas phase. The whole headspace gas injection method (WHSI) is put a whole headspace gas equilibrated in a vial and carried out with helium into the micro-trap. The micro-trap (11 cm) in their work was smaller than the US EPA method tube (at least 25 cm). The headspace was short purging time on Tenex TA for 15 second, and cooled to –30±2°C for ensuring no loss of VVOCs. The trap was immediately heated at 230°C for 2 minutes and the analytes were introduced to GC-MS. For this method a headspace purging time was only 15 seconds and the cooling of the trap at –30°C to prevent break-through was successful since only a small breakthrough was detected.

In the same year, 1998, Wolska *et al.* reported the method to determine volatile and semi-volatile compounds in marine water. This method was

simultaneous detected polar and semi-polar organic compounds by gas chromatograph with a cold on column injector and a nickle-63 electron capture detector. The direct aqueous injection (DAI) was used with a programmed temperature. RTX-624 with 6%cyanopropyl-94%dimethyl polysiloxane as capillary column was used for analysis. All twelve compounds were well separated.

Later, in the year 2000, the method to determine volatile and semi-volatile organic compounds in marine water was also developed by Huybrechts *et al.* This method could detect 27 VOCs included chlorinated alkanes and alkenes, monocyclic aromatic hydrocarbons and chlorinated monocyclic aromatic hydrocarbons at low ng/L concentration level while previous investigation can only 13 VOCs in marine water using GC-MS (Dewulf *et al.*,1995).

Recent advance in sample preparation for trace analysis is solid-phase microextraction (SPME) technique. This solvent-free extraction technique was developed in 1989 by Pawliszyn. The analytes are adsorbed directly from an aqueous or gaseous phase onto a fused-silica fiber coated with a polymeric phase. Hence sampling, extraction and concentration are achieved in a single step (Llompart *et al.*, 1999). SPME has become very popular in the last 2-3 years, especially in environmental analysis. Most SPME method developed until now are used in combination with gas chromatography, several coating is commercially available. The choice of a particular coating is chemical structure dependent (Alpendurada, 2000). A great number of application of SPME can be found in the environmental field such as air (Tuduri *et al.*, 2002), surface and groundwater (Kanatharana *et al.*, 2003, Klecka *et al.*, 1998 and Ketola et al., 1997), sea water (Castells *et al.*, 2003 and Masque *et al.*, 1998), soil (Llompart *et al.*, 1999) and food (Jaillais, 1999).

1,2-Dichloroethane was not detected in any samples of food in Canada that repeated analysis in 1991 and 1992. In U.S.A. was not found in 11 coffees and only one detected in ready-to-eat cereal (0.31 μg/kg) out of 19 table-ready

foods items. For food safety and health effect to human, 1,2-dichloroethane would be evaluated that might contaminate in packaged foods (CICADS, 1998). In Germany, the Federal Republic was reported the level of DCE concentration in milk products with added fruits at low concentration level 0.8 μg/kg. The reports on 1,2-dichloroethane residues in food are scarced (WHO, 2000). Most reports were for residues found when the compound has been used as an extractant or fumigant. However, 1,2-dichloroethane that can be migrate from packaging material into food are not reported. The aim of this is work to develop the sample preparation and analysis method of the 1,2-dichloroethane contamination in packaged food method.

1.4 Objectives

1.4.1 To develop the sample preparation technique, static headspace sampling, and compare between the lab-built and the automatic system and optimized the gas chromatographic with electron capture detector conditions.

1.4.2 Qualitative and quantitative analysis of trace 1,2-dichloroethane in packaging food.

Chapter 2

EXPERIMENTAL

2.1 Chemicals

2.1.1 1,2-Dichloroethane, DCE (purity > 99%, GC Grade, Fluka, Switzerland)

2.1.2 Methanol (AR Grade, Merck, USA)

2.1.3 Ultra pure water (Synthesis on Laboratory by Maxima, ELGA, England)

2.1.4 0.1% SP-1000 coated on carbopack C, 80/100 mesh (Supelco, USA)

2.1.5 Oxygen free nitrogen (99.99% purity)

2.1.6 Glass wool

2.2 Instruments and Apparatus

2.2.1 Shimadzu GC-14B Gas Chromatograph equipped with Electron Capture Detector and data processor model C-R7A chromatopac (Shimadzu, Japan)

2.2.2 Empty glass column, 1.8m x 2mm i.d.(Supelco)

2.2.3 Gastight syringe, 1 mL (Hamiton, Supelco, USA)

2.2.4 Syringe cleaner (Hamilton, Switzerland)

2.2.5 Stainless steel water bath with thermostate (Gallenkamb, UK)

2.2.6 Thermometer

2.2.7 Microlitter pipette (Gilson, France) and tips

2.2.8 Analytical balance (Denver Instrument Company, USA)

2.2.9 Ring stand and clamp utility

2.2.10 Crimp and decrimp (Shimadzu, Japan)

2.2.11 Propylene rubber

2.2.12 Aluminum cap

2.3 Glassware

2.3.1 Volumetric flask 10, 25, 50, 100, and 250 mL (Pyrex, USA)

2.3.2 Headspace vial, 27 mL (Shimadzu, Japan) with silicone septa and aluminum crimp cap

2.3.3 Beaker 50, 100, 250, 500, 1000 mL (Pyrex, USA)

2.3.4 Measurement pipette 1, 5, 10, 25 mL(Pyrex, USA)

2.3.5 Spetula and spoon

2.4 Methodology

2.4.1 Preparation of standard and aqueous solutions

2.4.1.1 The DCE standard stock solutions 1000 μg mL^{-1}

Standard stock solution of DCE was prepared with diluting an accurate volume of 21 μL DCE (with a microliter pipette) by methaol in a 25 mL volumetric flask. This was transferred to a glass bottle, sealed with PTFE-lined lid and stored at 4°C.

2.4.1.2 The DCE standard solutions

Standard working solution of DCE was prepared in methanol to obtain the concentrations over the range to 0.5 μg mL^{-1}.

2.4.1.3 The working standard solution

The working standard was prepared by pipetting a stock standard solution and spiked into ultra pure water to obtain the solution with a concentration of 0.5 μg mL^{-1}. The solution was used for testing optimum conditions.

2.4.2 Preparation of Packed column

The heart of a Gas Chromatograph is the column. In this studies, the packed column consisted of (1) the stationary phase: 0.1% SP-1000 on Carbopack C, 80/100 mesh (2) empty clean glass column, 1.8m x 2mm i.d. and (3) glass wool plugs as the packing retainer.

The clean column was filled with 0.1%SP-1000 on Carbopack C, 80/100 mesh packing material with appropriated tightness, that is, without fracturing or deforming of the particles. These are described as the followed:

Column preparation, an empty glass column 2mm i. d. x 1.8m was thoroughly cleaned with diluted acid, 0.5% sulfuric acid, and rinsed with distilled water to eliminate acid residues. Then, flushed with methanol, this served to replace –Cl with $-OCH_3$ and also wash out any HCl formed (Supina, 1974). Finally the column was purged with dry nitrogen.

Packing the column: A plug of glass wool was inserted into the end of the column intended for connecting to a detector. A rubber tubing was slipped over the end of column and was connected to a vacuum pump. A funnel was connected to the inlet end of the column and the stationary phase, 0.1%SP-1000, 80/100 mesh, was slowly added while the pump was applied at the other end. Carbopack C packing material has a high density and this can create back pressure. Caution must be taken account while packing the column. This was done as follows:

Connected the column to a vacuum pump and attach a funnel to the column inlet. Filled the funnel with an appropriate amount of stationary phase (Table 1). Moderately vibrated the column, starting the exit and slowly moved towards the inlet until the packing ceased to flow. Removed the vacuum pump hose and vibrated the column until the packing level dropped at the exit end. Reconnected the column to the vacuum pump. Capped the inlet and again vibrated the column, from the exit to the inlet until the packing stop flowing. Added enough additional stationary phase until it reached a proper inlet level, then slightly vibrated the column to settle the packing. Removed the vacuum hose and inserted the glass wool plug.

Table 3 Approximate Packing weight/Foot

Packing	1/8", SS	1/4", SS	2mm, Glass	4mm, Glass
Carbopack B	0.6g/ft.	2.7g/ft.	0.5 g/ft.	1.6 g/ft.
Carbopack C	1.0g/ft.	5.8g/ft.	0.8 g/ft.	3.3 g/ft.
Carbopack F*	0.8g/ft.	3.3g/ft.	0.7 g/ft.	2.8 g/ft.

*Carbopack F is available in packed column only.

SS = Stainless steel column, Glass = Glass column

ft = column length, foot unit

Column conditioning: The packed column must be conditioned before connecting to a detector, to purge all volatile compound components that would foul the detector and produce unsteady baseline and noise. For most column, heating the column overnight by flowing carrier gas at a temperature slightly above the propose operating temperature for the specific kind of packing. For most purpose, it is succificient to program from room temperature

upto 200 or 300°C by slowly changing the temperature. It would be better to raise the column from the low temperature to condition by temperature over period of several hours (Supina, 1974).

The packed column, 2mm i.d. x 1.8m with 0.1%SP-1000, on Carbopack C 80/100 mesh was conditioned by connecting the inlet end of the column to the injector side of Gas Chromatograph while the detector side was disconnected. The temperature program was set as: initial column temperature 50°C and hold for one hour, then ramped to 210°C with a ramp rate of 5°C/min and hold on the final temperature for 16 hours. The injector temperature was set at 170°C and the carrier gas flow rate was maintained at 20mL/min during conditioning (data sheet of SP-1000 packing material, Supelco).

2.4.3 Optimization of the GC-ECD conditions for DCE analysis

The optimum conditions of Gas Chromatography (GC) were based on various parameters *i.e.* the carrier gas flow rate, column temperature, injector temperature, and the detector temperature. In the studies of parameters working standard solution of DCE 0.5 μg mL^{-1}, 10 mL, in a sealed vial was placed in the lab-built water bath at 70°C. After 10 minutes, a gas-tight syringe was used to transfer the vapor phase and injected into gas chromatograph for analysis.

2.4.3.1 Carrier gas flow rate.

A 0.5 mL vapor phase of DCE working standard solution was injected into the GC. Other parameters were set as the recommended by Shimadzu GC-14B instrument manual. The temperature of column, injector and detector were isothermal *i.e.* 80°C, 190°C, and 260°C respectively. The optimum carrier gas flow rates was obtained by varied the nitrogen, flow rate at 10, 20, 30, and 40 mL min^{-1}. Five replications for each flow rate. The height equivalent to the theoretical plate (HETP) was determined from the

chromatogram response, retention time and the height at half width. A van-Deemter plot provided the optimum flow rate, at the lowest HETP in the van-Deemter plot.

2.4.3.2 Optimization of column temperature

The optimum column temperature is the temperature that increased detectability, sample throughput and reduced the operating time. The isothermal mode was used for the column temperature. The optimum was determined by varying the column temperature at 70, 80, 90, and 100°C. Five replications at each temperature were analyzed. Other conditions of gas chromatograph, were maintained as: injector temperature 190°C, detector temperature 260°C, and flow rate of carrier gas (N_2) 20 mL/min. The headspace conditions were, equilibration temperature 70°C, equilibration time 10 minutes, and volume of standard was 15 mL.

2.4.3.3 Optimization of injector temperature

The flow rate of carrier gas and column temperature were set from the results of 2.4.3.1 and 2.4.3.2. The optimization of injector temperature was investigated by varying the temperature from 90°C to 200°C at an increment of 10°C. Five replications at each temperature were analyzed. The temperature that gave the highest detectable response was the optimize injector temperature.

2.4.3.4 Optimization of detector temperature

The optimum detector temperature was determined by varying temperature of the detector at 200, 225, 250, 260, 270, 280 and 290°C

The other conditions of gas chromatograph were as 2.4.3.1-2.4.3.3 and the headspace conditions were set as in 2.4.3.2.

2.4.4 Optimization of headspace conditions

The responses of headspace technique depended on equilibration time, equilibration temperature, sample volume (phase ratio) and size of vial volume. In this study, the results from two headspace system, a lab-built water bath and an automate incubator, were compared. Autosampler vials with a volume of 27 mL were used. The analysis was done using the optimum conditions of gas chromatograph with electron capture detector (GC-ECD) were from experiment 2.4.3 as shown in Table 4.

