การพัฒนาดิสเพอร์ซีฟลิควิด-ลิควิดไมโครเอกซ์แทร็กชันที่ใช้ตัวทำละลายช่วยสำหรับการตรวจวัดสารตกค้างกลุ่มออร์แกโนฟอสฟอรัสและออร์แกโนคลอรีน

นางสาวพนิดา คำหนุน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2555

DEVELOPMENT OF DISPERSIVE LIQUID-LIQUID MICROEXTRACTION USING AN AUXILIARY SOLVENT FOR DETERMINATION OF ORGANOPHOSPHORUS AND ORGANOCHLORINE RESIDUES

Miss Panida Khamnoon

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2012

Thesis Title	DEVELOPMENT OF DISPERSIVE LIQUID-LIQUID MICROEXTRACTION USING AN AUXILIARY SOLVENT FOR DETERMINATION OF ORGANOPHOSPHORUS AND ORGANOCHLORINE RESIDUES
By	Miss Panida Khamnoon
Field of Study	Chemistry
Thesis Advisor	Puttaruksa Varanusupakul, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

……. Dean of the Faculty of Science

(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

……..Chairman

(Assistant Professor Warinthorn Chavasiri, Ph.D.)

…….. Thesis Advisor

(Puttaruksa Varanusupakul, Ph.D.)

……. Examiner

(Luxsana Dubas, Ph.D.)

……. External Examiner

(Apinya Navakhun, D.Sc.)

พนิดา คำหนุน: การพัฒนาดิสเพอร์ซีฟลิควิด-ลิควิดไมโครเอกซ์แทร็กชันที่ใช้ตัวทำละลายช่วยสำหรับการตรวจวัดสารตกค้างกลุ่มออร์แกโนฟอสฟอรัสและออร์แกโนคลอรีน (DEVELOPMENT OF DISPERSIVE LIQUID-LIQUID MICROEXTRACTION USING AN AUXILIARY SOLVENT FOR DETERMINATION OF ORGANOPHOSPHORUS AND ORGANOCHLORINE RESIDUES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ดร.พุทธรักษา วรานุศุภากุล, 68 หน้า.

งานวิจัยนี้ได้พัฒนาเทคนิคดิสเพอร์ซีฟลิควิด-ลิควิดไมโครเอกซ์แทร็กชันเพื่อวิเคราะห์หาปริมาณสารกำจัดศัตรูพืชกลุ่มออร์แกโนฟอสฟอรัสและกลุ่มออร์แกโนคลอรีนพร้อมกัน โดยใช้ตัวทำละลายที่มีความหนาแน่นสูงเป็นตัวทำละลายช่วยในการปรับความหนาแน่นของตัวทำละลายสกัดที่มีความหนาแน่นน้อยกว่าน้ำ ทำให้ความหนาแน่นของสารละลายผสมที่ได้มีความหนาแน่นสูงกว่าน้ำจึงง่ายต่อการแยกสารสกัดออกมา ในงานวิจัยนี้ ประสิทธิภาพในการเพิ่มความเข้มข้นและค่าการกลับคืนของการสกัดดีที่สุด เมื่อใช้ตัวทำละลายรวมของเฮปเทนซึ่งใช้เป็นตัวทำละลายสกัดและเตตระคลอโรเอทิลีนใช้เป็นตัวทำละลายช่วย ปริมาตร 40 ไมโครลิตร ผสมกับอะซิโตไนไตรล์ซึ่งใช้เป็นตัวทำละลายช่วยกระจายตัว ปริมาตร 1.4 มิลลิลิตร นำไปฉีดลงในตัวอย่างน้ำ ปริมาตร 5 มิลลิลิตร อย่างรวดเร็ว จะเกิดสารละลายขุ่น จากนั้นนำไปเซนตริฟิวก์เพื่อแยกชั้นตัวทำละลายสกัดให้อยู่ด้านล่างของหลอดทดลองและใช้เข็มฉีดดึงสารสกัดออกมาเพื่อนำไปวิเคราะห์ต่อด้วยแก๊สโครมาโทกราฟี-แมสสเปกโทรเมทรี การตรวจสอบความใช้ได้ของวิธีพบว่าร้อยละการกลับคืนของการสกัดสารกำจัดศัตรูพืชที่ความเข้มข้น 5.0 ไมโครกรัมต่อลิตรอยู่ในช่วง 40.7-90.5 และค่าการเบี่ยงเบนมาตรฐานสัมพัทธ์ในช่วง 6.6-10.6% (n=6) ขีดจำกัดของการตรวจวัดของวิธีอยู่ในช่วง 0.3-1.2 ไมโครกรัมต่อลิตร และมีประสิทธิภาพในการเพิ่มความเข้มข้นในช่วง 89-198 เท่า จากนั้นนำวิธีที่พัฒนาได้มาประยุกต์ใช้ในการวิเคราะห์สารกำจัดศัตรูพืชในตัวอย่างน้ำและมะเขือเทศ วิธีการวิเคราะห์นี้มีความง่าย เร็ว ราคาถูก ประสิทธิภาพในการเพิ่มความเข้มข้นสูงและใช้ปริมาณตัวทำละลายอินทรีย์น้อยซึ่งเป็นมิตรกับสิ่งแวดล้อม

ภาควิชา เคมี ลายมือชื่อนิสิต .....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา 2555

537 24050 23: MAJOR CHEMISTRY
KEYWORDS: DISPERSIVE LIQUID-LIQUID MICROEXTRACTION/ AUXILIARY SOLVENT/ ORGANOCHLORINE/ ORGANOPHOSPHORUS

PANIDA KHAMNOON: DEVELOPMENT OF DISPERSIVE LIQUID-LIQUID MICROEXTRACTION USING AN AUXILIARY SOLVENT FOR DETERMINATION OF ORGANOPHOSPHORUS AND ORGANOCHLORINE RESIDUES. ADVISOR: PUTTARUKSA VARANUSUPAKUL , Ph.D., 68 pp.

Dispersive liquid-liquid microextraction (DLLME) using a low-density organic solvent as an extraction solvent was developed for simultaneous determination of organophosphorus and organochlorine pesticides. A high-density organic solvent as an auxiliary solvent was used for adjusting the density of a low-density organic solvent as an extraction solvent. As a result, the density of mixed solvent was higher than water and a phase separation by centrifugation and recovery of the extract were simplified. In this work, enrichment factor and extraction recoveries were optimized when using 40 μL of a mixture of *n*-heptane as extraction solvent and tetrachloroethylene as auxiliary solvent (ratio 1:3) and 1.4 mL of acetonitrile as a disperser solvent. The mixed solution was injected into 5 mL of aqueous sample by syringe, rapidly forming a cloudy solution. After centrifugation, the sediment phase at the bottom was removed by microsyringe and directly analyzed by gas chromatography-mass spectrometry. For method validation, the extraction recoveries for 5.0 μg/L of pesticides were in the range of 40.7 – 90.5 % and relative standard deviations were between 6.6 and 10.6% (n=6). The limits of detections for the method were range from 0.3 to 1.2 μg/L. The enrichment factors were in the range of 89 – 198. The developed method was then applied for determination of pesticide residues in water and tomato sample. The proposed method is simple, cheap, rapid, high enrichment factor and reduced the use of toxic solvent in conventional DLLME, which is environmental friendly.

Department Chemistry Student's Signature .....
Field of Study Chemistry Advisor's Signature .....
Academic Year 2012

ACKNOWLEDGEMENTS

This successful thesis can be accredited to my advisor, Dr. Puttaruksa Varanusupakul, for her sincere carefulness, helpfulness, kind assistance, and valuable suggestion throughout this research. I would like to extend my thanks and respect to Assistant Professor Dr. Warinthorn Chavasiri, Dr. Luxsana Dubas and Dr. Apinya Navakhun for their valuable comments as thesis examiners.

I would like to express my appreciation to Dr. Kanet Wongravee for his advice and assistance about experimental design. In addition, I would like to thank all members in Chromatography and Separation Research Unit (ChSRU) and all members of 1205/1207 Laboratory, for their advices, encouragements, helps, and lovely friendship.

Finally, I would like to thank my family for their support, understanding and loves throughout my education.

CONTENTS

PAGE

PAGE

PAGE

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

LIST OF ABBREVIATIONS

mg/L	milligram per liter
µg/L	microgram per liter
µg/kg	microgram per kilogram
$CHCl_3$	chloroform
C_2Cl_4	tetrachloroethylene
DCM	dichloromethane
DLLME	dispersive liquid-liquid microextraction
ECD	electron-capture detector
EF	enrichment factor
EC	European commission
FID	flame ionization detector
FPD	flame photometric detector
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
HF-LPME	hollow fiber-liquid phase microextraction
HPLC	high-performance liquid chromatography
IL	Ionic liquid
L	liter
LDS	low density solvent
LLE	liquid-liquid extraction
LPME	liquid phase microextraction
LOD	limit of detection
LOQ	limit of quantitation
M	molar
mL	milliliter
MEKC	micellar electrokinetic chromatography
$MgSO_4$	magnesium sulfate
MRLs	maximum residue limits

MSD	mass selective detector
PAHs	polycyclic aromatic hydrocarbons
OCPs	organochlorine pesticides
OPPs	organophosphorus pesticides
s	second
SBSE	stir bar sorptive extraction
SDME	single drop microextraction
SIM	selected ion monitoring
SPME	solid phase microextraction

CHAPTER I

INTRODUCTION

1.1 Introduction

Pesticides are widely used in agriculture to control any pest by preventing, destroying, repelling or mitigating for ensure that a consistent supply of economical and high quality food. Large-scale use of pesticides began after World War II with the widespread use of organochlorine and organophosphorus compounds. Other chemical groups were subsequently developed and are used in agriculture e.g. carbamate compounds and synthetic pyrethroids. In spite of the several advantages, pesticides can be toxic to humans and animals. Their continuous application is causing serious problems for environmental and food contamination. Most pesticide residues occur in food as a result of the direct application of a pesticide to crop or farm animal or the post-harvest treatment of food commodities such as a grain to prevent attack. The small amounts of pesticides found in or on fruits, vegetables, grains, and other foods are called residues. However, they have no nutritional value and can potentially pose a risk to health in both short-term such as abdominal pain, dizziness, headaches, nausea, vomiting, as well as skin and eye problems and long term such as fertility problems, birth defects, brain tumors, breast cancer, prostate cancer, brain cancer, childhood leukemia for animals and humans. Government have a responsibility to regulate the food supply for ensure that foods offered to consumers are safe. In order to protect the health of the consumer, maximum residue limits (MRLs) has been established by the relevant authorities such as Codex Alimentarius [1], European Commission (EC) [2], United States Environmental Protection Agency (U.S.EPA) or national governments in Canada [3], Japan [4], Australia etc.

Pesticides can be classified according to the type of pests are herbicides, insecticides, fungicides, rodenticides etc. The most commonly used is insecticide, which can be grouped into chemical families include organochlorines, organophosphates, carbamates and pyrethroids.

Organochlorine pesticides (OCPs) were the first widely used group of synthetic insecticides, coming into use after World War II. The compounds contain carbon, chlorine, and hydrogen atoms. Their chlorine-carbon bonds are very strong which means that they do not break down easily. They are highly insoluble in water, but are attracted to fats. These chemicals were generally long-acting, controlling pests for an extended period of time, but many have been removed from the market due to their health and environmental effects and their persistence are potential to bioaccumulate [5]. Organochlorine pesticides have a wide range of both acute and chronic health effects, including cancer, neurological damage, and birth defects. Notable examples include DDT, dicofol, heptachlor, chlordane, aldrin, dieldrin and endrin.

Organophosphorus pesticides (OPPs) are the most widely used group of insecticides in the world. They are not persistent in the environment as they break down quickly. Because of their relatively fast rate of degradation, they have been a suitable replacement for the more persistent organochlorines. As well as being highly toxic to insects, they generally have quite high acute toxicity in mammals when be exposed to large amounts. Organophosphorus is the general name for esters of phosphoric acid. They act by inhibiting acetylcholinesterase, an enzyme which breaks down acetylcholine, a neurotransmitter chemical in both the central and peripheral nervous system [5]. Notable examples include parathion, malathion, methyl parathion, chlorpyrifos, diazinon, dichlorvos, fenitrothion and azinphos methyl.

In the present, pesticides have been registered with the EU about 1300 compounds and applied to agricultural crops. Therefore, pesticides can be residue in agricultural products and end up in food for human consumption. Government have a responsibility to regulate the food supply for ensure that foods offered to consumers are safe. In order to protect the health of the consumer, maximum residue limits (MRLs) of pesticide residues in food has been established [1, 2, 4]. The MRLs has been set at low level of pesticide in order to meet health concerns. For fruits and vegetables, MRLs of 10 μg/kg is applicable for all pesticides [1]. The low MRLs have encouraged the development of more sensitive analytical method to meet the requirement in complex samples. Therefore, it must be suitable analytical method for

monitoring the pesticide residues in agricultural products that have several classes of pesticide residues or multiresidue. Different extraction and quantification method are developed for estimation of multi class pesticide residues. The main criteria are that analytical method should be fast, easy, inexpensive and applicable to difference matrices.

Multiresidue analysis is difficult and complicate because of variety of polarities, solubilities, volatilities and pK_a values. Moreover, different chemical structures of each class of pesticide are required different detector. Gas chromatography is one of the technique used for separation of multiresidue to individual compounds, followed by detection with selective and sensitive detectors such as electron capture detector (ECD), nitrogen phosphorus detector (NPD), flame photometric detector (FPD) but each detector has selective for different classes of pesticides. Mass spectrometry is usually used as detector for multiresidue determination of multiclass pesticides because it is very sensitive for trace level and selective for a wide range of pesticide. Moreover, mass spectrometry is including both of qualitative- and quantitative- determination in one step.

The application of high sensitive analytical system like gas chromatography mass spectrometry (GC-MS) has been a powerful option. Nevertheless, a preconcentration of the compounds present mostly at trace levels prior to analysis is absolutely necessary for extraction, isolation and enrichment of analytes from sample matrix. Liquid- liquid extraction (LLE) is a classic preconcentration technique that has long been used for routine analysis of pesticides. Common extraction techniques used in preconcentration are liquid-liquid extraction (LLE) and solid-phase extraction (SPE), which are time-consuming, labor intensive and tedious. Moreover, LLE have required the use of large amount of organic solvent. Microextraction techniques, such as solid-phase microextraction (SPME) and liquid-phase microextraction (LPME), have been developed for preconcentration of pesticides. Solid-phase microextraction (SPME) is a solvent free technique that uses solid polymeric fiber for extraction and preconcentration of analytes directly from an aqueous and solid sample. This technique is fast, easy to use but its fiber is fragile and has limited lifetime.

Liquid-phase microextraction has been developed as solvent-minimized sample pretreatment procedure, which uses very little solvents and minimal exposure to toxic organic solvents. Single drop microextraction (SDME) is a one of such method that uses only one organic solvent drop for extraction. However, disadvantages of this technique are fast stirring which may break up the organic solvent drop, time-consuming for extraction and in most cases equilibrium is not attained even after a long time.

Dispersive liquid-liquid microextraction (DLLME) was developed by Razaee [6] from liquid phase micro-extraction, which used the extraction solvent in microliter level and high performance to pre-concentration. Nevertheless, the extraction solvents in DLLME, such as chloroform, dichloromethane, carbon tetrachloride, tetrachloroethylene and chlorobenzene, are extremely toxic and environmental unfriendly. Another DLLME method was then proposed by Leong [7], which based on a solidification of floating organic droplet (DLLME-SFO). This method used a lower density and toxicity organic solvent as an extraction solvent for determination of organochlorines. However, the process of DLLME-SFO is more complicated than DLLME and a sediment phase is difficult to separate causing a loss of analytes. In addition, Kocúrová et al. [8] developed another DLLME method based on the use of an auxiliary solvent for adjustment of density (DLLME-AS) for determination of gold. A low-density organic solvent as a extraction solvent and a high-density organic solvent as an auxiliary solvent for adjusting the density of a low-density organic solvent was used. As a result, the density of mixed solvent was higher than water and a phase separation by centrifugation and recovery of the extract were simplified same as the conventional DLLME.