Table 4 The GC-ECD conditions for the optimization of headspace conditions

Gas Chromatograph	Shimadzu GC-14B equipped with Electron Capture Detector and C-R7A Chromatopac recorder
Column	Glass column (2mm i. d x 1.8m, 0.1% SP-1000 on Carbopack C, 80/100mesh
Flow rate of carrier gas	20 ml/min
Column temperature	90°C
Injector temperature	170°C
Detector temperature	280°C
Injection volume (gas phase)	0.5 mL

2.4.4.1 Optimization of equilibration time

DCE working standard solution, 0.5 μg mL^{-1} 10 mL, was pipetted into 27 mL headspace vials. They were placed in a lab-built water bath at 70°C (Figure 2) for different period of time *i.e.* 5, 10, 15, 20 and 25 minutes. After each equilibration time was reached, 0.50 mL of the gas phase was taken from the headspace by a gas tight syringe and injected to the GC system operated at optimum conditions in Table 4. The peak area of the responses were plotted against the equilibration time. The equilibrium time was obtained as the time that the response was constant.

For the automatic headspace system (Figure 3), after the DCE working standard solution was filled into the headspace vials, the incubation times was set by as in the manual technique above.

2.4.4.2 Optimization of equilibration temperature

The vial with 10 mL of 0.5 μg mL^{-1} of DCE working standard solution was placed into the lab-built water bath for 10 minutes (2.4.4.1), at 50, 60, 70, 80 and 90°C respectively. Then, 0.50 mL gas from the headspace was transferred into GC system.

The optimum equilibration temperature was considered from plot of the responses (peak area) versus the temperatures. The equilibrate temperature was selected at the temperature that gave the highest response and low relative standard deviation (five replications for each temperature. The same procedures were also carried out using the automatic system.

2.4.4.3 Optimization of phase ratio

The phase ratio is the rate constant of the sample phase and gas phase that consist above the sample. In other word is also called sample volume that is the equilibrated partition of two haterogeneous phase between sample volume and vapor volume above sample. The best phase ratio

showed provide a high response and good precision. The volume of 0.5 μg mL^{-1} standard DCE solution, was varied at 5, 10, 15, and 20 mL for the 27 mL vial. Other conditions of the headspace were from the result in 2.4.4.1 and 2.4.4.2. The optimization was carried out for both the lab-built thermal bath and the automatic system.

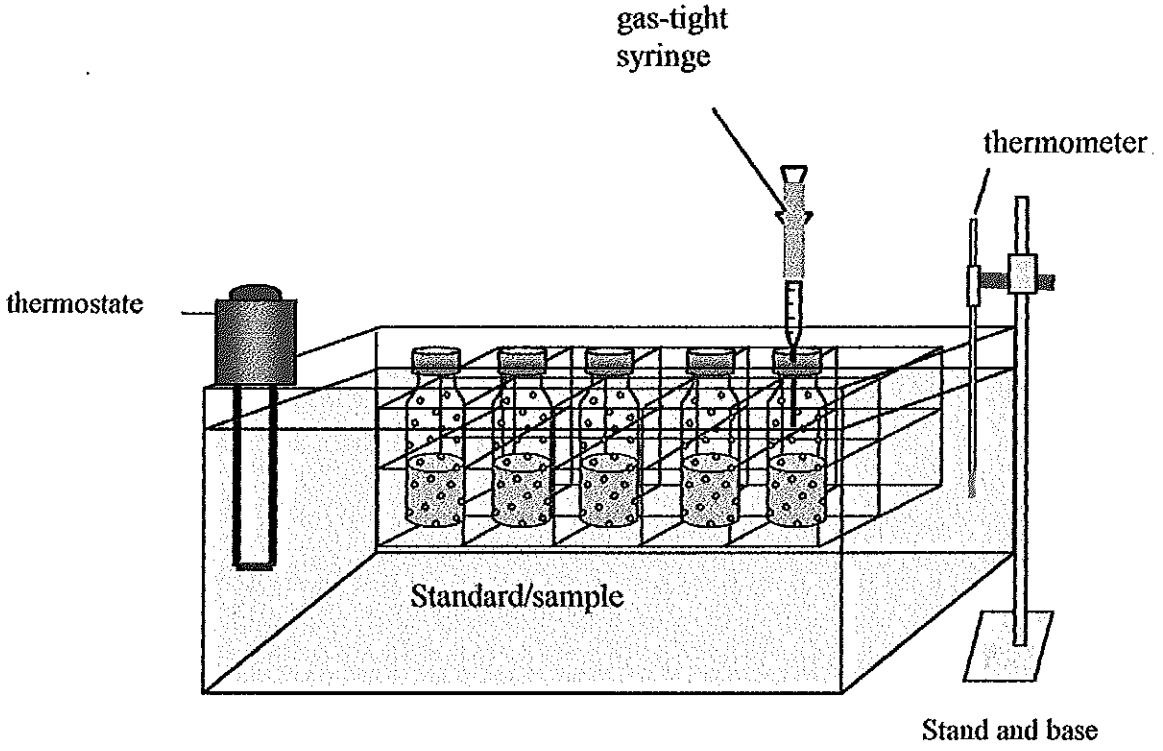

Figure 2 Lab-built water thermal system

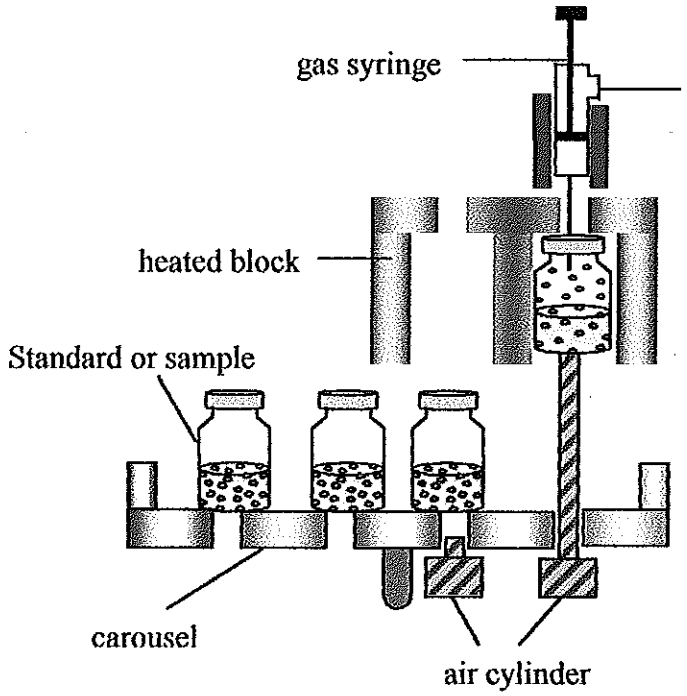

Figure 3 Automatic headspace system

2.4.5 Linear dynamic range

DCE standard solution, 0.5 to 1000 ng mL^{-1} were diluted from the stock standard solution, 1000 µg mL^{-1} with methanol and ultra pure water in the headspace vials with sealed cap. A 0.50 mL gas phase of each standard solution was injected into gas chromatograph set at the optimum conditions for GC-ECD and headspace system obtained from 2.4.3.1 to 2.4.4.4 as shown in Table 3. Five replications were done for all analysis.

The linear dynamic range obtained from plotting the peak area versus the concentration. The linearity of the responses was identified by considering the correlative coefficient.

Table 5 Conditions of GC-ECD and headspace system for determining linear dynamic range of DCE standard solution

Gas Chromatograph	Shimadzu GC-14B equipped with Electron Capture Detector and C-R7A Chromatopac recorder
Column	Glass column (2mm i. d x 1.8m, 0.1% SP-1000 on Carbopack C, 80/100mesh
Flow rate of carrier gas	20 ml/min
Column temperature	90°C
Injector temperature	170°C
Detector temperature	280°C
Equilibration time	10 minutes
Equilibration temperature	80
Phase ratio	0.55
Vial volume	27 mL

2.4.6 Limit of detection

The limit of detection is the lowest concentration of the analyte that an analytical process can be reliable detect (ACS definition). In this study, the determination of limit of detection was based on IUPAC definition.

From IUPAC defined the limit of detection as a concentration, C_L (or amount, q_L) derived from the smallest, X_L, that can be detected with reasonable certainly for a given analytical procedure (Long and

Winefordner, 1983). The limiting detection was determined by measuring blank response (X_B), mixture of methanol and ultra pure water, more than 20 times (n_B). A mean value of the blank response, $\bar{X}_B$, can be calculated as

$$\bar{X}_B = \sum_{j=1}^{n_B} X_{Bj} \qquad \text{.....(1)}$$

and standard deviation as

$$S_B^2 = \frac{\sum_{j=1}^{n_B} \left(X_{Bj} - \bar{X}_B \right)^2}{(n_B - 1)} \qquad \text{.....(2)}$$

In defining X_L, IUPAC state that

$$X_L = \bar{X}_B + kS_B \qquad \text{.....(3)}$$

Where k is a number factor chosen in accordance with the confidence limit desired. The C_L is a function of X_L and can be calculated as

$$C_L = \frac{\left(X_L - \bar{X}_B \right)}{m} \qquad \text{.....(4)}$$

Where m is the analytical sensitivity. Because the mean blank reading, $\bar{X}_B$, is not always 0, the signal must be background corrected. By substituting Equation 3 into 4, Equation 5 in obtained

$$C_L = \frac{kS_B}{m} \qquad \text{.....(5)}$$

This definition of C_L can be illustrated as shown in Figure 3. The limit of detection is found by relating kS_B to a concentration value by dividing by slope of calibration curve obtained from the linear regression analysis.

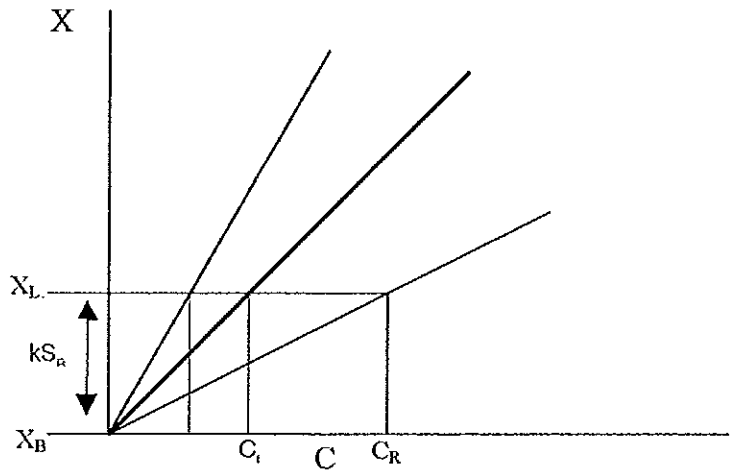

Figure 4 Analytical calibration curve of signal, X, concentration, showing the relationship of kS_B to the limit of detection, C_L (Long and Winefordner, 1983).

2.5 Sample Analysis

A static headspace technique combined to Gas Chromatography with electron capture detector was used for qualitative and quantitative analysis of chlorinated organic compounds, DCE, contaminated in packaged food. The conditions of GC-ECD and headspace system were set at their optimum conditions as investigated in 2.4.3.1 to 2.4.4.3.

2.5.1 Sampling

The samples were packaged food are well packed, *i.e.*, by laminar film sheet and/or contained in plastic box. Packaged food was sampling from supermarkets in Hat Yai district. A variety of packaged food were collected *i.e.* curry paste, ready to eat curry, milk, and other packaged food. All samples, were stored at 4°C and except ready to eat curry which was frozen. These are summaried in Table 3.

Table 6 Packaged food samples for DCE determination

Sample	**Number of variety**	**Number of brand**	**Total samples**
Curry paste	10	7	22
Salted pork	2	2	2
Milk	3	7	7
Curry ready to eat	6	3	6
Total samples	21	19	37

2.5.2 Sample preparation

Different type of packaged food sample contain different components. The sample preparation was divided into three categories *i.e.* wet-food, aqueous food and frozen food. Before analysis, samples were stand until they reach room temperature.

2.5.2.1 Wet-food samples

For wet-food samples *i.e.* curry paste and fermented pork, were prepared by weighting a 1-2 g aliquot of wet-food which was in contact with the packaging wrapped containing coated/laminar film. There was placed into a headspace vial then capped with septum and aluminum crimp cap.

2.5.2.2 Aqueous food samples

For an aqueous sample, milk, 15 g milk placed in the vials and immediately capped the vial with tight septum and cramped with aluminum cap.

2.5.2.2 Frozen food samples

For ready to eat curry in plastic box, this was packed with plastic wrapped and then frozen. Before eating, the frozen curry was to be heated by microwave (550 watt) for 4 minutes. The DCE, contaminant might be migrated into the aqueous solution of the curry. Therefore, the aqueous part of the curry was taken to determine for DCE contaminant. 15 g of curry was used for analysis in each.

2.5.3 Incubation time to minimize the effect of food matrix

The matrix of each type of food sample would be different because food consists of various components. To find the condition that would minimize the effect of food matrix the following experiment was carried out. Known amount of DCE standard solution, 50 ng, was spiked into headspace vials filled with the exact weight of food sample and stand at room temperature overnight. All sample vials were placed in the lab-built thermal bath and the automatic headspace system for various equilibration times *i.e.* 10, 15 and 20 minutes. The analysis for each sample was done for five replications at the GC optimum conditions obtained from 2.4.3.1 to 2.4.3.4. The equilibration time that gave the highest response with minimum interference from food matrix was selected for sample incubation throughout this work.

2.6 Qualitative and Quantitative analysis of real packaged food

2.6.1 Qualitative Analysis

Qualitative analysis was determined by the retention time data and confirmed with the DCE standard spiked technique. The retention time, t_R, is the threshold time from the injection of sample to the recording of the peak maximum.

2.6.2 Quantitative Analysis

The quantitative analysis was based on the response of chromatographic peak that was proportional to the amount of analyte. Two analytical standard methods, external (concentration) standard method and the standard addition method were implemented in this work. First, the external standard method was carried out by preparing the working standard solution of DCE at concentration closed to the unknown samples concentration. The

samples were analyzed under the optimum conditions in 2.4.3.1 to 2.4.4.3. The calibration curve was obtained by plotting the peak area versus concentration of DCE. DCE concentration of the samples were obtained by calculating from the calibration curve (Grob, 1985). For the second method, Standard addition method, each sample was analyzed by adding a series of known of amount DCE standard *i.e.* 1, 5, 25, 50 and 100 ng into 1 g of sample and stand overnight at room temperature. These samples were analyzed using GC-ECD at the optimum conditions.

2.6.3 Interference of matrix

In food analysis, the matrix could interfere with the analytes. In this work, the effect of matrix in food was studied by spiking known amount of DCE standard in range of 1 ppb to 75 ppb into 1 g of sample. DCE working standard solution was prepared by diluting the standard stock solution with ultra pure water under the same range. All the samples were placed at room temperature overnight before analysis. The responses, peak area, from the two groups were plotted against the known concentration. The slopes of the standards and the spiked sample were compared for matrix interferences.

Chapter 3

RESULTS AND DISCUSSION

The analysis of 1, 2-dichloroethane, DCE, in packaging food was carried out by Gas Chromatography with electron capture detector (GC-ECD). The stationary phase was 0.1%SP-1000 coated on Carbopack C, 80/100 mesh, packed in a column, of 2mm i.d. x 1.8m and used for DCE analysis.

3.1 Optimization of the GC-ECD analysis conditions

3.1.1 Carrier gas (N_2) flow rate

The optimum carrier gas flow rate was achieved (at the lowest HETP) from a van Deemter plot (that provided the highest column efficiency). The van Deemter equation considers the resistance to mass transfer between the two phase arising from diffusion. It was describes column performance by showing the height equivalent to the theoretical plate (HETP), assuming that there is a perfect equilibrium between the gas and liquid phases within each plate. The efficiency of a column is a function of several parameters. The general form for the van Deemter equation is

$$h = A + \frac{B}{u} + Cu \qquad \text{.....(1)}$$

where A = eddy diffusion term that the mobile phase diffused through the particles of the packing in the column. The result is shown in the velocity of mobile phase.

$= 2\lambda d_p$, λ is a constant characteristic of packing and d_p is a diameter particle of packing.

B = longitudinal or ordinary diffusion term resulted from the movement of molecules after collision in the column.

$= 2\lambda D_g$, λ is a factor characteristic of packing and D_g is a true molecular diffusion.

C = non-equilibration or resistant to mass-transfer term that a constant amount of the mass transfer.

$= (8/\pi^2)[k'/91+k')^2](d_f^2/D_l)$, k is a capacity factor, d_f is an effective film thickness of liquid phase and D_l is a diffusivity of solute in liquid phase.

The van Deemter equation shows the effect of h with changes in linear gas velocity. This equation represents a hyperbola that has a minimum velocity, at $u = (B/C)^{1/2}$ and a minimum *h* value (h min) at $A+2(BC)^{1/2}$. The constants can be calculated from an experimental plot of *h* versus linear gas velocity as shown in Figure 5.

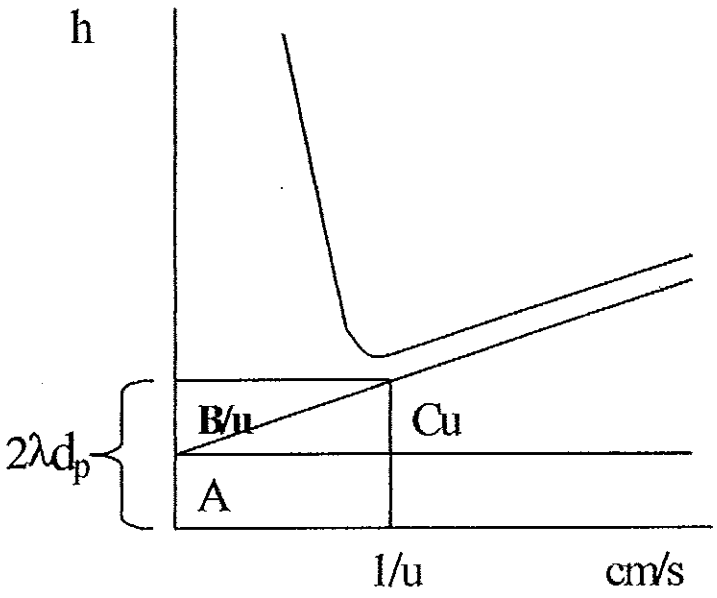

Figure 5 The van Deemter plot changes in h versus linear gas velocity, u:
$h_{min} = A+2(B/C)^{1/2}$, $u_{opt} = (B/C)^{1/2}$ (Grob, 1985)

In the practice, the term of A, B and C in equation, are difficult to know. However, the theoretical plates, that the column is divided into a number of zones and HETP is the zone thickness that can be calculated when the length of column is known n, that follow as:

$$h = HETP = \frac{L}{n} \qquad \text{.....(2)}$$

Where L is the known column length
n is a number of theoretical plates

A total number of theoretical plate (n) contained in the column are measured at the peak width of the chromatogram.

$$n = 16\left(\frac{t_R}{w}\right)^2 \qquad \text{.....(3)}$$

where w is the base width of the peak

t_R is the retention time of peak (Figure 2)

Sometime the efficiency of gas chromatography column was measured at the bandwidth at half-height $w_{0.5}$ of the number of plates.

$$w = \left(\frac{2}{\ln 2}\right)^{\frac{1}{2}} w_{0.5} \qquad \text{.....(4)}$$

Equation 3 expressed as equation 4 in term of $w_{0.5}$.

Therefore

$$n = 8 \ln 2\left(\frac{t_R}{w_{0.5}}\right)^2$$

$$= 8(2.30 \log 2)\left(\frac{t_R}{w_{0.5}}\right)^2$$

$$= 5.54\left(\frac{t_R}{w_{0.5}}\right)^2 \qquad \text{.....(5)}$$

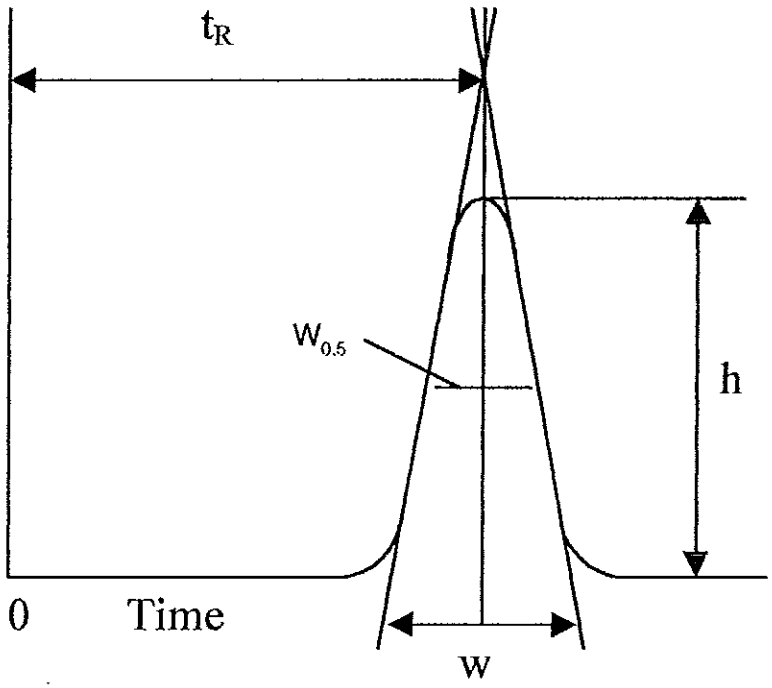

Figure 6 The chromatographic peak used in calculated total theoretical plates

From van Deemter equation, HETP is not only a function of column packing, but also depends on operating conditions and properties of the solute. This is why different values of HETP are obtained for various solutes (Grob, 1985).

In this work, equation (2) and equation (5) were used to calculate HETP. The number of the theoretical plates, n, at various carrier gas flow rates is summarized in Table 7. The relationship between HETP and the carrier gas flow rate for DCE is shown in Table 7 and Figure 8. The optimum carrier gas flow rate was obtained by the considering of the lowest flow rate of van Deemter plot in Figure 7 at 20 mL/min.

Table 7 The high equivalent to a theoretical plates, HETP, at various flow rate of carrier gas (N_2)

Flow rate (mL/min)	The number of theoretical plates (n)	The high equivalent of theoretical plates HETP (mm)
10	2366	0.072
20	3860	0.044
30	3238	0.052
40	2777	0.061

* 5 replications, RSD<4%

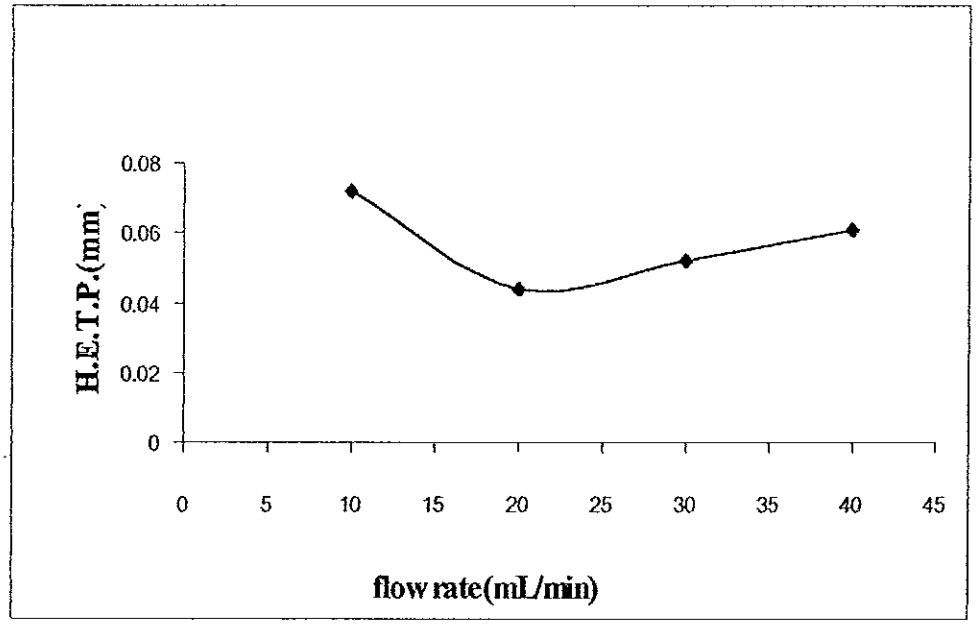

Figure 7 The van Deemter plot of 1, 2-dichloroethane (DCE)

3.1.2 Column temperature

The column temperature is one of the most important characteristic of good chromatographic isolation, since it would minimize the time used for elution components, increase detection and reduce time for analysis. The column temperature must provide a constant temperature that is high enough for components eluting without decomposing and uniform over the whole area of column. In this work, the isothermal temperature system was usedfor eluting 1, 2-dichloroethane. The results are shown in Table 8 and Figure 8. The column temperature that gave the highest response, 90°C was selected.

It should be noted that the packed column used in this work. The stationary phase, 0.1%SP-1000 is the liquid phase that used Carbopack C as a solid support. Carbopack column exhibit greater back pressure than those made with diatomite (data sheet of SP1000, Supelco). Longer column will exhibit greater back pressure and this be could noticed that when the column temperature increased, column back pressure also increased for the same flow rate.