Experimental design is the process of planning a study to meet specified objectives and was applied in analytical chemistry to obtain the optimum conditions for analysis and can be study many variables simultaneously, thus reducing the time spent in analysis. Xia et al. [9] used central composite design to study main parameters such as disperser solvent volume, extraction solvent volume and sample volume for determination of metacrate in water samples using dispersive liquid–liquid microextraction. Ravelo-Pérez et al. [10] used central composite design to study main

factors affecting the DLLME extraction yield such as sample pH, NaCl percentage, ionic liquid as extraction solvent amount and disperser volume for determination of pesticides in banana samples using ionic liquid based dispersive liquid–liquid microextraction.

In this research, dispersive liquid-liquid microextraction (DLLME) based on the auxiliary solvent was developed for determination of organochlorine and organophosphorus residues by gas chromatography-mass spectrometry (GC-MS). Several factors such as the type of extraction solvent, auxiliary solvent and disperser solvent; the ratio of extraction solvent and auxiliary solvent; the volume of extraction solvent, auxiliary solvent and disperser solvent; centrifugation time were optimized. Moreover, experimental design was used for studied some parameters affecting to extraction efficiency of DLLME.

1.2 Objectives of the research

To develop a dispersive liquid-liquid microextraction using an auxiliary solvent for adjusting the density of low-density extraction solvent as a sample preparation method for determination of organophosphorus and organochlorine residues.

1.3 Scope of this research

1.3.1 To optimize the parameters affecting the performance of DLLME using an auxiliary solvent method for determination of organophosphorus and organochlorine residues. Type of auxiliary, extraction and disperser solvent was selected by varying the method and volume of auxiliary, extraction and disperser solvent and centrifugation time were optimized by experimental design.

1.3.2 To evaluate the method and apply for determination of organophosphorus and organochlorine residues in real sample.

1.4 Benefit of this research

A sample preparation method for determination of multipesticide residues is obtained, that is simple, cheap, rapid, high enrichment factor and reduced the use of toxic solvent, which is suitable for determination of organophosphorus and organochlorine residues in real sample.

CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Sample preparation techniques for pesticide residues

2.1.1 Solvent extraction

Solvent extraction (SE) is a method to separate compounds based on their relative solubility. The extraction process depending on the sample type is liquid or solid. For liquid sample, solvent extraction is called liquid-liquid extraction (LLE). LLE is based on distribution of analytes between two immiscible liquid, usually aqueous sample and organic solvent. For solid sample, solvent extraction is called solid-liquid extraction (SLE). SLE is based on partitioning of analytes between solid phase and liquid phase, usually solid sample and organic solvent. An organic solvent is added to a solid sample. Insoluble material can be separated by gravity or vacuum filtration, and soluble material is extracted into the solvent. The extraction efficiency can be increased by selecting parameters such as type of organic solvent, salt addition for salting out effect, and adjusting pH of the sample.

However, solvent extraction has involved drawback e.g. complicate, time-consuming procedures, large amount of organic solvent, formation of emulsion, and discontinuous extraction. Despite these disadvantages, SE is also widely used in the extraction of the sample in the first step prior to analysis by other methods because of its simplicity, robustness and efficiency.

2.1.2 Solid phase extraction (SPE)

Solid phase extraction (SPE) is a simple preparation technique based on the partition between a liquid phase (sample solution) and a solid phase (sorbent). This sample preparation technique enables to concentrate and purify analytes from solution by sorption on a sorbent and purification of the extract after extraction. The general procedure is to load a sample solution into the SPE cartridge, wash to

eliminate the interference, and then wash off the analytes with another solvent into a collection tube. The benefits of SPE over solvent extraction are rapid, high selectivity, ease of removal of the sorbent from the sample solution, elimination of the formation of emulsion and low amount of organic solvent. However, SPE methods provide low recovery and poor reproducibility. SPE have many sizes, shapes and types of sorbent as shown in Figure 2.1. Selection of the suitable SPE type is important for extraction.

Figure 2.1 The various type and size of SPE cartridge

2.1.3 Matrix solid phase dispersion (MSPD)

Matrix solid phase dispersion (MSPD) is sample preparation procedure for extraction of various solid and semi-solid samples. MSPD involved direct mechanical blending of sample with a solid support (sorbent), usually florisil, C_{18}, alumina or silica. In this procedure, the sorbent serves as an abrasive that induce disruption of the sample architecture and acts as a bound solvent that assist in accomplishing complete sample disruption and dispersion. After homogenization, blended sample is packed into column and then eluted with suitable eluent. Steps in a typical MSPD extraction was shown in Figure 2.2.

The advantage of MSPD over SPE is type of sample, for SPE, sample must be in liquid state while MSPD can be used for both solid and viscous liquid sample.

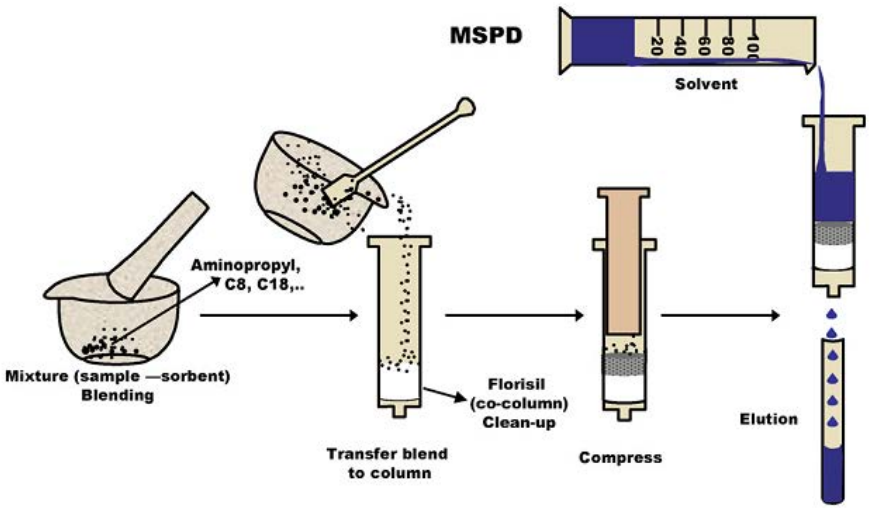


Figure 2.2 Steps in a typical MSPD extraction [11]

2.1.4 QuEChERS

QuEChERS is quick, easy, cheap, effective, rugged and safe and was developed by Anastassiades in 2003 for the multiclass and multiresidue analysis of pesticides in fruits and vegetables [12]. QuEChERS approaches typically use acetonitrile for extraction of well homogenized sample followed by using anhydrous magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) for phase separation. After centrifugation, take an aliquot of the organic phase and subject it to dispersive solid phase extraction (d-SPE) cleanup by mixing with anhydrous $MgSO_4$ and a primary secondary amine (PSA) and graphitized carbon black (GCB) sorbent for remove interferences such as fatty acid and pigment. After sample clean up step, the mixture is centrifuged and the supernatant can be directly analyze or subject to concentration. The advantages of QuEChERS procedure are high recovery, high sample throughput, low solvent uses, and ruggedness.

2.1.5 Liquid phase microextraction (LPME)

Liquid phase microextraction is a solvent-minimizes sample preparation procedure of LLE, in which only microliter level of solvent are required to concentrate analytes from sample rather than hundreds of milliliter needed in traditional LLE. In LPME, extraction normally takes place into a small amount of a water-immiscible solvent from an aqueous sample containing analytes.

2.1.5.1 Single drop microextraction (SDME)

Single drop microextraction is sample preparation procedure that use only one drop of an organic solvent at the tip of a microsyringe to extract analytes from sample. After extraction, the microdrop is retracted back into the syringe an transferred for further analysis. SDME is divided into two types that are direct-immersion (DI)-SDME and headspace (HS)-SDME as represented in Figure 2.3. SDME has some disadvantages, including the microdroplet unstable resulting in poor reproducibility and limitation by small droplet volume resulting in low sensitivity.

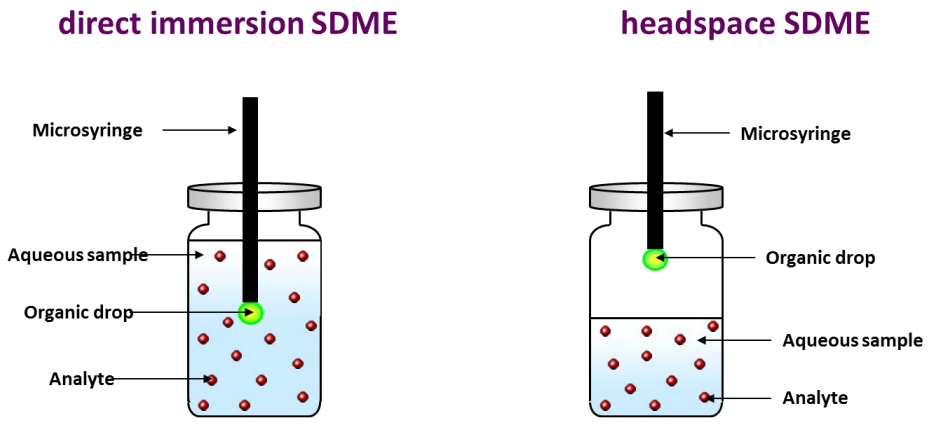


Figure 2.3 Single drop microextraction procedure.

2.1.5.2 Hollow fiber liquid phase microextraction (HF-LPME)

Hollow fiber liquid phase microextraction is the sample preparation procedure that uses the hollow fiber membrane for supporting the extraction solvent inside the porous wall. In HF-LPME system, the microvolume of the organic solvent is contained within the porous wall of the hollow fiber membrane, so the organic solvent is not directly contact with the sample solution. The major advantage of this technique is the stability of organic solvent, so it is not easily lost into the aqueous solution when stirred vigorously.

HF-LPME can be classified into two modes: three-phase and two-phase HF-LPME. In three-phase HF-LPME, an organic solvent is immobilized in the pores in the wall of the hollow fiber, and an aqueous acceptor solution is held within the lumen. The analytes are extracted into the organic phase and subsequently into the aqueous phase as shown in Figure 2.4 (a). Another mode of HF-LPME is based on a two-phase system in which the organic solvent is used to fill both the pores in the wall and the lumen of the hollow fiber membrane, as shown in Figure 2.4 (b).

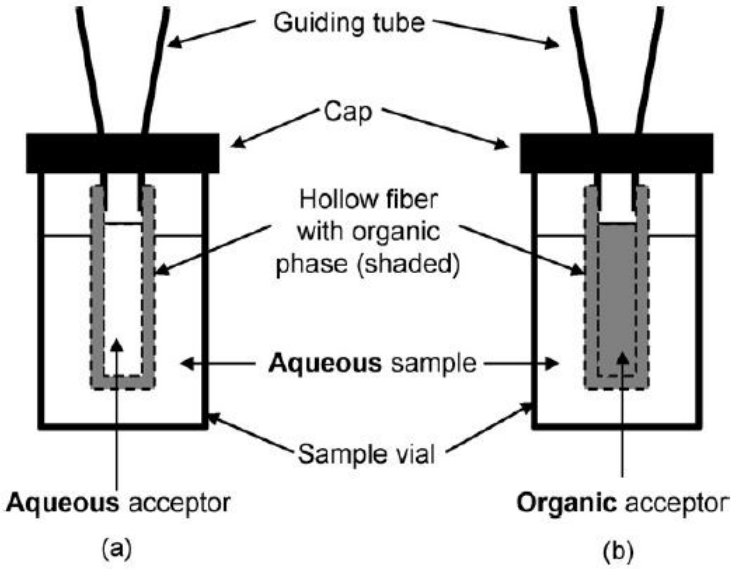


Figure 2.4 Hollow fiber liquid phase microextraction
(a) three-phase (b) two-phase HF-LPME [13]

2.2 Dispersive liquid-liquid microextraction

Dispersive liquid-liquid microextraction (DLLME) was developed by Razaee [6] from liquid phase microextraction for the determination of polycyclic aromatic hydrocarbons (PAHs) in water sample.

2.2.1 Conventional DLLME

DLLME is method using microliter level of organic solvent and high performance to pre-concentration, which is based on equilibrium distribution of the target analytes between sample solution and organic solvent. DLLME procedure is based on a ternary component solvent system in which the mixture of extraction solvent and disperser solvent is rapidly injected into aqueous sample. The extraction is enhanced by the formation of small droplets of extraction solvent in the aqueous sample. After centrifugation, the sediment phase at the bottom is removed by microsyringe and can directly analyzed by chromatography technique. The extraction steps of DLLME are illustrated in Figure 2.5.

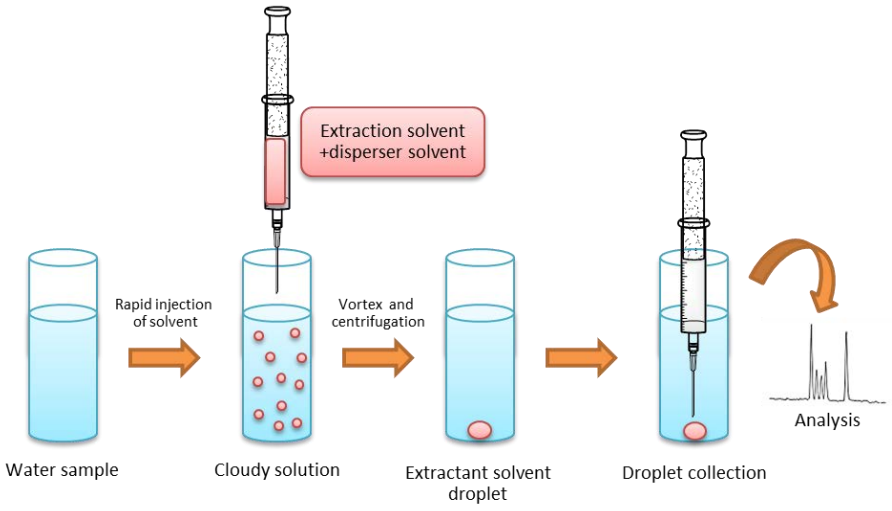


Figure 2.5 Conventional dispersive liquid-liquid microextraction procedure.

When the mixed solution of extraction and disperser was dispersed into the aqueous sample as very fine droplets, a cloudy state was formed. The analytes are enriched into the extraction solvent phase because of the large surface area between the extraction solvent and the aqueous sample, equilibrium state is achieved quickly and the extraction is independent of time. This is the most important advantage of this method. Moreover, the advantages of DLLME are simple, rapid, high enrichment factor and reduced the use of organic solvent.

There are many parameters affecting extraction efficiency of DLLME such as the type of extraction and disperser solvent, the volume of extraction and disperser solvent, and centrifugation time

2.2.1.1 Selection of extraction solvent

The main parameter of DLLME is the selection of extraction solvent. The extraction solvent for DLLME should be higher density than water, low solubility in water, extraction capability of interested compounds and good chromatographic behavior. The example of extraction solvents suitable for DLLME is chlorobenzene, chloroform, tetrachloroethylene and carbontetrachloride.

The extraction solvent volume has significant effect on the enrichment factor. The increase of the extraction solvent volume causes the increase of sedimented phase after centrifugation. Meanwhile, the target analyte concentration is decreased, that means enrichment factor is decreased. So the suitable extraction solvent volume should ensure that both the high enrichment factor and sufficient volume for determination after centrifugation.

2.2.1.2 Selection of disperser solvent

Disperser solvent for DLLME must be soluble in both extraction solvent and aqueous sample to be dispersed a fine droplet in the aqueous sample for the formation of cloudy solution. This can form the large surface area between extraction solvent and the target analytes for increasing of the extraction efficiency. Acetone, methanol and acetonitrile are usually selected as disperser solvents.

The disperser solvent volume directly involves the dispersion degree of the extraction solvent in aqueous phase, the formation of cloudy solution, and the extraction efficiency. Variation of disperser solvent volume changes the volume of sedimented phase. Consequently, the disperser solvent must have the appropriate volume to achieve a constant volume of sedimented phase.

2.2.2 Development of DLLME

DLLME has been widely applied for pesticide residue analysis. Many studies were reported. Assadi et al. developed another method of DLLME for the determination of organophosphorus pesticides [14], trihalomethanes [15], chlorophenols [16], polychlorinated biphenyls [17], heavy metal [18], etc. Nevertheless, the extraction solvents frequently used in conventional DLLME, such as chloroform, dichloromethane, carbon tetrachloride, tetrachloroethylene and chlorobenzene, are extremely toxic and environmental unfriendly.