Table 8 The response of 0.5 μg ml^{-1}, (headspace) DCE at various column temperature

Temperature (°C)	Response* x10^5, μV(%RSD)
70	9.35 (1.80)
80	9.64 (2.16)
90	10.44 (2.71)
100	9.97 (3.14)

*5 Replications, RSD<4%

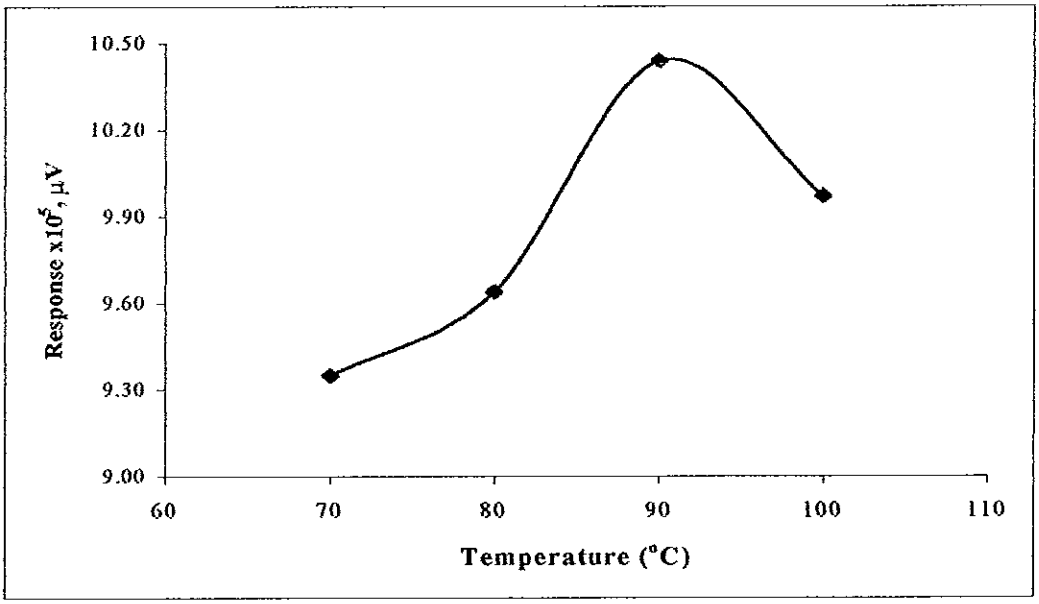

Figure 8 The response of 0.5 μg ml^{-1}, (headspace) DCE at various column temperature

3.1.3 Injector temperature

In a gas chromatographic technique, the injector temperature must be set higher than the boiling point of the analytes (Grob, 1985). The temperature at the injector must be very high or hot enough for sample rapid vaporization to vapor phase that ensures the reproducible retention time and good resolution. In this study, the analyte was equilibrated from the liquid phase into the gas phase before injected into the column and the injector temperature was tested in experiment 2.4.3.3. The results are shown in Table 9 and Figure 9 where the best temperature is 170°C.

Table 9 Responses of 0.5 μg ml^{-1}, (headspace) DCE at various injector temperature

Temperature (°C)	**Response (x 10^5), μV (%RSD)**
100	4.86 (3.20)
110	4.82 (3.80)
120	4.71 (2.92)
130	4.96 (1.49)
140	5.06 (3.78)
150	5.16 (2.50)
160	5.29 (3.41)
170	5.32 (2.56)
180	5.17 (1.53)

*5 replications, RSD<4%

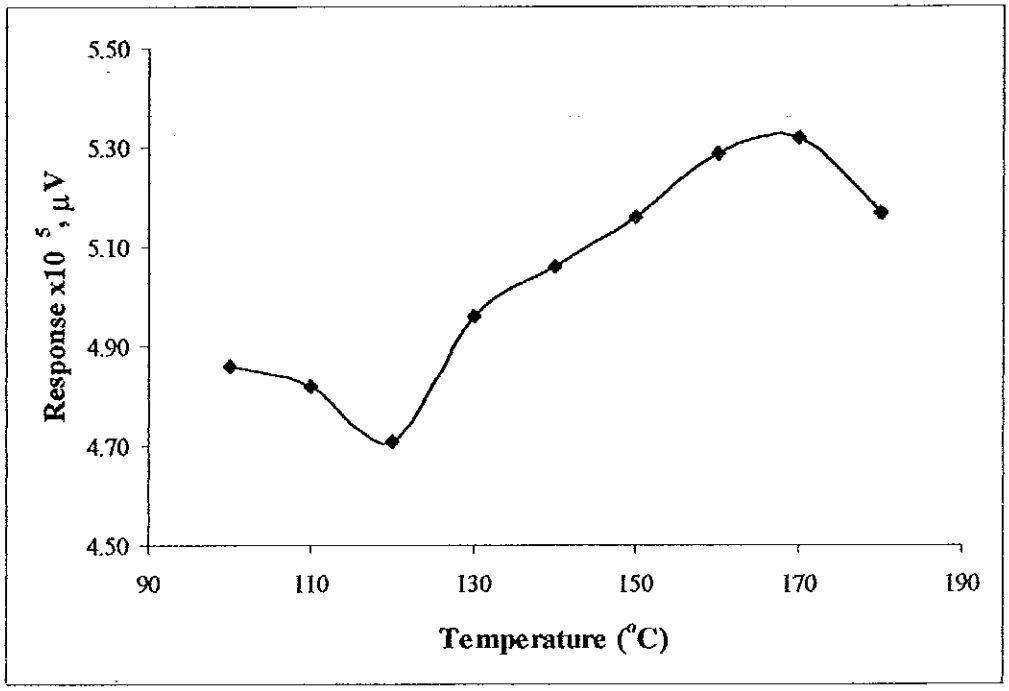

Figure 9 Response of 0.5 µg ml^{-1}, (headspace) DCE at various injector temperature

3.1.4 Detector temperature

The type of detector used depended on the application. The electron capture detector (ECD) was suitable for DCE. Detector temperature must always be set above 100°C to prevent water condensation. The capture process of the electron capture detector is high temperature sensitive. The sensitivity may either increase or decrease with an increase in temperature and depended on the analysis compound. Since detector temperature could affect the sensitivity so, it is possible to improve the analysis by operating at a different temperature. The radioactive source, determines the maximum temperature limit for the detector (Grob, 1985). The detector is held at a temperature above that of the oven to prevent solute condensation in the detector line and in the detector itself. The detector serves two purposes: it displays the quality of the

separation that has been achieved in the form of chromatograph and providing the detector linear response, furnished a mean of estimating the quantity of each component present in the sample.

The electron capture detector is one of detectors invented by Lovelock. A low energy β-ray source is used to produce electrons and ions. The compounds must amenable to the detector are the chlorinated hydrocarbons. In this research, Ni^{63}, is used as a source of β-ray. An electron from the carrier gas and the molecule enter the cell, the electrons are capture by the molecule and the molecule will become charged. The mobility of the captured electrons is much reduced compare with the free electrons and thus the signal can be measured from the falling dramatically of current.

The optimum detector temperature was investigated in the range of 250 to 290°C with 10°C increment. The response increased as temperature increased (Table 10 and Figure 10) and this agreed well with the detector characteristic (Grob, 1985). The optimum detector temperature was achieved by considering the sensitivity, analysis time and the lifetime of the stationary phase. The highest response that gave the lowest the analysis time was obtained at 280°C and this is the optimum detector temperature for DCE standard solution.

Table 10 Responses of 0.5 μg ml^{-1}, (headspace) DCE at various detector temperature

Temperature (°C)	Response ($x10^6$), μV (%RSD)
250	0.90 (1.99)
260	0.98 (2.70)
270	1.02 (2.83)
280	1.18 (1.12)
290	1.09 (3.09)

*5 replications, RSD<4%

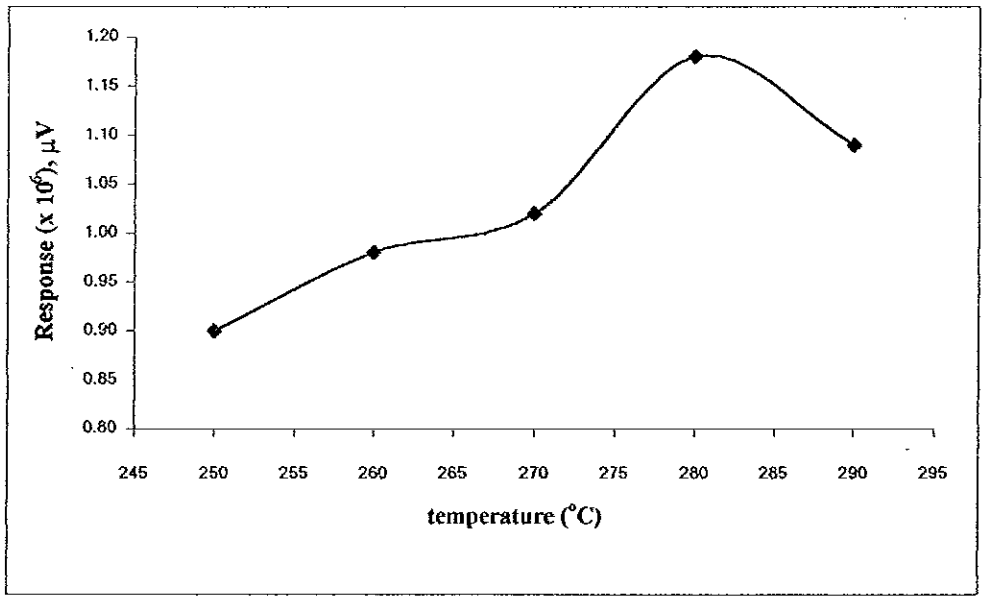

Figure 10 Responses of 0.5 μg ml^{-1}, (headspace) DCE at optimum detector temperature

3.2 Headspace analysis conditions

Headspace system is the analysis of gas phase of a binary heterogenous system at equilibration. One phase is the sample that could be either liquid or solid and the other is the vapor phase where the analytes will be distributed between two phases.

The static headspace analysis is a one step gas extraction method. A suitable method to determine gas vapor phase from vial headspace is gas chromatographic (GC) method.

The volatile DCE in standard solution would be presented in the gas phase which is in contact with the solution (Figure 11). Its relative concentrations in the gas phase depend on the partition pressure which in turn could be influenced by selection of temperature and time (Kolb and Ettre, 1997).

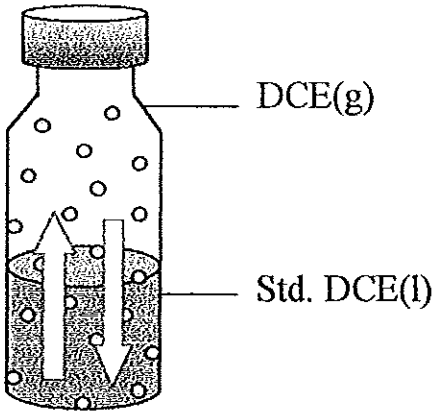

Figure 11 A headspace vial of standard DCE, DCE(g) = DCE in gas phase, Std.DCE(l) = DCE in liquid phase

3.2.1 Equilibrion time

The main parameters influencing the headspace sensitivity are the equilibration time and equilibration temperature. The equilibration time is depended on the distribution of the volatile components from and into the sample. For this work, headspace technique for the standard DCE solution is by placing the sample in a closed vial to establish on equilibrium condition between the sample (condense) phase and gas phase (Figure 7). Both thermal systems *i.e.* the lab built thermal bath and the automatic headspace system, were compared for equilibration of DCE standard. These were optimized in 2.4.4.1 and the results are shown in Table 11 and Figures 12-13.

When the gas extraction of DCE was extracted from the liquid phase (sample phase) into gas phase an equilibrium was reached when DCE in the gas phase equal to the DCE in the liquid phase. Then, the response would longer increase Figure 12 and 13 shown that 10 minutes was the equilibration time for both systems. Therefore, 10 minutes was selected to be the optimum equilibrated time.