2.2.2.1 Low density solvent-based DLLME (LDS-DLLME)

Another DLLME method was proposed by Leong, which based on a solidification of floating organic droplet (DLLME-SFO) [7]. This method used a lower density and toxicity organic solvent as an extraction solvent. The extraction steps of DLLME-SFO are shown in Figure 2.6.

However, the limitation of DLLME-SFO is extraction solvent must satisfy the following requirement: (i) it must have the lowest volatility possible in order to avoid losses of analytes during the extraction; (ii) it must have low solubility in the water possible; (iii) it must have a melting point near room temperature and more than melting point of water; and (iv) it must be compatible with the analytical instrumentation to used for determination of the analyte. Furthermore, DLLME-SFO requires an additional step that is cooling in an ice bath for solidification of the extraction phase.

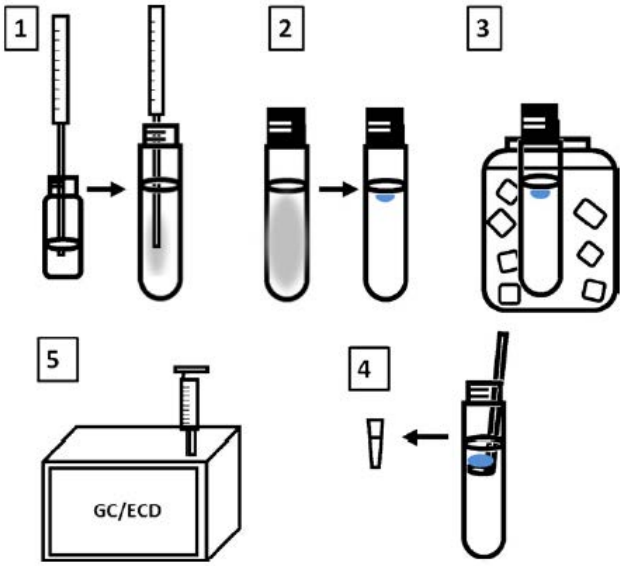


Figure 2.6 Dispersive liquid-liquid microextraction based on a solidification of floating organic droplet (DLLME-SFO) procedure [7].

In 2009, Farajzadeh et al. [19] designed a special vessel for extraction as illustrated in Figure 2.7. After centrifugation, the extraction phase is collected at the top of the aqueous phase, raised to the narrow part of the vessel by the injection of water via a septum at the bottom of extraction vessel. This approach eliminates the limitations of DLLME-SFO. After that, the vessel has been developed in different ways [20, 21] as shown in Figure 2.8. The extraction phase is always removed from the narrow parts of the devices. All of the devices also demonstrate the advantages and drawbacks in terms of ease of operation and difficulty of making extraction vessel.

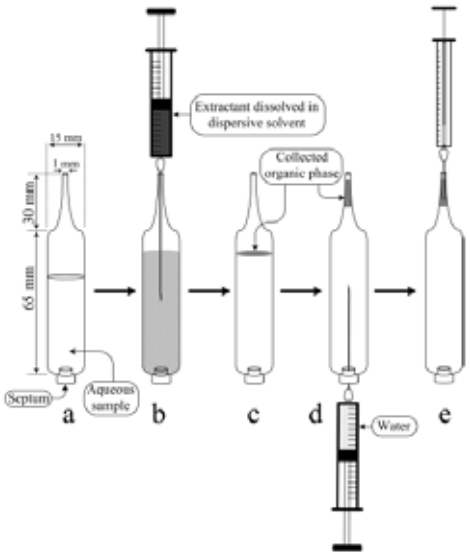


Figure 2.7 Dispersive liquid-liquid microextraction procedure using an extraction solvent lighter than water [19]. (a) the sample solution, (b) injection, (c) after centrifugation, (d) raise the sample surface by inject water into the extraction vessel, and (e) the collected phase was subjected using a syringe.

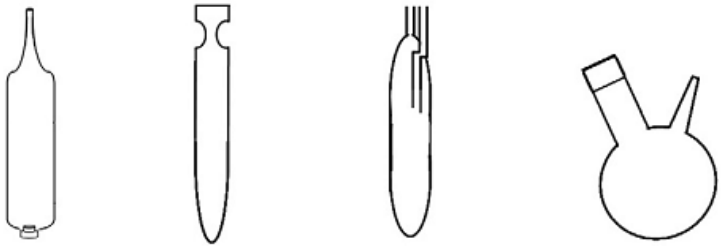


Figure 2.8 The design of extraction vessel for liquid-liquid micro extraction using low density solvent [22].

2.2.2.2 DLLME based on the use of an auxiliary solvent for adjustment of density

In 2010, Kocúrová et al. [8] developed a new method of DLLME based on the use of an auxiliary solvent for adjustment of density (DLLME-AS). This procedure was used a quaternary system consisting of a low-density organic solvent as a extraction solvent and a high-density organic solvent as an auxiliary solvent for adjusting the density of a low-density organic solvent that was mixed with a disperser solvent, then was injected into the aqueous sample. As a result, the density of mixed solvent was higher than water and a phase separation by centrifugation and recovery of the extract were simplified same as the conventional DLLME.

Furthermore, the benefits of this method are not required the use of special devices and the reduction of toxic organic solvent in conventional DLLME. The process of DLLME based on the use of an auxiliary solvent for adjustment of density is shown in Figure 2.9. The applications of DLLME for pesticide residues analysis are shown in Table 2.1.

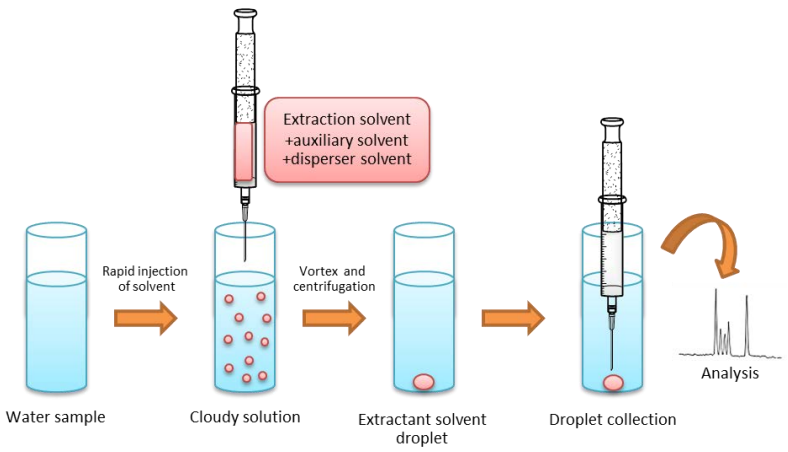


Figure 2.9 The process of DLLME based on the use of an auxiliary solvent for adjustment of density.

Table 2.1 The application of dispersive liquid-liquid microextraction for pesticides analysis.

Method	Analytes	Matrix	Extraction solvent	Disperser solvent	Detection	Enrichment factor (EF), limit of detections (LODs)	Ref.
DLLME	13 Organophosphorus pesticides	River, weel and farm water	Chlorobenzene	Acetone	GC-FPD	EF: 789-1070 LODs: 3-20 µg/L	[14]
DLLME	6 Organophosphorus pesticides	Watermelon and cucumber	Chlorobenzene	Acetonitrile	GC-FPD	EF: 41-50 LODs: 0.5-20 µg/kg	[23]
DLLME	8 Triazine herbicides	Water	Chlorobenzene	Acetone	GC-MS	EF: 151-722 LODs: 1 µg/L	[24]
DLLME	Methomyl	Natural water	Tetrachloroethane	Methanol	HPLC-UV	EF: 70.7	[25]
DLLME	2 Phenoxyacetic acid herbicides	Water	Chlorobenzene	Acetone	HPLC-UV	LODs: 0.16 µg/L	[26]
IL-DLLME	4 Heterocyclic insecticides	Water	$[C_6MIM][PF_6]$	Methanol	HPLC-UV	EF: 209-276 LODs: 0.53–1.28 µg/L	[27]

Table 2.1 The application of dispersive liquid-liquid microextraction for pesticides analysis (continued)

Method	Analytes	Matrix	Extraction solvent	Disperser solvent	Detection	Enrichment factor (EF), limit of detections (LODs)	Ref.
SPE-DLLME	7 Fungicides	Wine	1,1,1-trichloroethane	Acetone	GC-ECD, GC-MS	EF: 156-254	[28]
DLLME	3 Organophosphorus pesticides	Water	Cyclohexane	Acetone	GC-FID	EF: 100-110 LODs: 3-4 μg/L	[19]
IL-DLLME	4 Organophosphorus pesticides	Water	$[C_8MIM][PF_6]$	Methanol	HPLC-UV	EF: >200 LODs: 0.1-5.0 μg/L	[29]
IL-DLLME	4 Organophosphorus pesticides	Water	$[DBIM][PF_6]$	Methanol	HPLC-UV	LODs: 10-50 ng/L	[30]
QuEChERS-DLLME	8 Pesticides	Banana	$[C_6MIM][PF_6]$	Methanol	HPLC-UV	LODs: 0.320-4.66 μg/kg	[10]
QuEChERS-DLLME	8 Pesticides	Grapes and plums	$[C_6MIM][PF_6]$	Methanol	HPLC-UV	LODs: 0.651-6.33 μg/kg	[31]

Table 2.1 The application of dispersive liquid-liquid microextraction for pesticides analysis (continued)

Method	Analytes	Matrix	Extraction solvent	Disperser solvent	Detection	Enrichment factor (EF), limit of detections (LODs)	Ref.
UA-IL-DLLME	4 Benzoylurea Pesticides	Water	$[C_6MIM][PF_6]$	-	HPLC-UV	LODs: 0.21-0.45 µg/L	[32]
SBSE-DLLME	7 Triazole pesticides	Water	1,1,2,2-tetrachloroethane	Methanol	GC-FID	EF: 282-1792 LODs: 0.53-24 µg/L	[33]
DLLME	6 Carbamate pesticides	Apples	Chloroform	Acetone	MEKC	EF: 491-1834 LODs: 2-3 µg/kg	[34]
DLLME	5 N-methyl carbamate pesticides	Vegetables	Chloroform	Acetonitrile	HPLC-DAD	EF: 789-1070 LODs: 3-20 µg/L	[33]
DLLME	4 Organochlorine pesticides	Water	Carbon tetrachloride	Acetonitrile	HPLC-DAD	EF: 100 LODs: 0.32-0.51 µg/L	[35]

2.3 Experimental design

Experimental design is the process of planning a study to meet specified objectives. Planning an experiment properly is very important in order to ensure that the right type of data and a sufficient sample size and power are available to answer the research questions of interest as clearly and efficiently as possible.

Experimental design procedures are following:

(a) Problem statement
(b) Selection of factor and determine the level
(c) Selection of response
(d) Selection of experimental design type
(e) Perform the experiment
(f) Data analysis
(g) Conclusion

Central composite design is one type of experimental design that combine the three types of design consisting of a full factorial design, a star design and five replicates (Figure 2.10). A full factorial design is common experimental design which all input factors set at two levels each. These levels are called low and high. A design of full factorial has high/low combinations of all the input factors. A star design consists of the centre point and a point in the middle of each of the six faces of the cube. Finally, a five replicates is often important to estimate the error, and this is typically performed by repeating the experiment in the centre of the design five times [36]. The example of central composite design for three factors was represented in Table 2.2. The level of factor must be converted into a code for standardize. The low, medium and high levels are assigned as -1, 0 and +1, respectively. The star points are locate at $+\alpha$ and $-\alpha$ from the center of the experimental domain. The value of α depends on the number of experimental runs in the factorial portion of the central composite design is determined by $\alpha = [2^k]^{1/4}$, where k is the number of factors. After completion of the experiment, the information will be processed by the statistical program. The response surface plots were then obtained as example in Figure 2.11. The optimum condition can then select or observe from the graph.

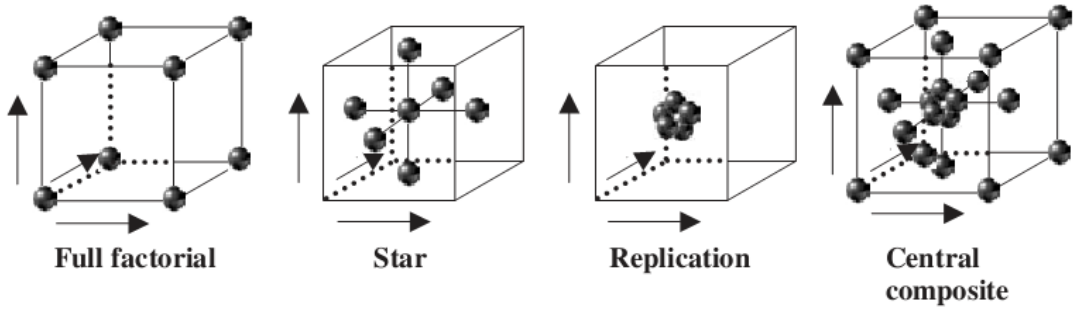


Figure 2.10 The construction of three factor central composite design [36].

Table 2.2 A three factor central composite design consisting of a full factorial design, a star design and replication for experimental.

Factor	A	B	C
Full factorial	-1	-1	-1
	-1	-1	1
	-1	1	-1
	-1	1	1
	1	-1	-1
	1	-1	1
	1	1	-1
	1	1	1
Star	-α	0	0
	α	0	0
	0	-α	0
	0	α	0
	0	0	-α
	0	0	α
	0	0	0
Replication	0	0	0
	0	0	0
	0	0	0
	0	0	0
	0	0	0

Factor	A	B	C
Central composite	-1	-1	-1
	-1	-1	1
	-1	1	-1
	-1	1	1
	1	-1	-1
	1	-1	1
	1	1	-1
	1	1	1
	-α	0	0
	α	0	0
	0	-α	0
	0	α	0
	0	0	-α
	0	0	α
	0	0	0
	0	0	0
	0	0	0
	0	0	0
	0	0	0
	0	0	0

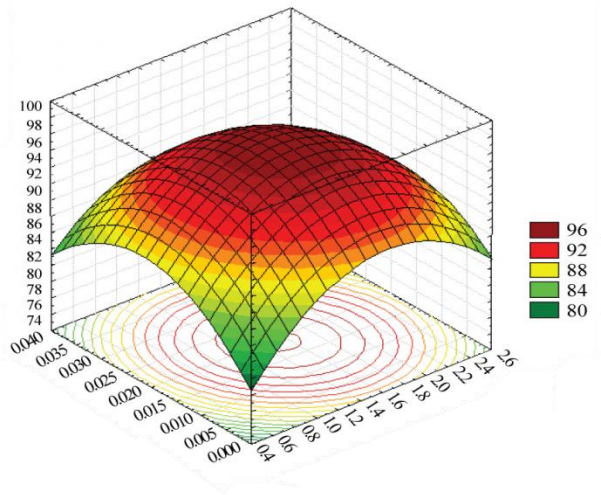


Figure 2.11 The example of response surface plots were obtained from experimental design

Experimental design was applied in analytical chemistry to obtain the optimum conditions for analysis and can be study many variables simultaneously, thus reducing the time spent in analysis. Xia et al. [9] used central composite design to study main parameters such as disperser solvent volume, extraction solvent volume and sample volume for determination of metacrate in water samples using dispersive liquid–liquid microextraction. Ravelo-Pérez et al. [10] used central composite design to study main factors affecting the DLLME extraction yield such as sample pH, NaCl percentage, ionic liquid as extraction solvent amount and disperser volume for determination of pesticides in banana samples using ionic liquid based dispersive liquid–liquid microextraction.

CHAPTER III

EXPERIMENTAL

Instruments, equipments, reagents, chemical, procedure and method modification are explained in this chapter.

3.1 Instruments and equipments

3.1.1 GC-MS instrumentation (Agilent, USA)

3.1.2 Food chopper (Moulinex, France)

3.1.3 Balance (Mettler-Toledo, USA)

3.1.4 Freezer

3.1.5 Homogenizer (Hettich, Germany)

3.1.6 15 mL centrifuge tube with screw caps

3.1.7 50 mL centrifuge tube with screw caps

3.1.8 Autopipettes and tips 0.5-10 µL, 10-200 µL, 100-1000 µL, and 1-10 mL (Eppendorf, USA)

3.1.9 Vortex mixer

3.1.10 Centrifuge (Hettich, Germany)

3.1.11 10 µL microsyringe (SGE, Australia)

3.1.12 3 mL disposable syringe (Nipro, Thailand)

3.1.13 Medical syringe needle with O.D. x length: 0.55 x 25 (mm) (Nipro, Japan)

3.1.14 2 mL vials

3.1.15 250 µL glass insert vial

3.1.16 Volumetric flask with stopper

3.1.17 Nitrogen evaporator

3.2 Chemicals and reagents

All chemicals and reagent for this work are shown in Table 3.1 and Table 3.2. Structure, octanol-water partition coefficient, and the regulation in water and tomato of studied pesticides are summarized in Table 3.3.

Table 3.1 List of pesticide standards

Pesticides	Concentration	Suppliers
1. Chlorpyrifos	100 µg/mL	Dr.Ehrenstorfer (Germany)
2. Diazinon	100 µg/mL	Dr.Ehrenstorfer (Germany)
3. alpha-Endosulfan	100 µg/mL	Dr.Ehrenstorfer (Germany)
4. beta-Endosulfan	100 µg/mL	Dr.Ehrenstorfer (Germany)
5. Endrin	100 µg/mL	Dr.Ehrenstorfer (Germany)
6. Fenitrothion	100 µg/mL	Dr.Ehrenstorfer (Germany)
7. Heptachlor	100 µg/mL	Dr.Ehrenstorfer (Germany)
8. Hexachlorobenzene (HCB)	100 µg/mL	Dr.Ehrenstorfer (Germany)
9. Malathion	100 µg/mL	Dr.Ehrenstorfer (Germany)
10. Pirimiphos-methyl	100 µg/mL	Dr.Ehrenstorfer (Germany)

Table 3.2 List of chemicals

Chemicals	Suppliers
1. Acetone	J.T.Baker (USA)
2. Acetonitrile	J.T.Baker (USA)
3. Carbon disulfide (CS_2)	J.T.Baker (USA)
4. Chloroform ($CHCl_3$)	J.T.Baker (USA)
5. Tetrachloroethylene (C_2Cl_4)	J.T.Baker (USA)
6. Cyclohexane	J.T.Baker (USA)
7. Dichloromethane (CH_2Cl_2)	J.T.Baker (USA)
8. *n*-Heptane	J.T.Baker (USA)
9. 1-Octanol	J.T.Baker (USA)
10. Toluene	J.T.Baker (USA)
11. *o*-Xylene	J.T.Baker (USA)
12. Magnesium sulfate anhydrous ($MgSO_4$)	Panreac (E.U.)
13. Sodium chloride (NaCl)	J.T.Baker (USA)
14. Primary secondary amine (PSA)	Supelco (USA)
15. Graphite carbon black (GCB)	Supelco (USA)

Table 3.3 Pesticide structure, octanol-water partition coefficient, and the regulation in water and tomato.

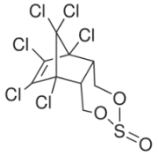
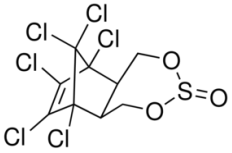
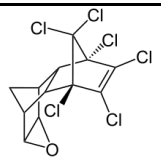
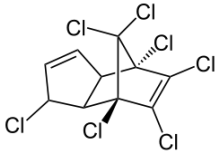
Pesticide name	Category	Structure	Log P_{ow}	Health value in drinking water (µg/L) [37]	MRLs in tomato (µg/kg) [2]
alpha-Endosulfan	Organochlorine		4.74	30	50
beta-Endosulfan	Organochlorine		4.79	30	50
Endrin	Organochlorine		5.34	2	10
Heptachlor	Organochlorine		5.27	0.4	10

Table 3.3 Pesticide structure, octanol-water partition coefficient, and the regulation in water and tomato. (continued)

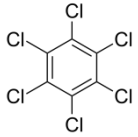
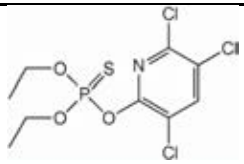
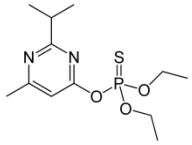
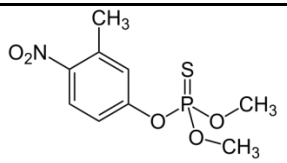
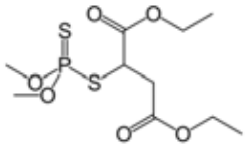
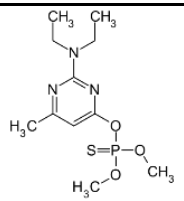
Pesticide name	Category	Structure	Log P_{ow}	Health value in drinking water (µg/L) [37]	MRLs in tomato (µg/kg) [2]
Hexachlorobenzene (HCB)	Organochlorine		5.20	1	10
Chlorpyrifos	Organophosphorus		4.96	20	500
Diazinon	Organophosphorus		3.81	0.6	10
Fenitrothion	Organophosphorus		3.30	10	10

Table 3.3 Pesticide structure, octanol-water partition coefficient, and the regulation in water and tomato. (continued)

Pesticide name	Category	Structure	Log P_{ow}	Health value in drinking water (µg/L) [37]	MRLs in tomato (µg/kg) [2]
Malathion	Organophosphorus		2.36	100	20
Pirimiphos-methyl	Organophosphorus		4.20	50	20

3.3 Chemical preparation

3.3.1 Intermediate mixed standard solution, 10 µg/mL

Intermediate mixed standard solution of 10 µg/mL was prepared by diluting the stock standard solution 100 µg/mL of each pesticide compound into a 10 mL volumetric flask with ethyl acetate. Calculate using equation (3.1)

$$C_1V_1 = C_2V_2 \quad (3.1)$$

C_1 = concentration of the stock solution (µg/mL)

V_1 = the volume of the stock solution (mL)

C_2 = final concentration (µg/mL)

V_2 = final volume (mL)

3.3.2 Working standard solutions

Standard mixture for calibration curve in the range of 0.1-2.0 µg/mL was prepared by diluting intermediate mixed standard solution 10 µg/mL into a 10 mL volumetric flask with ethyl acetate.

3.4 Instrumentation

Chromatographic analysis was performed on Agilent 6890 series (Agilent, USA) gas chromatography equipped with split/splitless injector and Agilent 5973N mass spectrometer. Separation was performed using a HP-5MS capillary column with 30 m x 0.25 mm I.D. and 0.25 µm film thickness (Agilent, USA). The carrier gas was helium (99.9995%) at constant flow rate of 1.6 mL/min. The injection port was set at 280 ºC in the splitless mode. The oven temperature was programmed as initially held at 100 ºC for 1 min, increased to 150 ºC at the rate of 10 ºC/min, then increased to 194 ºC at 8 ºC/min (held 1 min) and increased to 220 ºC at 10 ºC/min and held at 280 ºC for 10 min. the MS transfer line temperature was held at 280 ºC. Mass spectrometric parameter was set as follows: electron ionization (EI) energy, 70 eV; ion source temperature, 230 ºC and MS quadrupole temperature, 150 ºC. The selected ion monitoring (SIM) mode was used for determination of target compounds as shown in Table 3.4.

Table 3.4 Ion selected in SIM mode for pesticides analysis by GC-MS.

Pesticides	Retention time (min)	SIM ions (m/z)			
		Target ion	Q1	Q2	Q3
1. Hexachlorobenzene	11.434	284.0	286.0	282.0	288.0
2. Diazinon	12.648	179.0	137.0	152.0	199.0
3. Heptachlor	14.132	272.0	274.0	100.0	270.0
4. Fenitrothion	14.786	277.0	125.0	109.0	260.0
5. Pirimiphos-methyl	14.853	290.0	276.0	305.1	233.0
6. Malathion	15.096	173.1	127.0	125.0	93.0
7. Chlorpyrifos	15.356	197.0	199.0	314.0	97.0
8. alpha-Endosulfan	17.100	239.0	241.0	237.0	195.0
9. Endrin	18.626	263.0	281.0	261.0	265.0
10. beta-Endosulfan	19.018	195.0	237.0	241.0	239.0

3.5 Procedure

3.5.1 DLLME step

3.5.1.1 Extraction solvent and auxiliary solvent were added into disperser solvent, and then shake the tube by vortex to combine.

3.5.1.2 The mixed solvent from 3.5.1.1 was injected into 5 mL of water in 15 mL centrifuge tube. A cloudy solution was formed.

3.5.1.3 The centrifuge tube was shaken by vortex for 1 min. Then was centrifuged the tube at 5000 rpm for 7 min.

3.5.1.4 The extracted phase was sedimented in the bottom of centrifuge tube. The sedimented phase was removed by 10 μL of microsyringe and directly analyzed by GC/MS.

3.5.2 Selection of auxiliary solvent

Tetrachloroethylene (C_2Cl_4), chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2) and carbon disulfide (CS_2) were studied as auxiliary solvent. 30 µL of auxiliary solvent was mixed with 10 µL of toluene as extraction solvent and 1.4 mL of acetonitrile as disperser solvent.

3.5.3 Selection of extraction solvent

Toluene, *o*-xylene, cyclohexane, *n*-heptane and 1-octanol were studied as extraction solvent. 30 µL of auxiliary solvent was mixed with 10 µL of extraction solvent and 1.4 mL of acetonitrile as disperser solvent.

3.5.4 Selection of mixed solvent ratio

A1:0, 1:1, 2:1 and 3:1 ratios of C_2Cl_4: *n*-heptane were studied as mixed solvent ratio. 40 µL of mixed solvent of C_2Cl_4: *n*-heptane was mixed with 1.4 mL of acetonitrile as disperser solvent.

3.5.5 Selection of disperser solvent

Acetonitrile, acetone, methanol and ethanol were studied as disperser solvent. 30 µL of C_2Cl_4 as auxiliary solvent was mixed with 10 µL of toluene as extraction solvent and 1.4 mL of disperser solvent.

3.6 Experimental design

A central composite design was selected to study the factors affecting the DLLME extraction recovery such as mixed solvent volume, disperser solvent volume and centrifugation time. The value of each level was defined by set the range of study. The low and high values were represented by -1 and +1 code, and then the central level was represented by 0 code. The star points are locate at +α and −α from the center of the experimental domain. The value of α depends on the number of experimental runs in the factorial portion of the central composite design is determined by $\alpha = [2^k]^{1/4}$, where k is the number of variables. In this work, 3 variables were determined. So the α value is 1.682. The design matrix for the 2^3 central composite designs was shown in Table 3.5.

The resulting 20 experiments were carried out randomly as shown in Table 3.6, using 5 mL of spiked Milli-Q water at concentration of 5 µg/L of each pesticide, mixed solvent of tetrachloroethylene as auxiliary solvent and *n*-heptane as extraction solvent with 3:1 ratio, acetonitrile as disperser solvent.

Table 3.5 Design matrix for the 2^3 central composite design

Factors	**Levels**			**Star point α=1.682**	
	Low (-1)	**Central (0)**	**High (+1)**	**-α**	**+α**
Mixed solvent volume (µL) (X_1)	30	40	50	23.18	56.82
Disperser solvent volume (mL) (X_2)	0.8	1.4	2.0	0.39	2.41
Centrifugation time (min) (X_3)	4	7	10	2	12

Table 3.6 The 20 experiments of DLLME for determination of pesticide residues

experiment	X_1 (μL)	X_2 (mL)	X_3 (min)	experiment	X_1 (μL)	X_2 (mL)	X_3 (min)
1	30	0.8	4	11	40	0.39	7
2	30	0.8	10	12	40	2.41	7
3	30	2.0	4	13	40	1.4	2
4	30	2.0	10	14	40	1.4	12
5	50	0.8	4	15	40	1.4	7
6	50	0.8	10	16	40	1.4	7
7	50	2.0	4	17	40	1.4	7
8	50	2.0	10	18	40	1.4	7
9	23.18	1.4	7	19	40	1.4	7
10	56.82	1.4	7	20	40	1.4	7

3.7 Data analysis

The sample concentration, extraction recovery, enrichment factor (EF), standard deviation (SD) and relative standard deviation (RSD) were calculated from the equation below:

$$C_0 = \frac{C_{sed} \times V_{sed}}{V_{sample}} \quad (3.2)$$

$$C_{sed} = \frac{y-b}{m} \quad (3.3)$$

$$Enrichment\ factor = \frac{C_{sed}}{C_0} \quad (3.4)$$

$$\%\ Extraction\ recovery = \frac{C_{sed} \times V_{sed}}{C_0 V_{sample}} \times 100 \quad (3.5)$$

C_0 = Concentration of pesticide residues in sample

C_{sed} = Concentration of pesticide residues in sedimented phase

V_{sed} = Final volume (sedimented phase)

V_{sample} = Sample volume

y = Peak area of analyte

b = y-intercept of standard calibration curve

m = Slope of standard calibration curve

3.8 Method evaluation

The performance of the DLLME for determination of pesticide residues was evaluated. Linearity, accuracy, precision, limit of detection and enrichment factor were explained.

3.8.1 Linearity

The linear calibration curve between the concentration of fortified sample and the peak area was established for the concentration ranging from 1- 50 µg/L. The linear regression method was used to obtain slope, intercept and R^2.

3.8.2 Accuracy

Six replicates of fortified sample blank at concentration of 5 µg/L were determined under the same condition and same time for method accuracy. The expected recovery from AOAC was shown in Table 3.7.

Table 3.7 Expected recovery as a function of analyte concentration[1]

Concentration of analyte	%Recovery
100%	98-102
10%	98-102
1%	97-103
0.1%	95-105
100 ppm	90-107
10 ppm	80-110
1 ppm	80-110
100 ppb	80-110
10 ppb	60-115
1 ppb	40-120

[1] Table excerpted from "AOAC Peer-Verified Methods Program, Manual on Policies and Procedures", Journal of AOAC INTERNATIONAL (1998)

3.8.3 Precision

Six replicates of fortified sample blank at concentration of 5 µg/L were tested under the same condition and same time for method precision. The standard deviation (SD) of each analyte and each concentration was used to calculate the relative standard deviation (RSD_r) by equation (3.6).

The repeatability was calculated by Horwitz's equation (3.7) to found predicted relative standard deviation (PRSD(R)) for resulting in Horwitz ratio or HORRAT by equation (3.8).

$$RSD_r = \frac{SD}{\bar{X}} \times 100 \qquad (3.6)$$

$$PRSD(R) = 0.66 \times 2^{(1-0.5 \log C)} \quad (3.7)$$

$$HORRAT = \frac{RSD_r}{PRSD} \quad (3.8)$$

3.8.4 Limit of detection

Six replicates of fortified sample blank at lowest acceptable concentration that can be observed from background signal were determined. Limit of detection were calculated from three times of SD.

$$LOD = 3SD \quad (3.9)$$

3.8.5 Limit of quantitation

Six replicate of fortified sample blank at lowest acceptable concentration that can be observed from background signal were determined. Limit of detection were calculated from ten times of SD

$$LOQ = 10SD \quad (3.10)$$

3.9 Application of real sample

3.9.1 Water sample

The water sample was collected from Chao Phraya River, Rama VIII Park, Bangkok and filtered through a membrane filter (0.45 μm) to get the clear water before analysis.

3.9.2 Tomato sample

Sample preparation followed by Codex Alimentarius (CAC/GL 41) guidelines. Whole tomato commodity after removal of stems was blended for homogeneous sample. Tomato sample was extracted by QuEChERS method before further extracted by DLLME in 3.5.1. The QuEChERS method are as followed;

3.9.2.1 15 ± 0.1 g of blended tomato sample was weighted into a 50 mL centrifuge tube and fortified with a volume of an appropriate standard mixture solution.

3.9.2.2 15 mL of acetonitrile were added and shaken the tube by vortex mixer for 1 min.

3.9.2.3 The mixture of 6 g of anhydrous $MgSO_4$ and 1.5 g of NaCl was added and shaken the tube by vortex mixer for 1 min.