Table 11. The influence of the equilibration time on the response of DCE 0.5 $\mu g\ mL^{-1}$

Time (min.)	**Response*x10^5, μV(%RSD)**	
	Lab built thermal bath	**Automatic headspace system**
5	5.37 (1.20)	4.69 (1.03)
10	6.23 (0.45)	4.81 (2.15)
15	5.94 (2.15)	4.51 (1.80)
20	5.95 (3.37)	4.41 (2.42)

*5 replications, RSD<4%

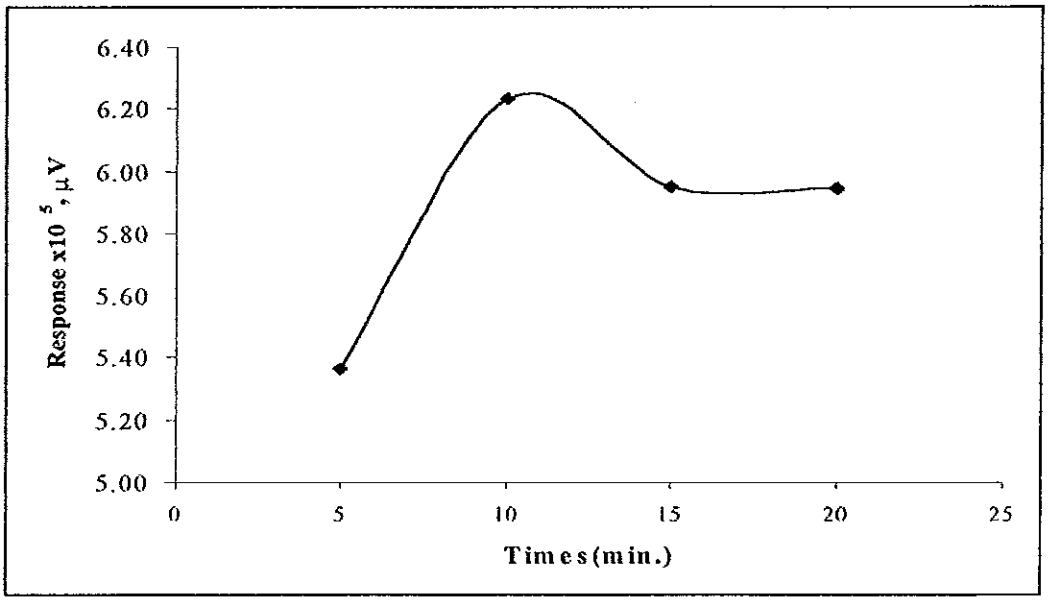

Figure 12 The relationship between equilibration time and response of DCE 0.5 µg mL^{-1} for the lab built thermal system

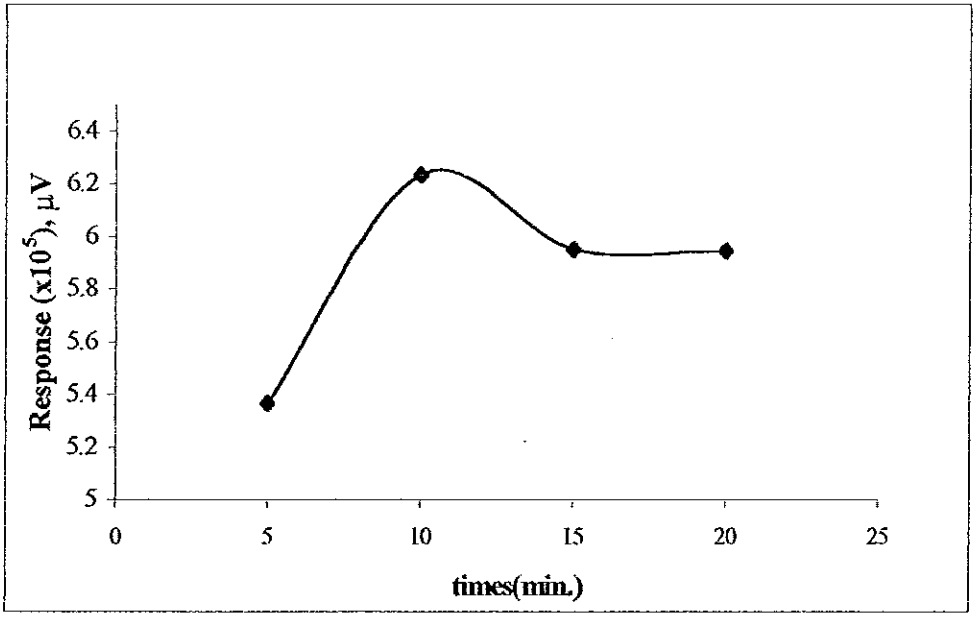

Figure 13 The relationship between equilibration time and response of DCE 0.5 µg mL^{-1} for automatic headspace system

3.2.2 Equilibration temperature

The equilibration temperature that influence headspace sensitivity is studied. In static headspace analysis increasing temperature to enhance sensitivity can cause condensation problem from the evaporation of the analyte into gas phase. Therefore, the equilibration temperature should be optimized at the lowest temperature to avoid the condensation.

The results from both headspace systems are shown in Table 12 and Figures 14-15. The equilibration temperature affected the sensitivity of DCE. The response increased when the equilibration temperature increased. For both headspace systems 80°C gave the highest response/sensitivity.

For the lab built thermal system, at temperature higher than 80°C there was condensation of analyte in the sample vial and the syringe. This was because the thermal bath was an open system also the temperature of the gas phase changed during the transfer to the syringe. To avoid the syringe condensation, ASTM standard practice recommended the syringe should be heated it in the oven at 90°C before sampling (Kolb and Ettre, 1997).

Table 12 . The equilibration temperature and the response of DCE 0.5 μg mL^{-1} for both thermal systems

Temperature(°C.)	Response*x10^5, μV(%RSD)	
	Lab built thermal bath	**Automatic headspace system**
60	8.85 (1.42)	4.69 (1.03)
65	9.22 (1.59)	4.81 (2.15)
70	10.92 (2.78)	4.51 (1.80)
75	11.51 (3.38)	4.41 (2.42)
80	12.57 (3.49)	4.81 (2.15)
85	-	4.51 (1.80)

*5 replications, RSD<4%

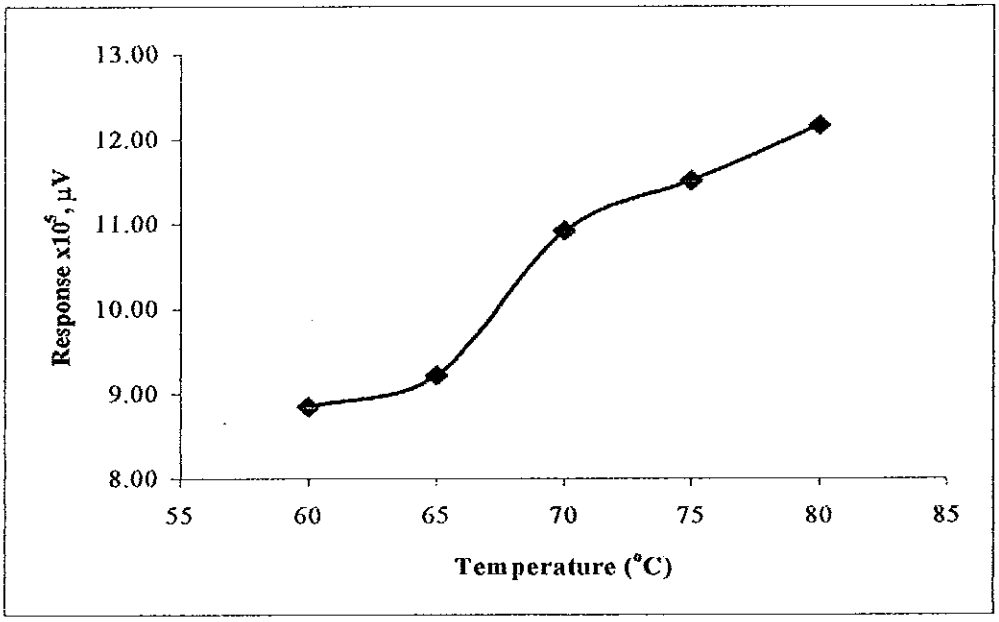

Figure 14 The equilibration temperature and the response of DCE 0.5 μg mL^{-1} for lab built thermal system

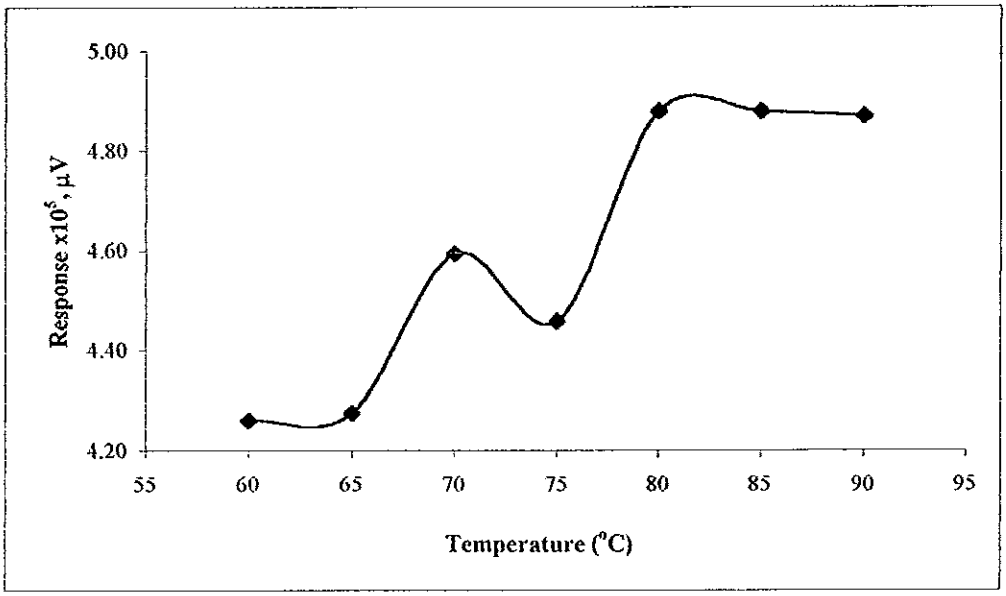

Figure 15 The equilibration temperature and the response of DCE 0.5 µg mL^{-1} for automatic headspace system

3.2.3 The phase ratio

The headspace sensitivity does not depend only on partition coefficient (equilibration time) but also on the phase ratio. The phase ratio, β, is the ratio of the volume of the two phase, that is the volume of gas phase in the vial to the volume of the sample phase and is expressed as

$$\beta = \frac{V_G}{V_S} \qquad \text{.....(6)}$$

Where V_G = volume of the gas phase

V_S = volume of the sample (condense) phase

V_V = volume of the total

Since

$$V_V = V_G + V_S \qquad \text{.....(7)}$$

Equation (6) can be expressed as

$$\beta = \frac{V_V - V_S}{V_S} \qquad \text{.....(8)}$$

and

$$V_S = \frac{V_V}{1 + \beta} \qquad \text{.....(9)}$$

That is

$$\beta = \frac{V_G}{V_S} = \frac{V_V - V_S}{V_S} = \frac{V_G}{V_V - V_G} \qquad \text{.....(10)}$$

From equation (10), the phase ratio will increase when the sample volume decrease or gas phase increase.

Headspace analysis is related to the distribution of analyte between two phases upon equilibrium that is expressed by a thermodynamic constant an equilibration condition partition (distribution) coefficient, K, and is express as

$$K = \frac{C_S}{C_G} \qquad \text{.....(11)}$$

Where K is the partition coefficient related to the mass distribution in two-phase system. It depends on the solubility of the analyte in the sample phase, that is, compounds with high solubility in the sample phase have a high concentration in sample phase related to the gas phase.

This work used a headspace analysis technique and the response of DCE using GC-ECD was measured as the peak area for the DCE concentration in the sample. The relation of the concentration of sample and peak area is expressed as

$$A \propto C_G = \frac{C_0}{K + \beta} \qquad \text{.....(12)}$$

Where A = peak area

C_G = the concentration of analyte in headspace

C_o = original sample concentration of the analyte

K = partition (distribution) coefficient

β = phase ratio of two-phase

From equation (12) the headspace sensitivity is related to the peak area which is depended on the distribution coefficient (K) and the phase ratio (β). The sensitivity of the headspace will increase as the distribution coefficient and the phase ratio decrease.

In 2.4.4.3 the phase ratio for two the thermal systems *i.e.* lab built thermal bath and automatic headspace system was investigated. The results are shown

in Table 13 and Figures 16-17 where the phase ratio increased as the sensitivity decreased. The optimum phase ratio of both headspace techniques was 0.80.

Table 13 The effect of phase ratio of the response of DCE 0.5 μg mL^{-1}

Phase ratio	Response* $x10^5$, μV (%RSD)	
	Lab built thermal bath	**Automatic headspace system**
0.35	10.08(3.30)	4.58 (1.89)
0.80	10.22(2.39)	4.91 (2.01)
1.70	8.62(3.26)	3.58 (3.07)
4.40	7.03(1.43)	3.07 (3.31)

*5 replications, RSD<4%

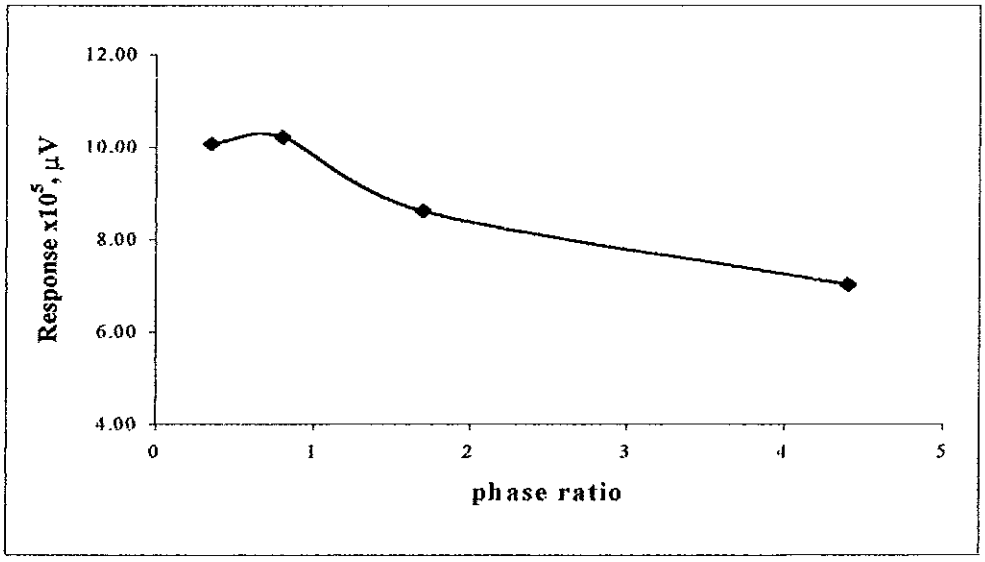

Figure 16 The phase ratio and the response of DCE 0.5 μg mL^{-1} for lab built thermal system

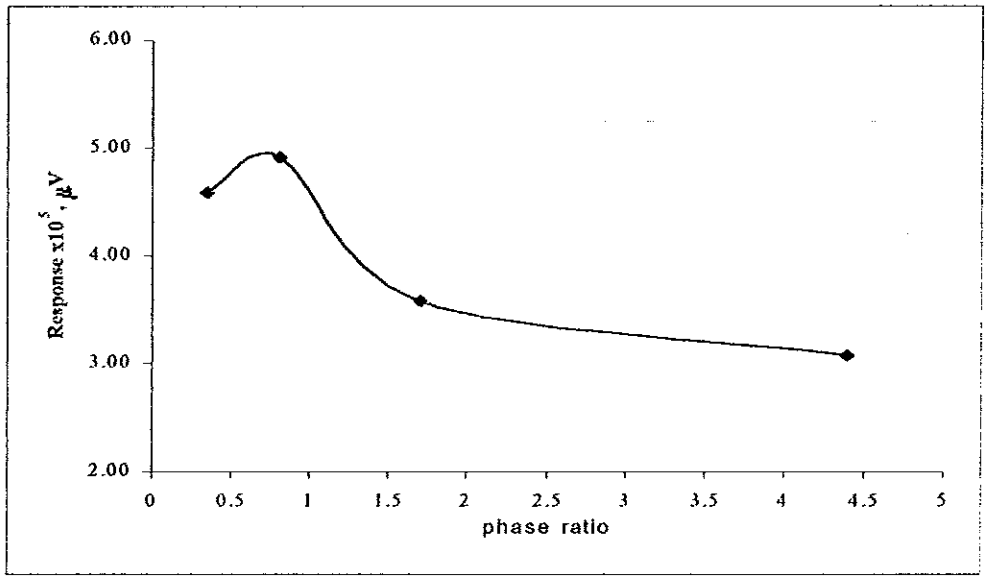

Figure 17 The phase ratio and the response of DCE 0.5 μg mL^{-1} for automatic headspace system

The optimum headspace conditions for DCE analysis using the glass column, 1.8m x2mm i.d., 0.1%SP-1000 on Carbopack C, 80/100 mesh, are summarized in Table 14 and Figure 18 (the chromatogram of DCE by using HS-GC-ECD).

Table 14 The optimum conditions of headspace GC-ECD

GC conditions	
Carier gas flow rate of N_2	20 mL min^{-1}
Column temperature	90°C
Injector temperature	170°C
Detector temperature	280°C
Headspace conditions	
Equilibration time	10 min.
Equilibration temperature	80°C
phase ratio	0.80

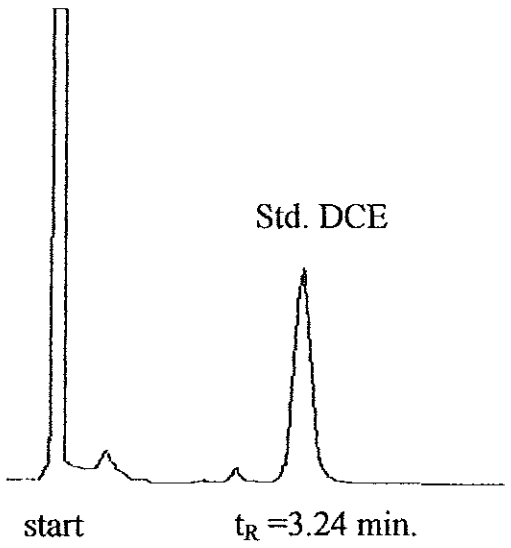

Figure 18 The chromatogram of DCE 0.5 μg mL^{-1}at the optimum conditions

3.3 The linear dynamic range (Linearity)

The linear dynamic range of the headspace is the relationship between the original concentration (C_o) of analyte in the sample and its concentration in the headspace. This can be found by investigating the relationship between C_o and peak area (A) obtained when the analyte reached the equilibrium. The linear dynamic range of DCE was determined in 2.4.5 for both systems *i.e.*, the lab built thermal bath and the automatic headspace system. The responses of DCE at various concentrations are shown in Table 15 and Figures 19-20. For each concentration, five replications were done and high precision was obtained since the relative standard deviation (RSD) were all lower than 4%. Both headspace systems showed a wide linear dynamic range from 1 to 750 ng mL^{-1} with a good correlation coefficient, R^2>0.99.

Table 15 The relationship between the response and the various DCE concentration (ng mL^{-1})

DCE concentration (ng mL^{-1})	**Response*x10^5, μV (%RSD)**	
	Lab built thermal bath	**Automatic headspace system**
1	0.27(3.81)	0.12(1.79)
5	0.63(2.22)	0.32(1.63)
10	1.03(2.23)	0.46(0.31)
25	1.62(2.69)	0.63(2.35)
50	2.15(2.01)	1.08(2.31)
100	4.33(3.05)	1.63(3.27)
250	10.56(3.11)	3.11(1.50)
500	19.81(3.98)	5.46(2.81)
750	34.07(3.14)	7.91(1.26)

*5 replications, RSD<4%

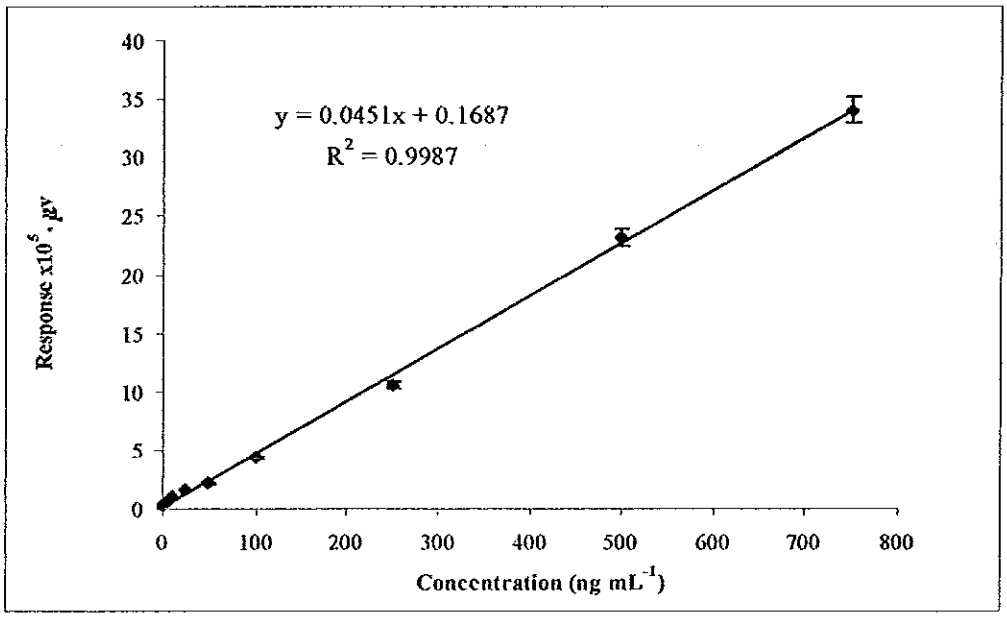

Figure 19 The linear dynamic range of DCE for lab built thermal system

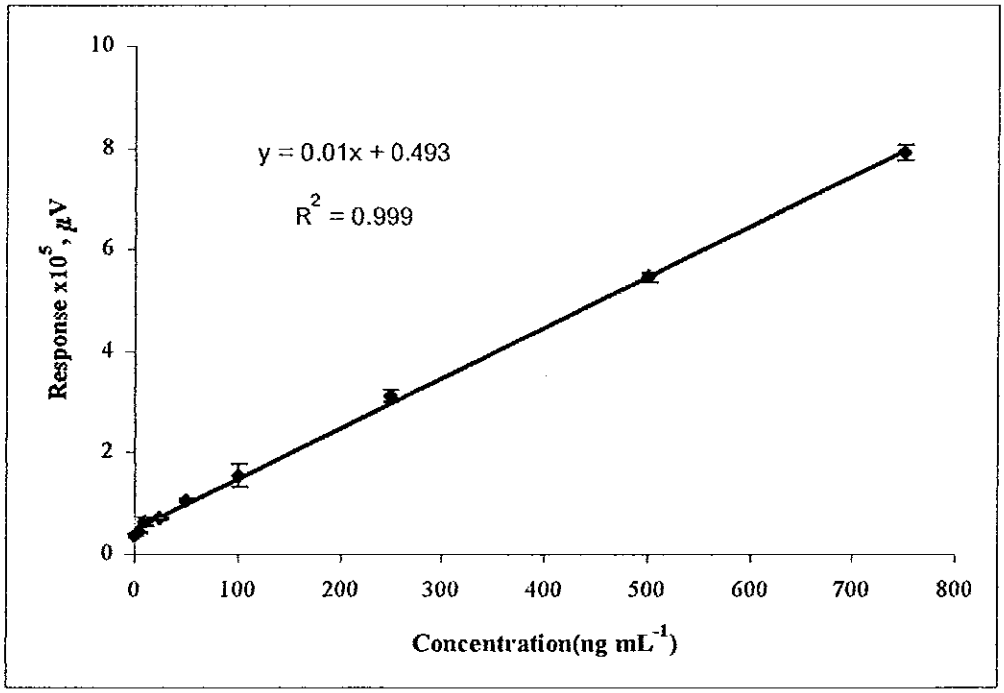

Figure 20 The linear dynamic range of DCE for automatic headspace system

3.4 The limit of detection

The limit of detection is the lowest concentration of the analyte that an analytical process can be reliable detect (Long and Winefordner, 1983). The limit of detection of DCE was investigated base on the IUPAC method. The responses of blanks which appeared at various retention time were measured. The maximum detectable blank signals are shown in Table 16 for the automatic headspace system. The limit of detection of both systems were calculated from the standard calibration curve in Table 17 and Figure 21. The limit of detection of lab built system was obtained at 0.92 ng mL^{-1} and the automatic headspace system at 0.72 ng mL^{-1}. The results of both headspace system were summarized in Table 18. The results of both headspace systems showed the different 22%.

The automatic headspace system showed better (lower) limit of detection than the lab-built system. This may due to two reasons could drawn in two. One was the heating system, the automatic headspace system was a closed system, *i.e.* the temperature was constant, while the lab built thermal system was a open system. The temperature of the latter system could fluctuate during the transfer of sample to the column. The uncontrolled temperature could affect the headspace sensitivity that is depended on the temperature (K constant) in equation (12). The other reason was that the automatic headspace system was always heated. The syringe usual constant heated, vapor of analytes was not condensed. The lather provided the uncontrolled temperature of vapor phase above the sample that the effective of the phase ratio in turn affected the headspace sensitivity (equation 12). The concentration of the lowest detectable was calculate from the equation

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

Where the C_L is the concentration of the detectable, k is the constant value at the confidence limit, S_B as the standard deviation of blank, for twenty repeatability and m is the slope (sensitivity) of the calibration curve. For this case, the limit of detection also depended on the standard deviation and the slope of the calibration curve.