3.9.2.4 The mixture was centrifuged at 5000 rpm for 5 min.

3.9.2.5 Aliquot of acetonitrile layer (upper layer) was transferred to use as disperser solvent in DLLME step in section 3.5.1.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Optimization of DLLME

Dispersive liquid-liquid microextraction (DLLME) using an auxiliary solvent was developed for the determination of organophosphorus and organochlorine residues in water. The parameters influenced the extraction efficiency were studied including type of auxiliary, extraction and disperser solvent as well as volume of auxiliary, extraction and disperser solvent and centrifugation time. Type of auxiliary, extraction and disperser solvent was selected by varying the method and volume of auxiliary, extraction and disperser solvent and centrifugation time were optimized by experimental design. For all experiment, the water sample containing 5 µg/L of each pesticide was used.

4.1.1 Selection of auxiliary solvent

Auxiliary solvent was used for adjustment the density of a low-density extraction solvent. The solvents as auxiliary were selected on the basis of density, miscibility with extraction solvent and disperser solvent, solubility on water, extraction capability and chromatographic behavior. The solvent should be high density comparing to water, miscible with extraction solvent and disperser solvent, low solubility in water, extraction capability of interested compounds and good chromatographic behavior. Therefore, tetrachloroethylene (C_2Cl_4), chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2) and carbon disulfide (CS_2) were studied as auxiliary solvent. The properties of studied auxiliary solvents are shown in Table 4.1. The batches of fortified water sample were experimented by using 10 µL of toluene as extraction solvent, 30 µL of auxiliary solvent and 1.4 mL of acetonitrile as disperser solvent. In preliminary experiments, 3:1 ratio of auxiliary solvent and extraction solvent was chosen for studies to ensure that a density of mixed solvent was higher than water.

From the experiment, each type of auxiliary solvent gave different sediment phase volume. The sediment phase volume using C_2Cl_4 and $CHCl_3$ were 21 and 12 µL, respectively, while the sediment phases of CH_2Cl_2 and CS_2 were not achieved at the bottom of the tube. Moreover, the extraction recovery of organophosphorus and organochlorine residues was highest for all pesticide residues when using C_2Cl_4 (34.6-67.6%) as shown in Figure 4.1. Therefore, C_2Cl_4 was selected for further studies as an auxiliary solvent.

Table 4.1 The properties of studied auxiliary solvents.

	C_2Cl_4	**$CHCl_3$**	**CH_2Cl_2**	**CS_2**
Density (g/cm^3)	1.623	1.483	1.330	1.261
Solubility in water (g/100 mL, 25ºC)	0.30	0.50	1.3	0.29

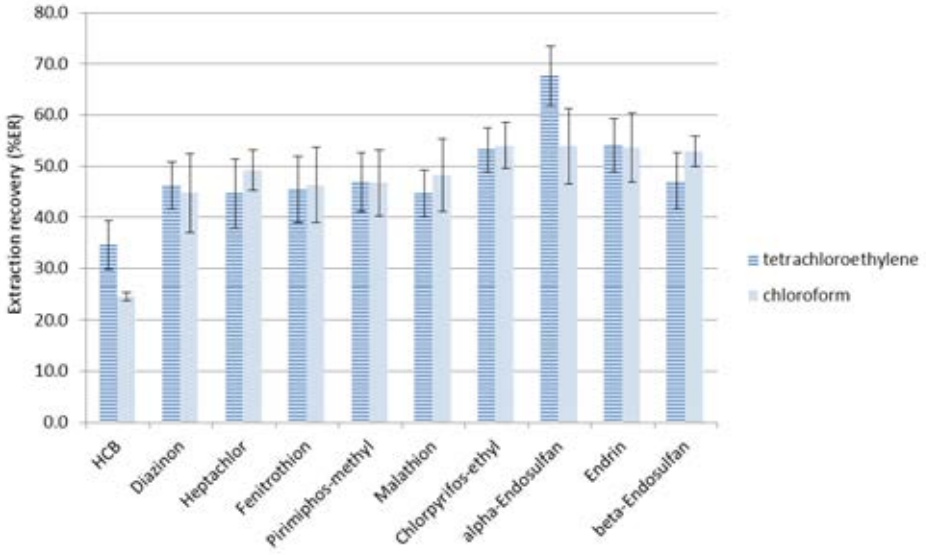


Figure 4.1 Effect of various types of auxiliary solvent on extraction recovery of pesticide residues obtained from DLLME using an auxiliary solvent in fortified water samples at concentration of 5 µg/L. Extraction conditions: sample volume, 5 mL; auxiliary solvent, 30 µL; extraction solvent, 10 µL toluene; disperser solvent, 1.4 mL acetonitrile; centrifugation, 5000 rpm for 7 min.

4.1.2 Selection of extraction solvent

The requirements for the extraction solvent are the same as in DLLME-SFO, namely low solubility in water and high efficiency for extraction of the target analytes. In this study, toluene, *o*-xylene, cyclohexane, *n*-heptane and 1-octanol were studied. The properties of selected extraction solvents were shown in Table 4.2.

The batch of fortified water sample was performed by using a mixture of 10 µL of extraction solvent, 30 µL of C_2Cl_4 as auxiliary solvent and 1.4 mL of acetonitrile as disperser solvent. As shown in Figure 4.2, the extraction recovery of the extraction of organophosphorus and organochlorine residues obtained from *n*-heptane was highest (44.0-79.6%) and lowest standard deviation. Therefore, *n*-heptane was selected for further studies as an extraction solvent.

Table 4.2 The properties of studied extraction solvents.

	toluene	***o*-xylene**	**cyclohexane**	***n*-heptane**	**1-octanol**
Density (g/cm^3)	0.8660	0.8802	0.7786	0.6838	0.8258
Solubility in water (g/100 mL, 25ºC)	-	0.02	0.01	-	0.06

4.1.3 Selection of mixed solvent ratio

The mixed solvent is the mixture of auxiliary solvent and extraction solvent. A ratio of auxiliary solvent and extraction solvent can alter a density of mixed solvent which affected a sediment phase volume and an extraction efficiency. Therefore, 1:0, 1:1, 2:1 and 3:1 ratios of C_2Cl_4: *n*-heptane were studied by specific volume of mixed solvent of 40 µL. The extraction recovery, enrichment factor and sedimented phase volume were shown in Figures 4.3-4.5. Even though the ratio of 1:1 and 2:1 gave higher enrichment factor but lower extraction recovery was obtained. Therefore, the ratio of 3:1 was selected for further studies as the highest extraction recovery, good reproducibility with acceptable enrichment factor and sedimented phased volume.

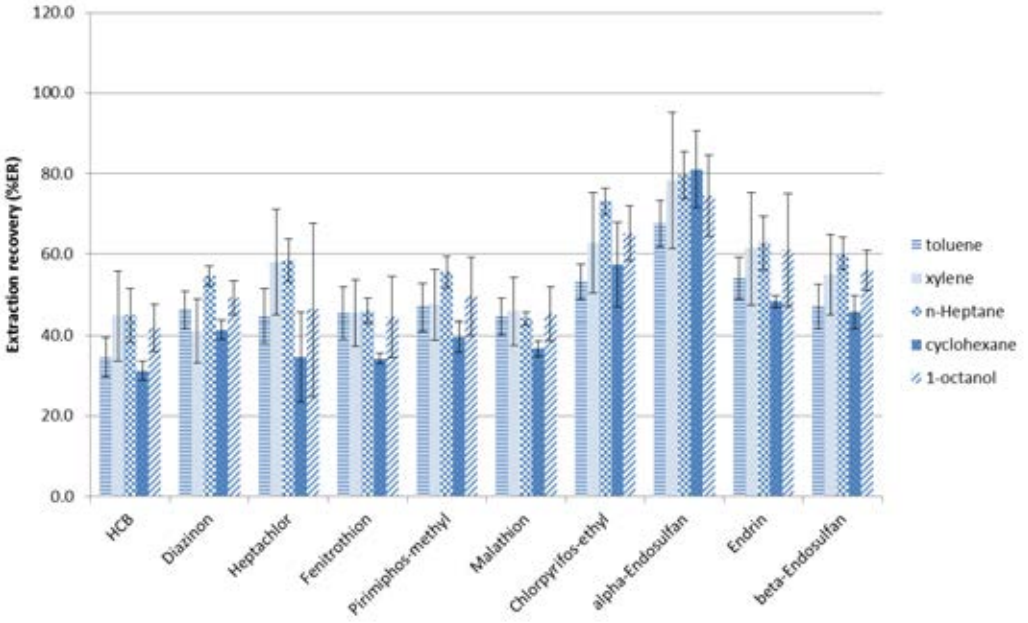


Figure 4.2 Effect of various types of extraction solvent on extraction recovery of pesticide residues obtained from DLLME using an auxiliary solvent in fortified water samples at concentration of 5 µg/L. Extraction conditions: sample volume, 5 mL; auxiliary solvent, 30 µL C_2Cl_4; extraction solvent, 10 µL; disperser solvent, 1.4 mL acetonitrile; centrifugation, 5000 rpm for 7 min.

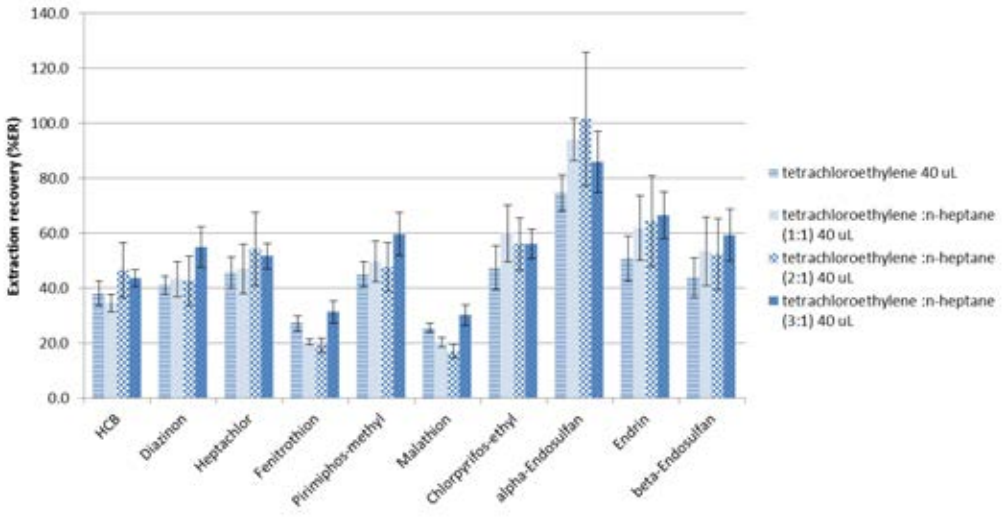


Figure 4.3 Effect of different ratio of auxiliary solvent and extraction solvent on extraction recovery of pesticide residues obtained from DLLME using an auxiliary solvent in fortified water samples at concentration of 5 µg/L. Extraction conditions: sample volume, 5 mL; auxiliary solvent, C_2Cl_4; extraction solvent, *n*-heptane; disperser solvent, 1.4 mL acetonitrile; centrifugation, 5000 rpm for 7 min.

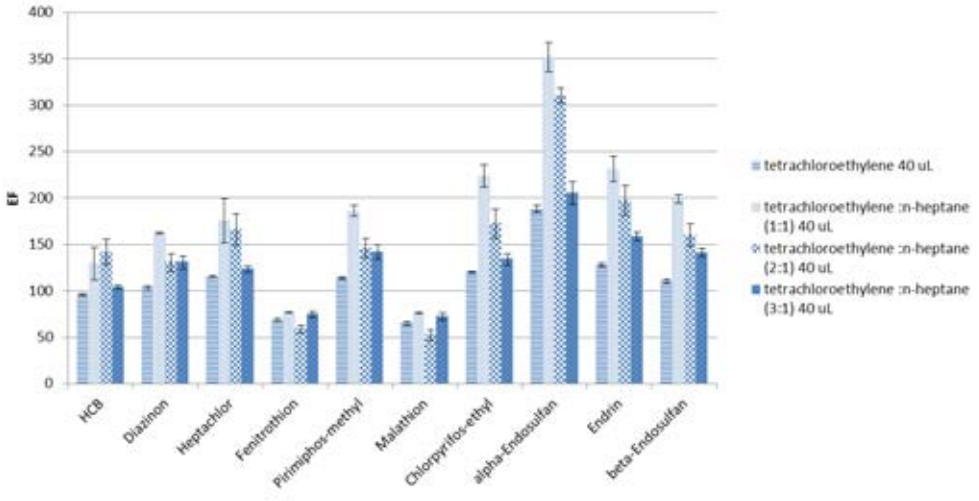


Figure 4.4 Effect of different ratio of auxiliary solvent and extraction solvent on enrichment factor of pesticide residues obtained from DLLME using an auxiliary solvent in fortified water samples at concentration of 5 µg/L. Extraction conditions: sample volume, 5 mL; auxiliary solvent, C_2Cl_4; extraction solvent, *n*-heptane; disperser solvent, 1.4 mL acetonitrile; centrifugation, 5000 rpm for 7 min.

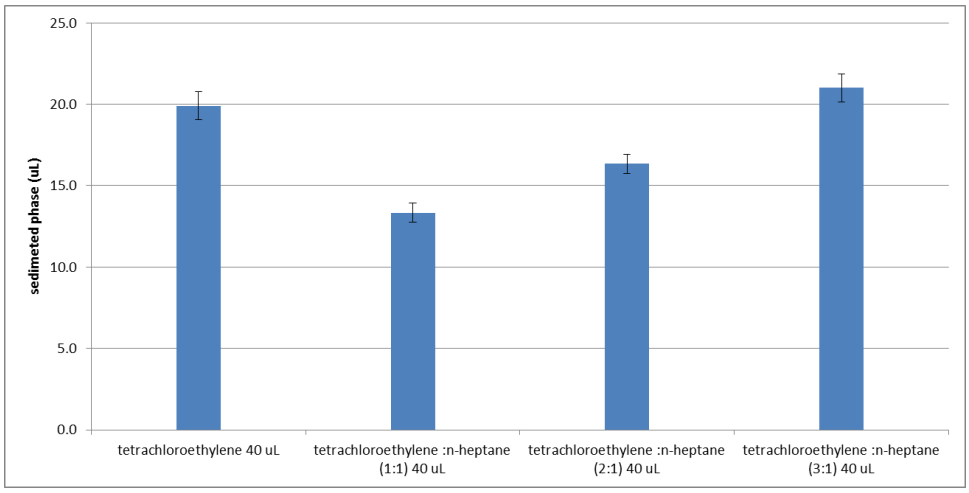


Figure 4.5 Effect of different ratio of auxiliary solvent and extraction solvent on sedimented phase volume obtained from DLLME using an auxiliary solvent in fortified water samples at concentration of 5 µg/L. Extraction conditions: sample volume, 5 mL; auxiliary solvent, C_2Cl_4; extraction solvent, *n*-heptane; disperser solvent, 1.4 mL acetonitrile; centrifugation, 5000 rpm for 7 min.

4.1.4 Selection of disperser solvent

The main criterion for selecting the disperser solvent is its miscibility with the extraction solvent, auxiliary solvent and aqueous sample for the formation of cloudy solution. Acetonitrile, acetone, methanol and ethanol were selected to study as disperser solvent.

The batch of spiked aqueous sample were performed by using a mixture of 10 µL of *n*-heptane, 30 µL of C_2Cl_4 and 1.4 mL of studied disperser solvent. As shown in Figure 4.6, the extraction recovery of the extraction of organophosphorus and organochlorine residues obtained from different type of extraction solvent. It was found that acetonitrile provided the highest extraction efficiency (44.0-79.6%). Therefore, acetonitrile was selected for further studies as disperser solvent.

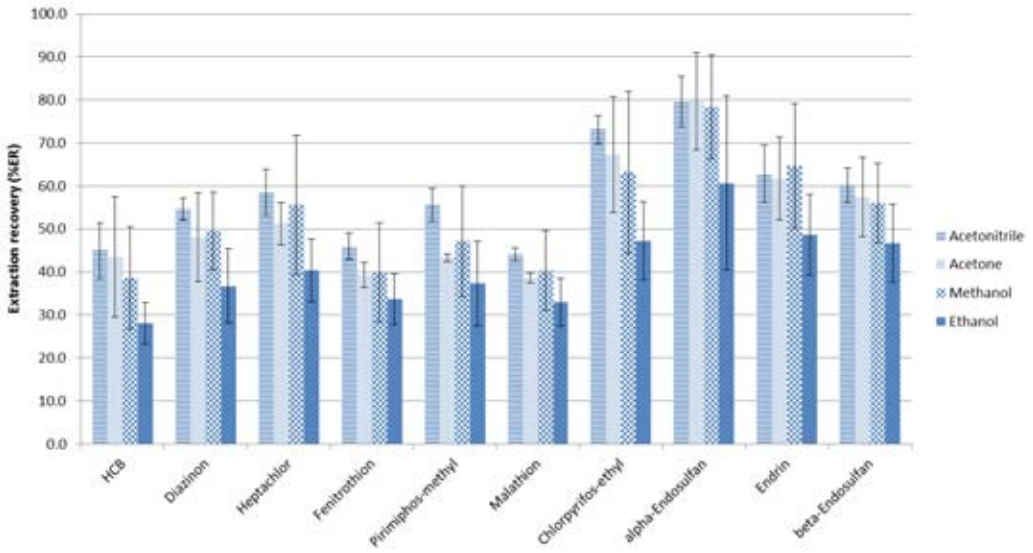


Figure 4.6 Effect of various type of disperser solvent on extraction recovery of pesticide residues obtained from DLLME using an auxiliary solvent in fortified water samples at concentration of 5 µg/L. Extraction conditions: sample volume, 5 mL; auxiliary solvent, 30 µL C_2Cl_4; extraction solvent, 10 µL *n*- heptane; disperser solvent, 1.4 mL; centrifugation, 5000 rpm for 7 min.

4.2 Experimental design

A central composite design was selected with the aim of appropriately optimizing the main factors affecting the DLLME extraction recovery such as mixed solvent volume (X_1), disperser solvent volume (X_2) and centrifugation time (X_3).

For mixed solvent volume (X_1), the minimum value was defined by the sedimented phased volume enough for analysis and the maximum value was defined by reducing the volume of toxic solvent in conventional DLLME [38]. For disperser solvent volume (X_2) and centrifugation time (X_3), the ranges were defined by commonly used in DLLME.

The result as extraction recovery (%ER) and enrichment factor (EF) of 20 experiments using 5 mL of fortified water sample at concentration of 5 µg/L of each pesticide, mixed solvent of C_2Cl_4 as auxiliary solvent and *n*-heptane as extraction solvent with 3:1 ratio, acetonitrile as disperser solvent were shown in Table 4.3.

Table 4.3 Extraction recovery (%ER) and enrichment factor (EF) of 20 experiments DLLME for determination of pesticide residues

experiment	X_1 (µL)	X_2 (mL)	X_3 (min)	%ER	EF
1	30	0.8	4	21.9	93
2	30	0.8	10	42.9	146
3	30	2.0	4	33.4	208
4	30	2.0	10	32.6	147
5	50	0.8	4	46.2	94
6	50	0.8	10	78.9	119
7	50	2.0	4	60.7	131
8	50	2.0	10	57.9	113

Table 4.3 Extraction recovery (%ER) and enrichment factor (EF) of 20 experiments DLLME for determination of pesticide residues (continued)

experiment	X_1 (μL)	X_2 (mL)	X_3 (min)	%ER	EF
9	23.18	1.4	7	19.1	157
10	56.82	1.4	7	74.2	93
11	40	0.39	7	25.2	81
12	40	2.41	7	46.6	145
13	40	1.4	2	45.7	141
14	40	1.4	12	66.0	110
15	40	1.4	7	68.1	145
16	40	1.4	7	65.4	149
17	40	1.4	7	59.2	129
18	40	1.4	7	61.3	133
19	40	1.4	7	56.4	125
20	40	1.4	7	60.9	130

The mean extraction recovery of all the pesticides was introduced separately as the response in statistical program (MATLAB). Data analysis gave a semi-empirical expression of extraction recovery (%ER) and enrichment factor (EF) with following equation:

$$\%ER = 61.83 + 15.04X_1 + 2.24X_2 + 6.16X_3 - 5.05X_1^2 - 8.83X_2^2 - 1.78X_3^2 - 0.98X_1X_2 + 1.21X_1X_3 - 7.16X_2X_3$$

$$EF = 134.71 - 17.99X_1 + 18.58X_2 - 3.85X_3 - 1.17X_1^2 - 5.59X_2^2 - 1.01X_3^2 - 10.54X_1X_2 + 1.94X_1X_3 - 19.71X_2X_3$$

The response surfaces plots described the suitable condition of mixed solvent volume, disperser solvent volume and centrifugation time that maximize the extraction recovery and enrichment factor. The response surface plots of %ER and EF versus significant variables were shown in Figure 4.7 and 4.8, respectively. According to optimization study, the increase of mixed solvent volume increased the extraction recovery while decreased the enrichment factor. On the other hand, the extraction recovery increased when disperser volume increased before a maximum value from which extraction recovery decreased. In case of centrifugation time has a little effect on %ER and EF.

The optimized method conditions can be selected based on the responses that meet the requirements from the response surface plots. The criteria are high values of both %ER and EF. On the response surface plots, 50 µL of mixed solvent gave the highest %ER but low EF. So, 40 µL of mixed solvent was chosen due to give higher EF and acceptable %ER. While the suitable disperser solvent volume for 40 µL of mixed solvent was 1.4 mL which gave high %ER. Because centrifugation time has a little effect on %ER and EF, 7 min was selected as appropriate centrifugation time.

All of the above is the reason to choose 40 µL of mixed solvent (CH_2Cl_4: *n*-heptane, 3:1), 1.4 mL of disperser solvent (acetonitrile) and 7 min of centrifugation time for further studies.

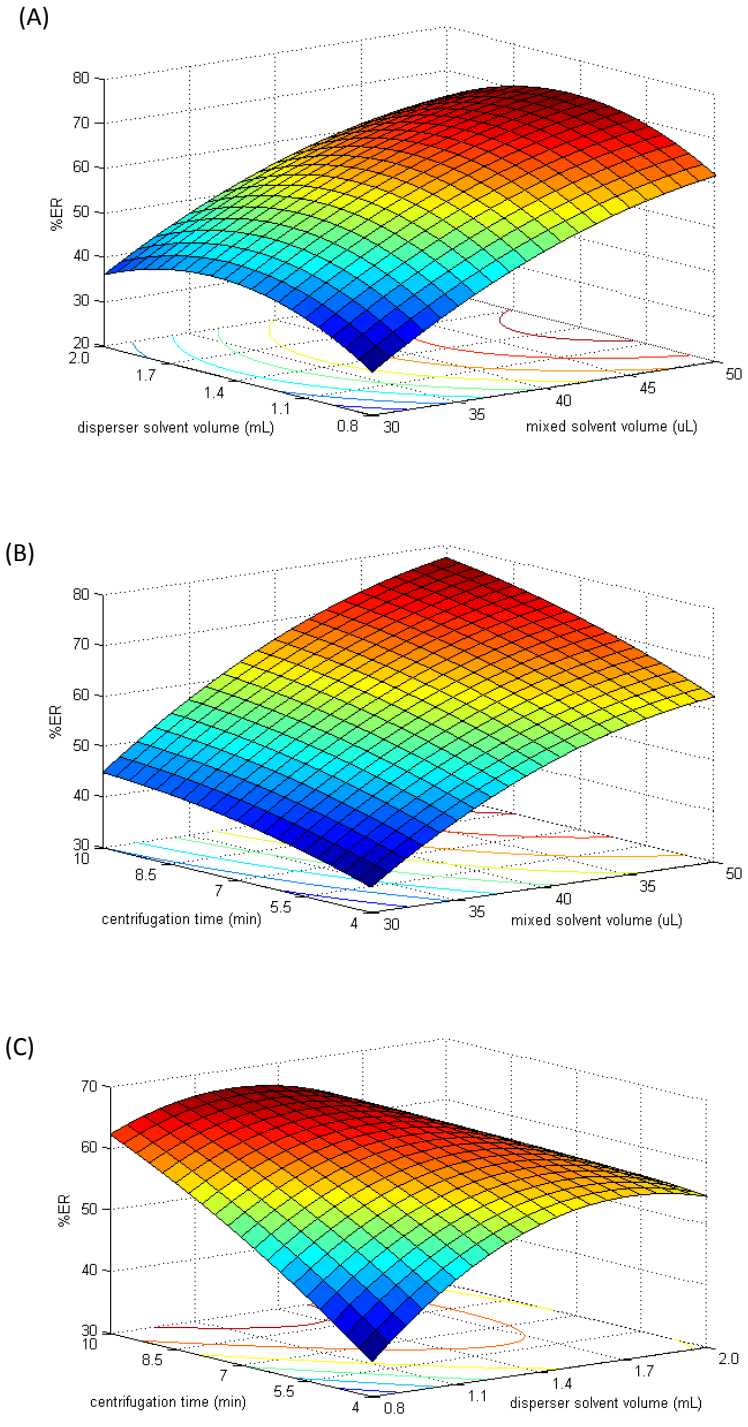


Figure 4.7 Response surfaces of mean extraction recovery for the central composite design of DLLME using an auxiliary solvent. (A) Mean extraction recovery, mixed solvent volume and disperser solvent volume. (B) Mean extraction recovery, mixed solvent volume and centrifugation time. (C) Mean extraction recovery, disperser solvent volume and centrifugation time

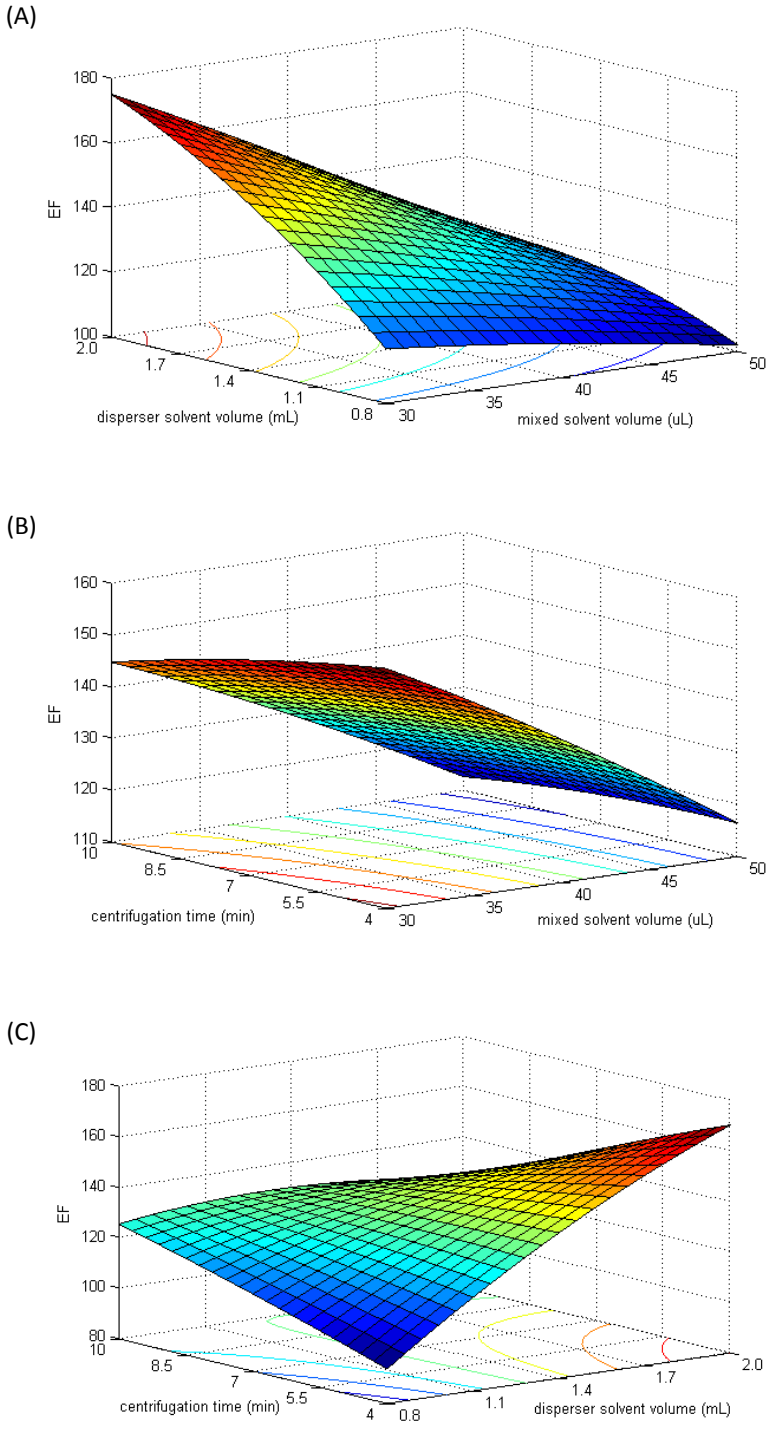


Figure 4.8 Response surfaces of mean enrichment factor for the central composite design of DLLME using an auxiliary solvent. (A) Mean enrichment factor, mixed solvent volume and disperser solvent volume. (B) Mean enrichment factor, mixed solvent volume and centrifugation time. (C) Mean enrichment factor, disperser solvent volume and centrifugation time

4.3 Method evaluation

4.3.1 Linearity

Five levels concentration of fortified sample were analyzed and plotted between the concentration of fortified sample and the peak area. The chromatogram of mixed standard of 10 pesticides in Figure 4.9 showed a clear separation and determination. The calibration plots were shown in Figure 4.10. The coefficients of determination (R^2) for all pesticides were in the range of 0.9913-0.9992 (Table 4.4).

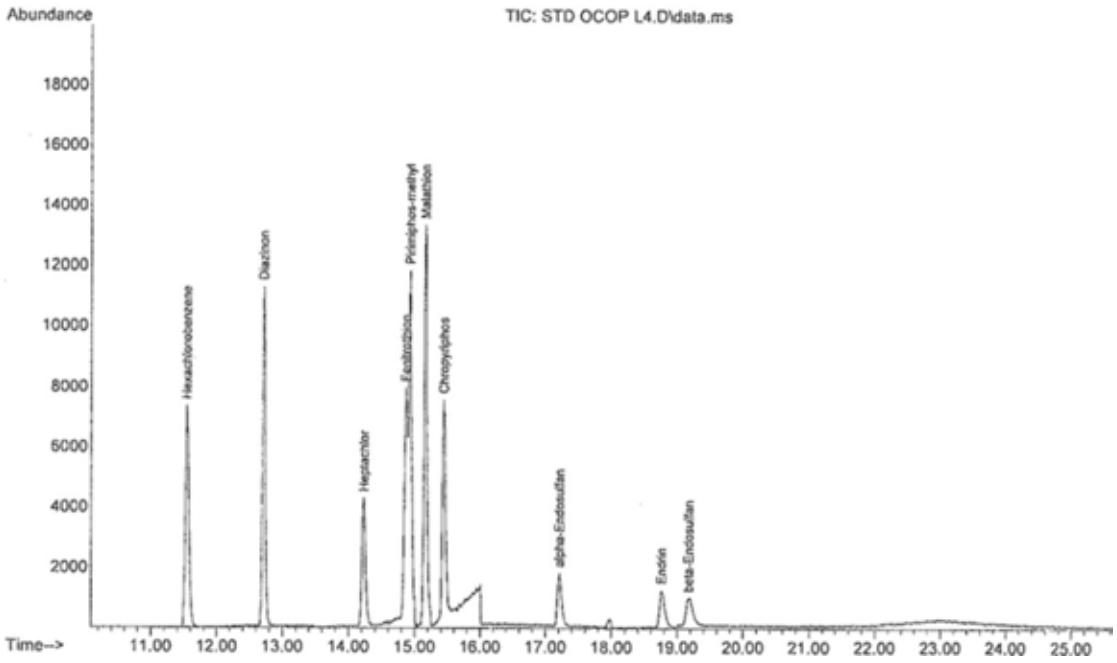


Figure 4.9 Chromatogram of mixed standard solution.

Table 4.4 Linearity of fortified sample.