Table 16 The data of the blank measurements by automatic headspace system, $n_B = 20$

t_R	**Maximum response, μV**
2.83	9834
2.83	9289
2.83	7368
2.83	7548
2.83	9261
2.83	10246
2.83	7316
2.82	7816
2.83	7408
2.82	10428
2.83	7226
2.81	10229
2.82	7664
2.83	6977
2.81	7664
2.82	9464
2.81	7594
2.81	8650
2.83	7177
2.82	9566
	$\overline{X}$ = 8436
	S_B^2 = 1466521

Table 17 The relation between the response of DCE and various concentration for automatic headspace system

Concentration (ng mL^{-1})	Response* $x10^4$, μV (%RSD)
1.0	0.99(2.42)
2.5	1.77(2.13)
5.0	3.12(2.26)
7.5	4.30(0.81)
10.0	5.55(1.36)

*5 replications, RSD<4%

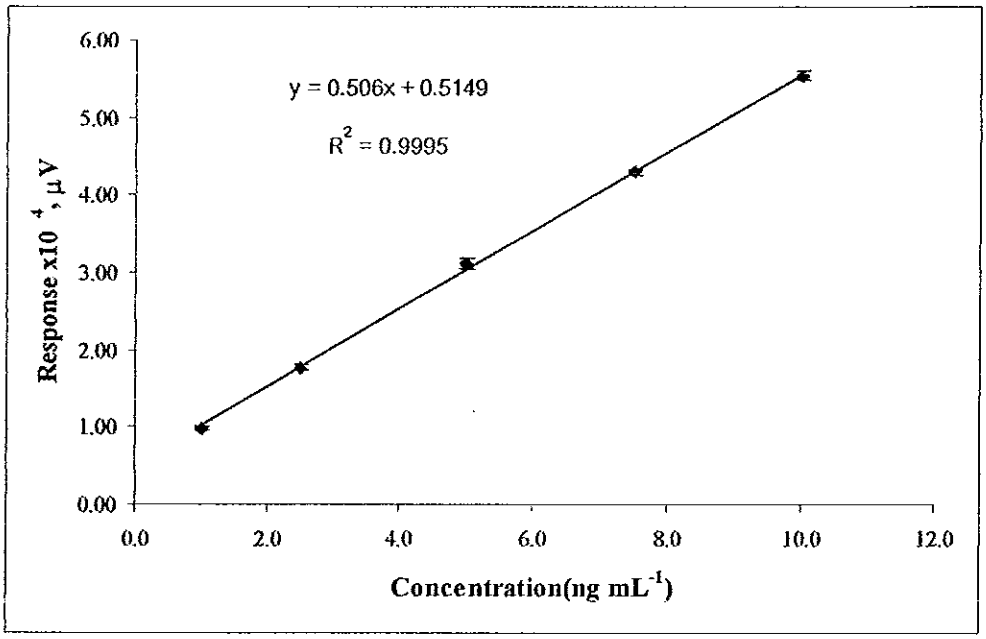

Figure 21 The calibration curve of DCE for automatic headspace system

Table 18 The limit of detection for DCE standard solution with optimum conditions of HS-GC-ECD

Parameters	**Results**	
	Lab built thermal bath	**Automatic headspace system**
$\overline{X}$	2040	8436
S_B^2	270400	1466521
S_B	520	1212
m	1693	5060
C_L (ng mL^{-1})	0.92	0.72

3.5 Sample Analysis

3.5.1 Sampling

Packaged food samples that contam food which is in close contact with the plastic container or plastic laminar/thin film was selected and analyzed using the optimum conditions of gas chromatography with electron capture detector combined with headspace technique as the preparation sample. A total of 37 samples of packaged food were sampling from supermarkets in Hat Yai, Songkhla (Table 20). All samples of the same type, and the same brand, but different time of packing were re-collected and re-analysed.

Table 19 Packaged food samples

Sample	Number of type of packaged food	Number of brand of packaged food	Total of samples
Curry paste	10	7	22
Salted pork	2	2	2
Milk	3	7	7
Curry ready to eat	6	3	6
Total of all samples	21	19	37

3.5.2 Sample preparation

The samples were divided in to three groups *i.e.* wet-food, aqueous food and frozen food. From the physical properties of each group the sample preparation was differed and the results discussed in 3.6.2.

3.5.3 Minimization of food matrix

In food analysis the composition of food is not exactly known so the matrix effect can be minimized by evaporated the analyte completely in vial. Therefore, the factor affected the headspace sensitivity must be investigated since packaged food consists of various component and the matrix would differ from type to type. The composition of the sample phase would have most influence on the partition of the analyte (Kolb and Ettre, 1997). Therefore, the time needed for the equilibration depended on the diffusion of the analyte, DCE. The investigation was carried out in 2.4.3. The time that was used for food sample to reach the equilibrium is shown in Table 20 and Figure 22. 10 minutes, the highest response was obtained and the response become constant at the beyond 10 minutes. Therefore, 10 minutes was enough time for the sample preparation of the packaged food. The result indicated that the

equilibration time at 10 minutes for food sample is the same as for the DCE standard solution.

The equilibration time depend on the sample type, in general the solid sample takes longer equilibration time than the liquid sample (Kolb and Etrre, 1997). In this work, the packaged food sample has large water in their components (Risbo, 2003), this may be the reason why the same equilibration time of DCE in standard solution and in packaged food were obtained.

Table 20 The equilibration time for analysis packaged food

Time(min.)	Response* $x10^4$, μV(%RSD)	
	Lab built thermal system	Automatic headspace system
5	1.21 (3.71)	0.94 (2.36)
10	1.60 (2.94)	1.12 (2.87)
15	1.59(3.91)	1.10(3.94)
20	1.58(3.28)	1.09(3.06)

*5 replications, RSD<4%

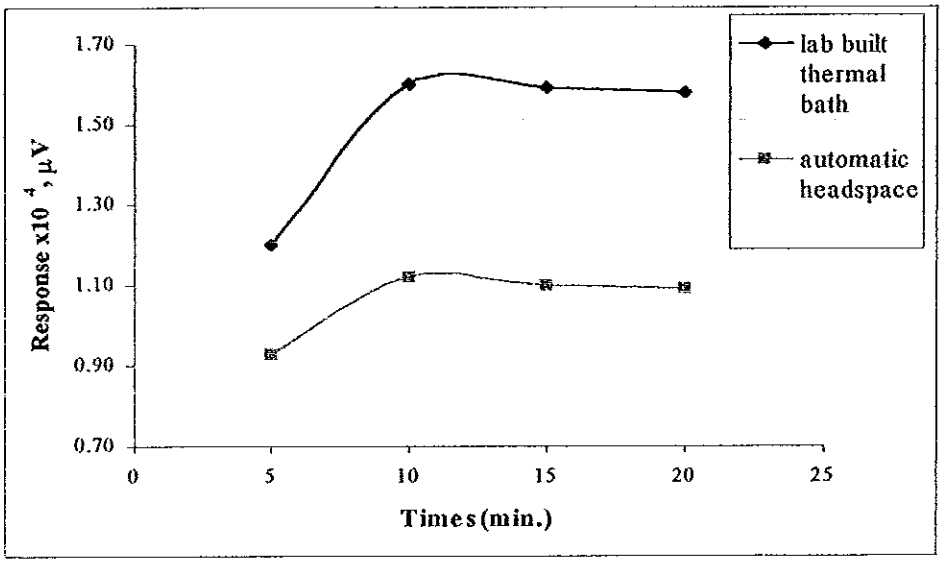

Figure 22 The equilibration time for analysis packaged food

3.6 Qualitative and Quantitative analysis of packaged food

3.6.1 Qualitative Analysis

The optimum conditions of GC-ECD were used to analyse DCE in packaged food. For the qualitative analysis, the retention time, t_R was used. The average t_R of DCE was 3.20 minutes

3.6.2 Quantitative Analysis

The quantitative analysis of DCE was done by considering the response *i.e.* peak area that was related to the concentration of the analyte. In this work, the analytical standard method, *i.e.* external standard method and standard addition method were used to quantify DCE in packaged food. First the external standard method was used for the determining of DCE contaminant. The calibration curve of DCE standard solution was used to determine the DCE concentration in packaged sample as 2.6.1. The three

groups of packaged food were analysed by using the optimum headspace conditions. The results were:

The wet-food provided two groups of sample, one was curry paste and the other fermented pork. For fermented pork no DCE was detected in both samples. For curry paste, DCE was found in seven out of twenty-two curry paste samples. DCE was found in the range from not-detectable to 29.42 ng g^{-1} from different type of curry paste of the same brand. All curry paste samples were re-sampling and re-analysed. The results are the same as the first analysis. The results were shown in Table 21. The concentrations of DCE contaminated in packaged food were similar to the 34 groups of samples reported in Canada in 1992 that is less than 5 μg/Kg for the solid food and less than 1 μg/Kg for liquid food (CICADS, 1998). DCE was also found in milk with added fruit as reported by German FDA (WHO, 2000). The DCE contaminant in this work could either migrate from the plastic or the thin laminar film that is in close contact with the food, or from the food process that used DCE in cleaning PVC product in manufacture (EHC 176, 1995). In addition DCE could also be contaminated from the vegetation surface such as galangal, shallot and/or garlic that were used as a raw materials for the curry paste. These plants can also adsorb the VOCs on their surfaces that depended on the type of plants (Alvarado *et. al.*, 2003).

Table 21 DCE anlysed from of curry paste samples using HS-GC-ECD

Types of curry paste	**No. of sample**	**DCE*, ng g^{-1} (number found)**
Green curry paste	5	n.d-4.02 (1)
Red curry paste	3	n.d-0.79 (1)
Yellow curry paste	1	n.d
Masman curry paste	2	n.d-8.86 (1)
Panang curry paste	3	n.d-0.79 (1)
Tom Yam	1	n.d
Tom Kha	1	n.d-1.69 (1)
Holy Basil seasoning paste	1	1.01 (1)
Satay seasoning	2	n.d-29.42 (1)
Soup powder	3	n.d

*5 replications, n.d.= not-detectable

In another groups of sample *i.e.* milk and curry ready to eat, did not found DCE contamination in three samples, shown in Table 23-25.

Table 22 DCE analysed from Fermented pork samples using HS-GC-ECD

Types of salted pork	**No. of sample**	**DCE***
Fermented pork (Nam)	2	n.d.

*5 replications, n.d.= not-detectable

Table 23 DCE analysed from milk samples using HS-GC-ECD

Types of milk sample	No. of sample	DCE*
Yoghurt	4	n.d.
Milk mixed fruit	2	n.d.
Fresh milk	1	n.d.

*5 replications, n.d.= not-detectable

Table 24 DCE analysed from ready to eat curry samples using HS-GC-ECD

Types of ready to eat curry	No. of sample	DCE*
Chicken green curry	2	n.d.
Savoury pork burger	1	n.d.
Roast pork with gravy	1	n.d.
Chicken musman curry	1	n.d.
Tom Yam (sour and spicy prawn soup)	1	n.d.

*5 replications, n.d.= not-detectable

The concentration of DCE in some packaged foods were not detected by the external standard method, it is possible that the amount of DCE of these sample were lower than the limit of detection (0.72 ng mL^{-1}, the automatic system and 0.92 ng mL^{-1}, the lab-built system). The confirmation was carried out by standard addition method with the sample that represented the group of samples that gave non-detectable results. The results of standard addition of these samples are in Tables 25-26 and Figures 23-24. The DCE concentrations of represented samples determined by standard addition method are shown in Table 27. The results obtained (Table 27) agreed well with the hypothesis *i.e.* less than the limit of detection, non-detectable-0.76 ng g^{-1}. The not detectable DCE of the packaged food could be because DCE did not migrate from the plastic/thin laminar film into packaged food or migrated with trace amount in specific packaged food sample.