Pesticides	Range (µg/L)	Equation	R^2
Hexachlorobenzene	1-50	y = 14198x + 32311	0.9976
Diazinon	1-50	y = 16738x + 5430.3	0.9913
Heptachlor	1-50	y = 7293.5x + 13254	0.9992
Fenitrothion	1-50	y = 8297.1x + 5715.1	0.9938
Pirimiphos-methyl	1-50	y = 13858x + 8316.2	0.9940
Malathion	1-50	y = 11628x + 6041.3	0.9930
Chlorpyrifos-ethyl	1-50	y = 15423x + 37659	0.9934
alpha-Endosulfan	1-50	y = 3864.6x + 14905	0.9938
Endrin	1-50	y = 6219.5x + 9918	0.9981
beta-Endosulfan	1-50	y = 4943.3x + 6234.1	0.9945

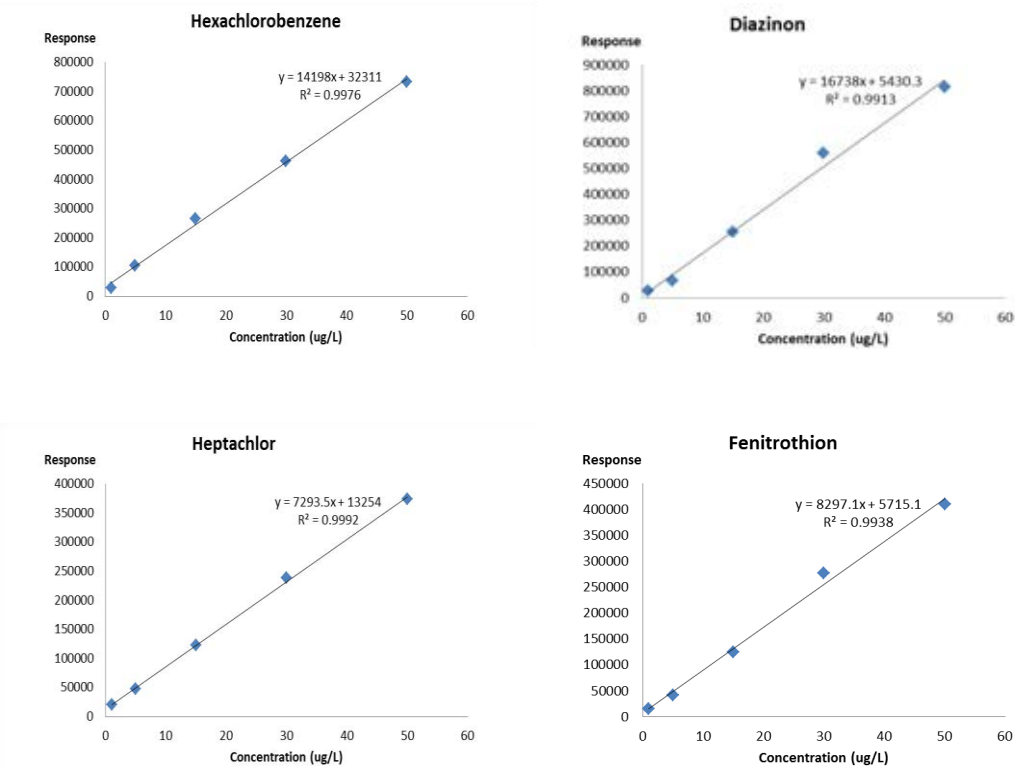


Figure 4.10 Linearity of organophosphorus and organochlorine residues.

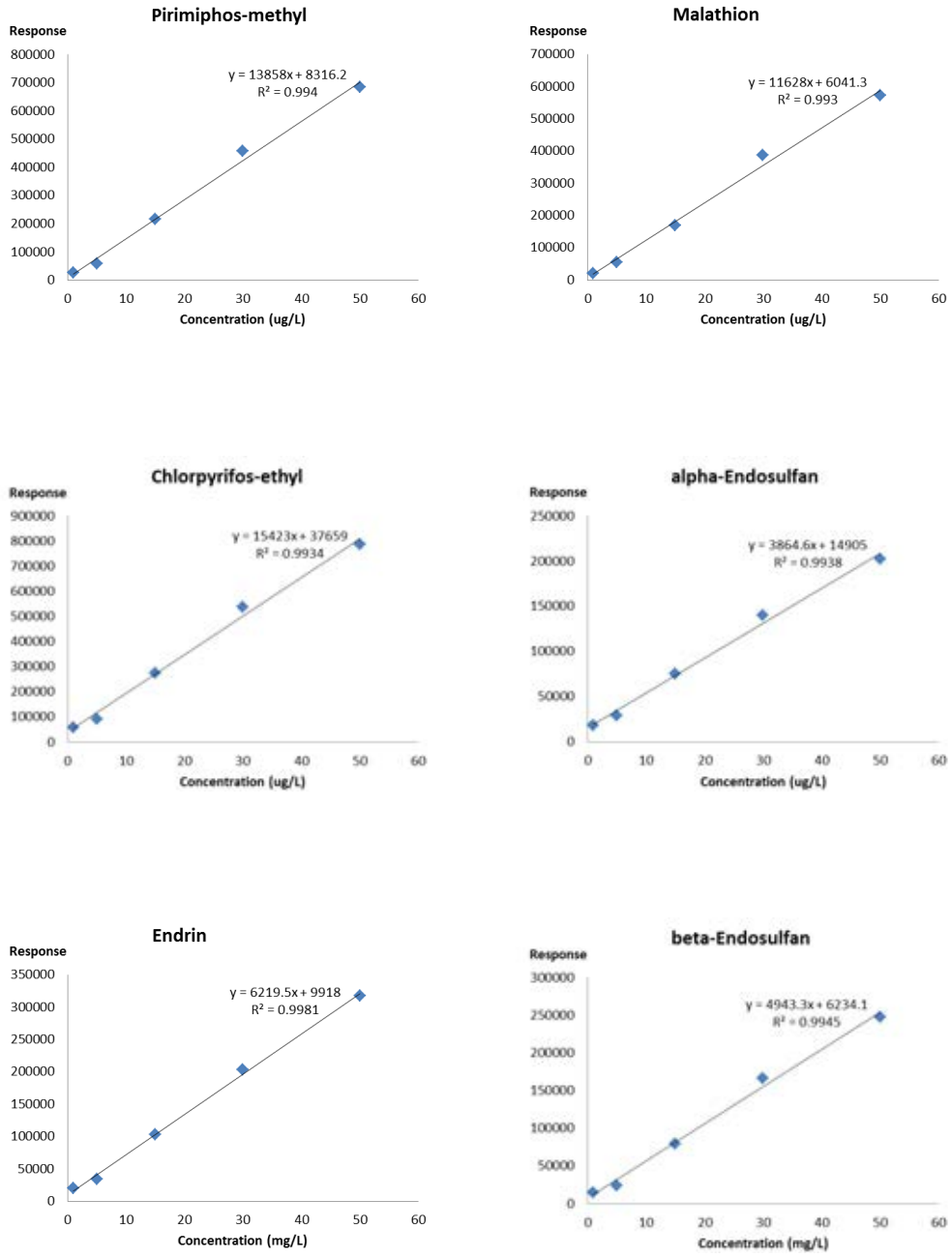


Figure 4.10 Linearity of organophosphorus and organochlorine residues (continue).

4.3.2 Accuracy

Reagent blank, sample blank and fortified sample at concentration of 5 µg/L were analyzed. The extraction recoveries were in the range of 40.7-90.5% (Table 4.5) which is accepted for the accuracy criteria of AOAC at 1 ppb (40-120%). The enrichment factors were between 89 and 198.

Table 4.5 The extraction recovery and enrichment factor (EF) for extraction of pesticide residues by DLLME using an auxiliary solvent.

Pesticides	**Extraction recovery (%) (n=6)**	**EF (n=6)**
Hexachlorobenzene	52.2±5.5	114
Diazinon	52.9±4.4	115
Heptachlor	69.8±5.2	152
Fenitrothion	42.9±3.6	94
Pirimiphos-methyl	55.7±4.5	122
Malathion	40.7±4.2	89
Chlorpyrifos-ethyl	77.1±5.2	168
alpha-Endosulfan	90.5±6.9	198
Endrin	73.4±4.8	160
beta-Endosulfan	63.5±6.6	139

4.3.3 Precision

Reagent blank, sample blank and fortified sample at concentration of 5 µg/L were analyzed. The result in terms of repeatability, the HORRAT values were in the range of 0.28-0.45 which is accepted for AOAC (HORRAT < 2) as shown in Table 4.6.

Table 4.6 The extraction recovery and relative standard deviations for extraction of pesticide residues by DLLME using an auxiliary solvent.

Pesticides	**Concentration (µg/L) (n=6)**				
	Mean	**SD**	**RSD_r**	**$PRSD_r$**	**HORRAT**
Hexachlorobenzene	2.61	0.28	10.6	23.44	0.45
Diazinon	2.64	0.22	8.4	23.44	0.36
Heptachlor	3.49	0.26	7.5	23.44	0.32
Fenitrothion	2.15	0.18	8.4	23.44	0.36
Pirimiphos-methyl	2.79	0.23	8.1	23.44	0.35
Malathion	2.03	0.21	10.3	23.44	0.44
Chlorpyrifos-ethyl	3.86	0.26	6.8	23.44	0.29
alpha-Endosulfan	4.53	0.34	7.6	23.44	0.32
Endrin	3.67	0.24	6.6	23.44	0.28
beta-Endosulfan	3.18	0.33	10.4	23.44	0.44

4.3.4 Limit of detection (LOD) and limit of quantitation (LOQ)

Reagent blank, sample blank and fortified sample at low end of the concentration range were analyzed. The results were demonstrated in Table 4.7. The LODs were 0.3 – 1.2 µg/L and LOQs were 1.1 – 4.1 µg/L which meet the regulation limits of these pesticide residues, for example regulation limits in drinking water by IUPAC and regulation limits in tomato by EU (Table 3.3).

Table 4.7 Limit of detection (LOD) and limit of quantitation (LOQ) for extraction of pesticide residues by DLLME using an auxiliary solvent.

Pesticides	**Concentration (µg/L)**	
	LOD	**LOQ**
Hexachlorobenzene	0.6	1.9
Diazinon	0.5	1.6
Heptachlor	1.0	3.2
Fenitrothion	0.3	1.1
Pirimiphos-methyl	0.6	2.1
Malathion	0.3	1.1
Chlorpyrifos-ethyl	1.2	4.0
alpha-Endosulfan	0.9	3.1
Endrin	1.2	4.1
beta-Endosulfan	1.1	3.5

4.4 Comparison between the proposed DLLME and other DLLME methods

The extraction performances of the proposed DLLME method for determination of pesticide residues was compared with conventional DLLME method by Zhou [34] and low-density solvent-based DLLME (LDS-DLLME) method by Farajzadeh [16]. The proposed method performed as followed: 5 mL of fortified sample contained 5 µg/L of each pesticide was put into 15 mL centrifuge tube. A mixture of 30 µL C_2Cl_4, 10 µL *n*-heptane and 1.4 mL acetonitrile was directly injected into fortified aqueous sample. After centrifugation at 5000 rpm for 7 min, the sediment phase at the bottom was removed by microsyringe and directly analyzed by GC-MS.

The comparison of analytes, type and volume of extraction solvent and disperser solvent, enrichment factor and limit of detection (LOD) was shown in Table 4.8. The extraction efficiency of proposed method was comparable to conventional DLLME but lower volume of toxic solvent was used which is more environmental friendly. Moreover, the proposed method has lower limits of detection than LSD-DLLME.

Table 4.8 Comparison between the purposed DLLME, conventional DLLME and LDS-DLLME method for determination of pesticide residues.

Properties	**The purpose DLLME**	**Conventional DLLME [35]**	**LDS-DLLME [19]**
Analytes	5 organophosphorus, 5 organochlorine pesticides	4 organochlorine pesticides	3 organophosphorus pesticides
Extraction solvent	C_2Cl_4 30 µL+ *n*-heptane 10 µL	CCl_4 50 µL	Cyclohexane 100 µL
Disperser solvent	acetonitrile 1.4 mL	acetonitrile 0.6 mL	acetone 2 mL
Enrichment factor (EF)	89-198	100	100-110
Extraction recovery (%ER)	40.7-90.5	85.58-119.6	80-94
Limit of detection (µg/L)	0.3-1.2	0.32-0.51	3-4

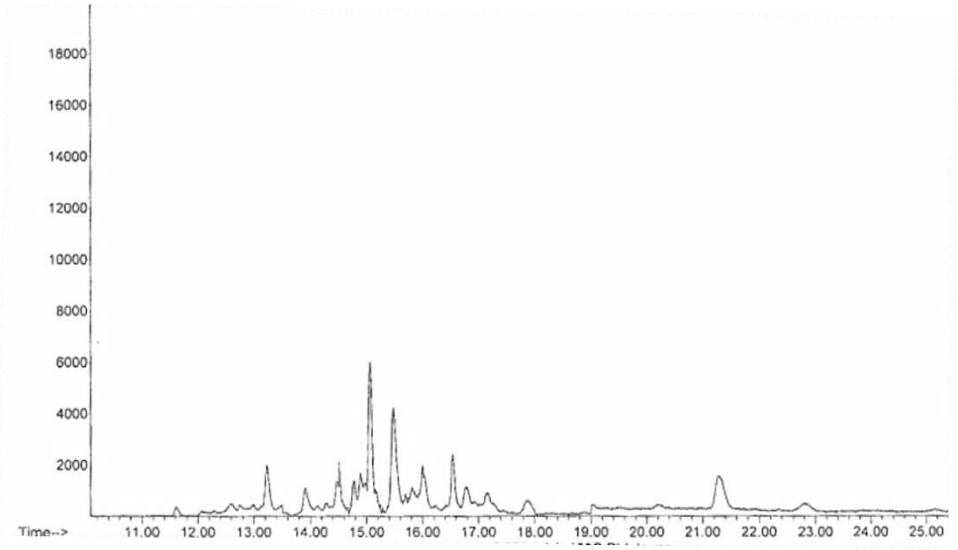
4.5 The application of purposed method in real samples

The proposed method was applied for determination of organophosphorus and organochlorine residues in real sample. The water sample and fortified water sample at concentration of 5 μg/L of each pesticide were analyzed. Moreover, the tomato sample and fortified tomato sample at concentration of 20 μg/L of each pesticide were analyzed by followed the extraction step in section 3.5.1 and 3.5.2 before DLLME procedure. The results were shown in Table 4.9. The chromatogram of water sample and tomato sample were shown in Figure 4.11-12. Satisfied extraction recoveries of all pesticides both in water and tomato samples were achieved.

Table 4.9 The extraction recovery for determination of organophosphorus and organochlorine residues in real water sample and tomato sample.