Table 25 The results of standard addition in curry paste sample

Spiked concentration (ng/g)	**Response* (x10^{4}), μV (%RSD)**		
	Green curry paste	Panang curry paste	Red curry paste
25	1.33 (3.38)	1.41 (2.75)	1.27 (2.17)
50	2.76 (0.48)	2.87 (2.27)	2.50 (2.83)
75	3.87 (1.64)	4.11 (1.99)	3.80 (2.76)
100	5.31 (0.89)	5.62 (3.53)	5.15 (2.49)

*5 replications

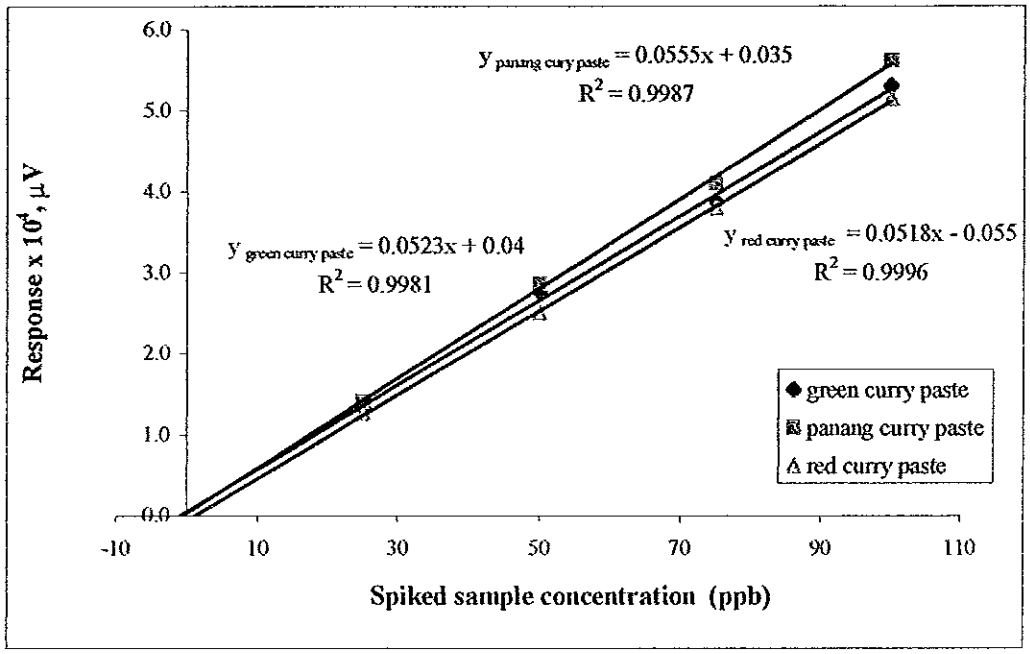

Figure 23 The results of standard addition in curry paste sample

Table 26 The results of standard addition in milk sample

DCE concentration added (ng/mL)	Response*(x10^4), μV (%RSD)	
	Yoghurt	Fresh milk
10	0.33 (3.51)	0.30 (3.54)
25	0.64 (3.73)	0.62 (3.62)
50	1.19 (3.91)	1.48 (3.94)
100	2.63 (3.67)	2.90 (3.97)

*5 replications

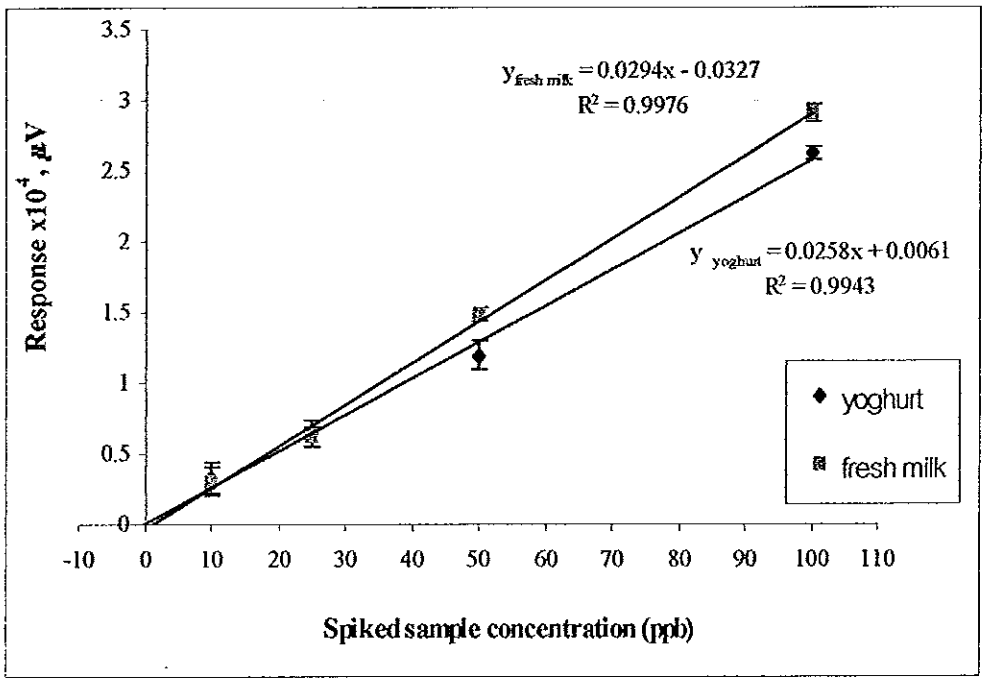

Figure 24 The results of standard addition in milk sample

Table 27 The DCE concentration of represented samples determined by standard addition method

Represented samples	Concentration of DCE found in sample* (ng g^{-1})
Green curry paste	0.76
Red curry paste	n.d.
Panang curry paste	0.63
Yoghurt milk	0.23
Fresh milk	n.d.

*5 replications, n.d. = not detectable

3.6.3. Matrix interference

In food analysis, the various compositions in food could interfere with the interest analyte. If the analysts are not aware of these interferences, these interferences can lead to a number of the effect. They may have the effect of apparently enhancing the concentration of the analyte by contributing to the signal attribution to the analyte. Interference would usually affect the slope of the calibration curve, so that it will be differently from the slope of the analyte of interest, so the slope of the calibration curve in the method of additions may affect the linearity of the curve. This effect has the potential to indicate the possible present of a hidden interferences (Eurachem guide, 1998).

In this study, two types of packaged food were used to study the interferences in samples. Tom Yam curry paste was the representation of wet-food and yoghurt milk as the aqueous sample. The standard addition method was used for this study by adding the various amounts of known standard into the sample, (2.6.3). The optimum conditions were set for determining the addition sample and standard DCE. The results of the addition are shown in Table 28 and Figure 25. The slopes of the spiked sample curve were very close to the slope of the standard. Therefore, the interferences from the food matrix in these packaged foods can be neglected.

Table 28 The results of standard addition in some packaged food sample

Concentration (ppb)	Response ($x10^4$), μV (%RSD)		
	Standard DCE	**DCE in Tom Yam paste**	**DCE in milk sample**
10	2.49 (2.18)	1.05 (2.60)	0.30 (3.12)
25	3.00 (1.52)	1.42 (3.88)	0.62(3.97)
50	3.92 (1.46)	2.53 (2.40)	1.48 (3.32)
100	5.76 (2.33)	4.26 (1.32)	2.90 (3.98)

*5 replications

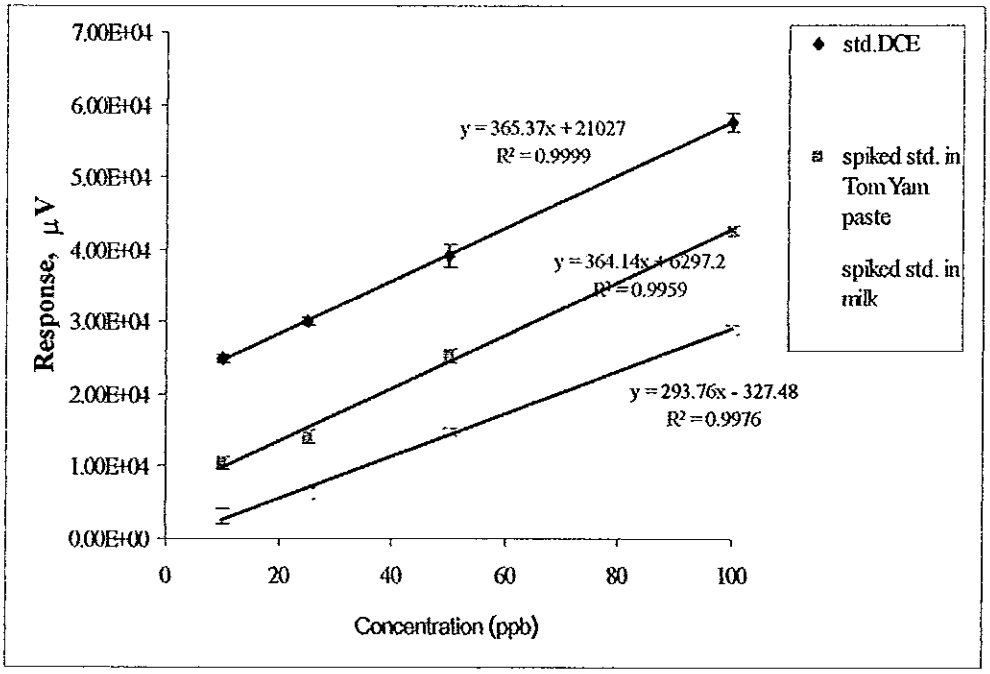

Figure 25 The results of standard addition in some packaged food sample

Chapter 4

CONCLUSIONS

Contaminants in various environmental samples are the main topics of interest while the contaminated solvents in food are often overlooked, even if their effect may influence of human health. The methods of food analysis have many steps because of the food matrix is complicated for analysis. Most of the techniques used nowaday for food samples preparation are extraction by solvent, purging with inert gas and trapping on sorbent before analysis. Most of three techniques were time consuming, high cost, and need large amount of solvent. In this work, the static headspace technique was developed for packaged food samples analysis.

The method used in this work consisted of two part *i.e.* sample preparation technique, and gas chromatographic analysis. Static headspace technique was used as the sample preparation technique and two systems, *i.e.* the lab built water bath and the automatic headspace system were compared.

First, GC analysis was investigated to obtained the best and high efficiency responses. The gas liquid chromatography consisted of 1.8m x 2mm i.d., glass column, 0.1%SP1000 on Carbopack C, 80/100 mesh equipped with an electron capture detector (ECD). The optimum conditions obtained were: the carrier gas flow rate at 20 mL min^{-1}, the isothermal column temperature, 90°C, the injector temperature and detector temperature, 170°C and 280°C respectively.

The headspace technique was compared between the lab built thermal system and automatic headspace system (commercial). The optimum conditions were; equilibration time 10 minutes, equilibration temperature 80°C

and the phase ratio, 0.8 for both headspace systems. These optimum conditions used only a short period of time within 10 minutes, and gave limit of detections at 0.72 and 0.92 ng mL^{-1} and wide range of linearity at 1-750 ng mL^{-1} with a high precision (RSD<4%) and a linear regression (R^2) more than 0.99.

The sample preparation techniques, the static headspace conditions, were investigated for both the lab built and the automatic headspace systems. The results showed insignificant difference between two headspace systems with high precision (RSD<4%), low limit of detection and wide linear dynamic range. Nevertheless, the packed column and the headspace preparation technique were suitable for analysis the high volatile compounds and the HS-GC-ECD with packed column was selective method for analysis DCE. The sample preparation procedure was simple and less time consuming *i.e.* only placing a reasonable amount of sample placed in a headspace vial then closed and crippled with tightly septum and aluminum cap.

The 37 samples of packaged food were sampling from the supermarket in Hat Yai, Songkhla for the qualitative and quantitative analysis of the DCE concentration. The samples (same brand and type) were recollected and re-analysed at different manufacturing time. DCE contaminated in packaged food were found in some samples. Seven out of twenty-two of curry paste packaged food samples were contaminated by DCE in the range of 0.79 ng g^{-1} to 29.42 ng g^{-1}. All seven contaminated samples were from the same brand, but difference types of curry paste. In other types of packaged food, milk and ready to eat curry, no DCE detected. The concentration of DCE in trace level, lower than limit of detection, was confirmed by the standard addition method. The results showed in the range of not detectable to 0.76 ng g^{-1}. The effect of matrix interference was also studied the results showed that for this proposed method, the matrix of packaged food was negligible.

In conclusions, static headspace technique coupled with gas chromatographic method with an electron capture detector was suitable for

trace DCE contaminant analysis. This method could also be used for analysis of chlorinated compounds in various media or other types of sample. The lab built thermal system for headspace is economical and could provide the same results as the commercial, automatic headspace system. The advantages of this technique are, simply sample preparation, samples can be analysed without the use of organic solvent, low cost, and simple to use for the analysis of trace DCE less than ng mL^{-1} and ng g^{-1}in various samples.

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