Pesticides	Extraction recovery (%) (n=3)	
	Water sample	Tomato sample
Hexachlorobenzene	40.1±1.9	71.7±14.2
Diazinon	50.8±1.8	106.1±11.0
Heptachlor	38.9±9.5	65.1±20.1
Fenitrothion	31.3±1.1	69.3±9.9
Pirimiphos-methyl	41.4±3.7	88.0±9.1
Malathion	34.7±1.6	78.7±8.7
Chlorpyrifos-ethyl	65.4±4.0	115.9±12.9
alpha-Endosulfan	84.9±8.3	140.1±19.2
Endrin	59.5±8.7	71.7±105.7
beta-Endosulfan	61.2±4.7	106.1±115.7

(A)



(B)

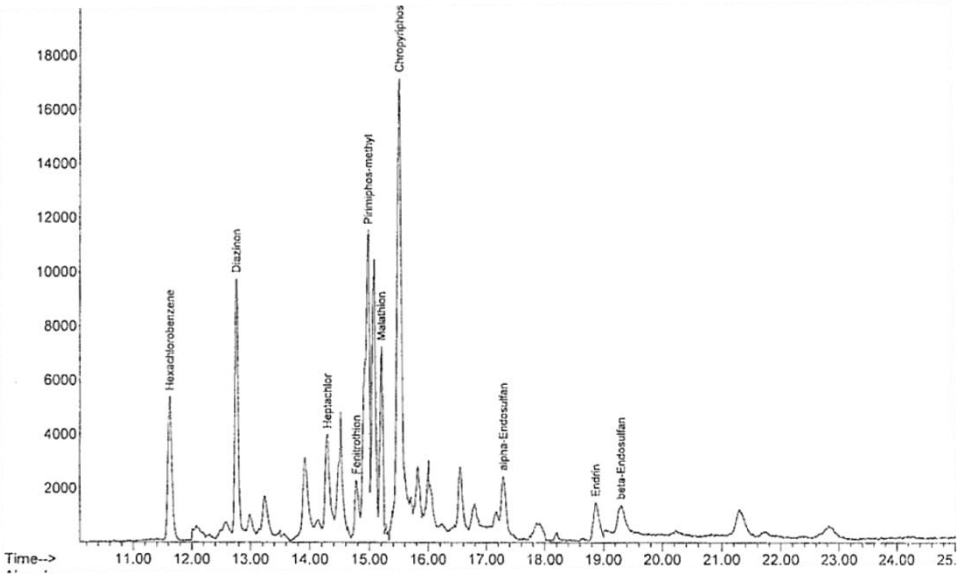
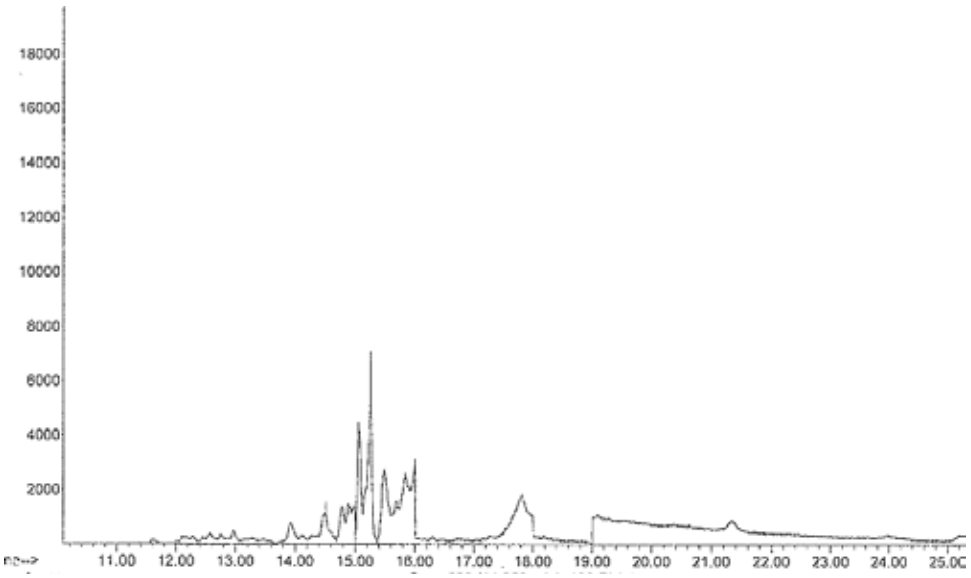


Figure 4.11 Chromatogram of (A) water sample and (B) fortified water sample.

(A)



(B)

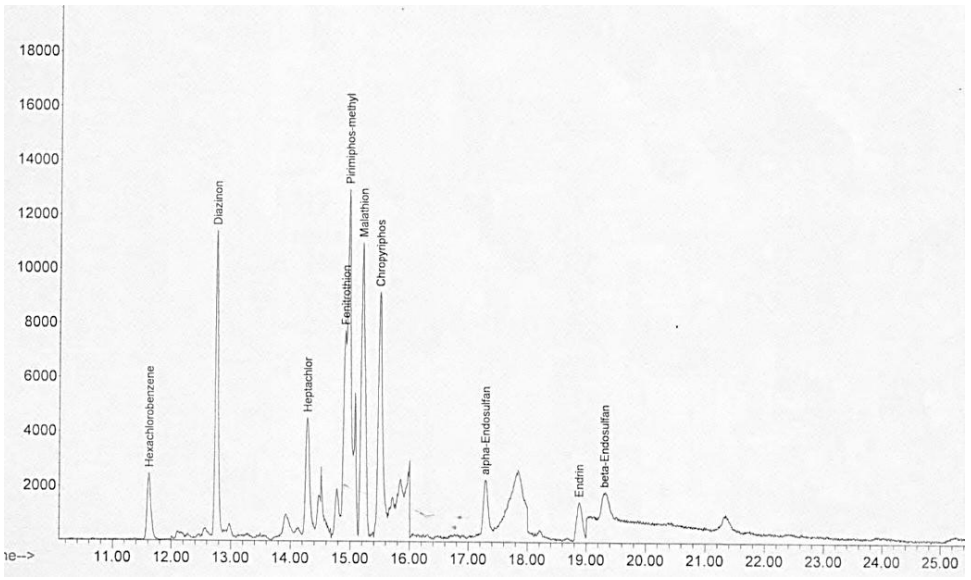


Figure 4.12 Chromatogram of (A) tomato sample and (B) fortified tomato sample.

CHAPTER V

CONCLUSION

5.1 Conclusion

Dispersive liquid-liquid microextraction using an auxiliary solvent was developed for the determination of 10 organophosphorus and organochlorine residues namely hexachlorobenzene, diazinon, heptachlor, fenitrothion, pirimiphos-methyl, malathion, chlorpyrifos, α-endosulfan, endrin and β-endosulfan by gas chromatography-mass spectrometry. The DLLME using an auxiliary solvent procedure was used a quaternary system consisting of a low-density organic solvent as a extraction solvent and a high-density organic solvent as an auxiliary solvent for adjusting the density of a low-density organic solvent that was mixed with a disperser solvent, then was injected into the aqueous sample.

The parameters influenced the extraction efficiency were studied including type of auxiliary, extraction and disperser solvent as well as volume of auxiliary, extraction and disperser solvent and centrifugation time. Type of auxiliary, extraction and disperser solvent was selected by varying the method and volume of auxiliary, extraction and disperser solvent and centrifugation time were optimized by experimental design. The optimal conditions for extraction were 30 µL tetrachloroethylene (C_2Cl_4) as auxiliary solvent, 10 µL *n*-heptane as extraction solvent, 1.4 mL acetonitrile as disperser solvent and 7 min centrifugation time at 5000 rpm.

The purposed method provided good linearity in the concentration range of 1-50 µg/L with correlation coefficient (R^2) greater than 0.99. The extraction recoveries for 5.0 µg/L of pesticides were in the range of 40.7 – 90.5 % and relative standard deviations were between 6.6 and 10.6% (n=6) which indicated an acceptable accuracy and precision of the method. The limits of detections (LODs) and limits of quantitation (LOQs) for the method were 0.3 – 1.2 µg/L and 1.1 – 4.1 µg/L,

respectively. The LODs of this proposed method were lower than the regulation limits of these pesticide residues in water regulated by IUPAC and in tomato regulated by EU. The enrichment factors were in the range of 89-198. In addition, this proposedmethod was applied for determination of organophosphorus and organochlorine residues in real water sample and tomato extracted sample. Satisfied extraction recoveries of all pesticides both in water and tomato samples were achieved.

In comparison with other DLLME methods, the proposed method were simultaneous analyzed organophosphorus and organochlorine residues while the extraction performance was comparable to conventional DLLME and low-density solvent-based DLLME (LDS-DLLME) method. Moreover, the use of toxic solvent was lower than that in conventional DLLME which is more environmental friendly. In addition, the proposed method has lower limits of detection than LSD-DLLME.

5.2 Suggestion of future work

The idea of dispersive liquid-liquid microextraction using an auxiliary solvent may be apply to other auxiliary solvent and extraction solvent for extraction efficiency and widely used in various analytes. Furthermore, this method should be applied for other samples.

REFERENCES

[1] Codex Maximum Residue Limits for Pesticides. CODEX Alimentarius [Online]. 2012. Available from: http://www.codexalimentarius.net/pestres/data/index.html [March 11, 2013]

[2] EU Pesticides Database. European Commission [Online]. 2013. Available from: http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=homepage&CFID=8673624&CFTOKEN=82aa3b57dd599ef-4C5EAD31-D9DC-9449-A27A7EB1859DF685&jsessionid=2405633995f117f5c1e4TR [March 12, 2013]

[3] Maximum Residue Limits for Pesticides. Health Canada [Online]. 2013. Available from: http://pr-rp.hc-sc.gc.ca/mrl-lrm/index-eng.php [March 22, 2013]

[4] The Japanese Positive List System for Agricultural Chemical Residues in Foods. The Japan Food Chemical Research Foundation [Online]. 2013. Available from: http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/MRLs-p [March 19, 2013]

[5] Hamilton, D. and Crossley, S. Pesticide Residues in Food and Drinking Water: Human Exposure and Risks. Chichester, UK: John Wiley & Sons, Ltd, 2004.

[6] Rezaee, M., et al. Determination of organic compounds in water using dispersive liquid-liquid microextraction. Journal of Chromatography A 1116 (2006): 1-9.

[7] Leong, M.-I. and Huang, S.-D. Dispersive liquid-liquid microextraction method based on solidification of floating organic drop combined with gas chromatography with electron-capture or mass spectrometry detection. Journal of Chromatography A 1211 (2008): 8-12.

[8] Kocúrová, L.; Balogh, I. S.; Škrlíková, J.; Posta, J. and Andruch, V. A novel approach in dispersive liquid-liquid microextraction based on the use of an auxiliary solvent for adjustment of density: UV-VIS spectrophotometric and graphite furnace atomic absorption spectrometric determination of gold based on ion pair formation. Talanta 82 (2010): 1958-1964.

[9] Xia, J.; Xiang, B. and Zhang, W. Determination of metacrate in water samples using dispersive liquid-liquid microextraction and HPLC with the aid of response surface methodology and experimental design. Analytica Chimica Acta 625 (2008): 28-34.

[10] Ravelo-Perez, L. M.; Hernandez-Borges, J.; Asensio-Ramos, M. and Rodriguez-Delgado, M. A. Ionic liquid based dispersive liquid-liquid microextraction for the extraction of pesticides from bananas. Journal of Chromatography A 23 (2009): 7336-45.

[11] Gilbert-López, B.; García-Reyes, J. F. and Molina-Díaz, A. Sample treatment and determination of pesticide residues in fatty vegetable matrices: A review. Talanta 79 (2009): 109-128.

[12] Anastassiades, M.; Lehotay, S. J.; Stajnbaher, D. and Schenck, F. J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International 86 (2003): 412-431.

[13] Lee, J.; Lee, H. K.; Rasmussen, K. E. and Pedersen-Bjergaard, S. Environmental and bioanalytical applications of hollow fiber membrane liquid-phase microextraction: A review. Analytica Chimica Acta 624 (2008): 253-268.

[14] Berijani, S.; Assadi, Y.; Anbia, M.; Milani Hosseini, M.-R. and Aghaee, E. Dispersive liquid-liquid microextraction combined with gas chromatography-flame photometric detection: Very simple, rapid and sensitive method for the determination of organophosphorus pesticides in water. Journal of Chromatography A 1123 (2006): 1-9.

[15] Rahnama Kozani, R.; Assadi, Y.; Shemirani, F.; Milani Hosseini, M. R. and Jamali, M. R. Determination of Trihalomethanes in Drinking Water by Dispersive Liquid-Liquid Microextraction then Gas Chromatography with Electron-Capture Detection. Chromatographia 66 (2007): 81-86.

[16] Fattahi, N.; Samadi, S.; Assadi, Y. and Hosseini, M. R. M. Solid-phase extraction combined with dispersive liquid-liquid microextraction-ultra preconcentration of chlorophenols in aqueous samples. Journal of Chromatography A 1169 (2007): 63-69.

[17] Rezaei, F.; Bidari, A.; Birjandi, A. P.; Milani Hosseini, M. R. and Assadi, Y. Development of a dispersive liquid-liquid microextraction method for the determination of polychlorinated biphenyls in water. Journal of Hazardous Materials 158 (2008): 621-627.

[18] Shokoufi, N.; Shemirani, F. and Assadi, Y. Fiber optic-linear array detection spectrophotometry in combination with dispersive liquid-liquid microextraction for simultaneous preconcentration and determination of palladium and cobalt. Analytica Chimica Acta 597 (2007): 349-356.

[19] Farajzadeh, M. A.; Seyedi, S. E.; Shalamzari, M. S. and Bamorowat, M. Dispersive liquid-liquid microextraction using extraction solvent lighter than water. Journal of Separation Science 32 (2009): 3191-3200.

[20] Hashemi, P.; Beyranvand, S.; Mansur, R. S. and Ghiasvand, A. R. Development of a simple device for dispersive liquid-liquid microextraction with lighter than water organic solvents: isolation and enrichment of glycyrrhizic acid from licorice. Analytica Chimica Acta 655 (2009): 60-5.

[21] Saleh, A.; Yamini, Y.; Faraji, M.; Rezaee, M. and Ghambarian, M. Ultrasound-assisted emulsification microextraction method based on applying low density organic solvents followed by gas chromatography analysis for the determination of polycyclic aromatic hydrocarbons in water samples. Journal of Chromatography A 1216 (2009): 6673-6679.

[22] Kocúrová, L.; Balogh, I. S.; Šandrejováa, J. and Andruch, V. Recent advances in dispersive liquid-liquid microextraction using organic solvents lighter than water. A review. Microchemical Journal 102 (2012): 11-17.

[23] Zhao, E.; Zhao, W.; Han, L.; Jiang, S. and Zhou, Z. Application of dispersive liquid-liquid microextraction for the analysis of organophosphorus pesticides in watermelon and cucumber. Journal of Chromatography A 1175 (2007): 137-140.

[24] Nagaraju, D. and Huang, S.-D. Determination of triazine herbicides in aqueous samples by dispersive liquid-liquid microextraction with gas chromatography-ion trap mass spectrometry. Journal of Chromatography A 1161 (2007): 89-97.

[25] Wei, G.; Li, Y. and Wang, X. Application of dispersive liquid-liquid microextraction combined with high-performance liquid chromatography for the determination of methomyl in natural waters. Journal of Separation Science 30 (2007): 3262-7.

[26] Farhadi, K.; Matin, A. and Hashemi, P. LC Determination of Trace Amounts of Phenoxyacetic Acid Herbicides in Water after Dispersive Liquid-Liquid Microextraction. Chromatographia 69 (2009): 45-49.

[27] Liu, Y.; Zhao, E.; Zhu, W.; Gao, H. and Zhou, Z. Determination of four heterocyclic insecticides by ionic liquid dispersive liquid-liquid microextraction in water samples. Journal of Chromatography A 6 (2009): 885-91.

[28] Montes, R.; Rodriguez, I.; Ramil, M.; Rubi, E. and Cela, R. Solid-phase extraction followed by dispersive liquid-liquid microextraction for the sensitive determination of selected fungicides in wine. Journal of Chromatography A 17 (2009): 5459-66.

[29] He, L., et al. Ionic liquid-based dispersive liquid-liquid microextraction followed high-performance liquid chromatography for the determination of organophosphorus pesticides in water sample. Analytica Chimica Acta 655 (2009): 52-59.

[30] He, L.; Luo, X.; Jiang, X. and Qu, L. A new 1,3-dibutylimidazolium hexafluorophosphate ionic liquid-based dispersive liquid-liquid microextraction to determine organophosphorus pesticides in water and fruit samples by high-performance liquid chromatography. Journal of Chromatography A 1217 (2010): 5013-20.

[31] Ravelo-Pérez, L. M.; Hernández-Borges, J.; Herrera-Herrera, A. V. and Rodríguez-Delgado, M. Á. Pesticide extraction from table grapes and plums using ionic liquid based dispersive liquid-liquid microextraction. Analytical and Bioanalytical Chemistry 395 (2009): 2387-2395.

[32] Zhou, Q.; Zhang, X. and Xiao, J. Ultrasound-assisted ionic liquid dispersive liquid-phase micro-extraction: a novel approach for the sensitive determination of aromatic amines in water samples. Journal of Chromatography A 15 (2009): 4361-5.

[33] Farajzadeh, M. A.; Djozan, D.; Nouri, N.; Bamorowat, M. and Shalamzari, M. S. Coupling stir bar sorptive extraction-dispersive liquid-liquid microextraction for preconcentration of triazole pesticides from aqueous samples followed by

GC-FID and GC-MS determinations. Journal of Separation Science 33 (2010): 1816-1828.

[34] Zhang, S., et al. Application of dispersive liquid-liquid microextraction combined with sweeping micellar electrokinetic chromatography for trace analysis of six carbamate pesticides in apples. Analytical Methods 2 (2010): 54-62.

[35] Zhou, Q.; Pang, L. and Xiao, J. Trace determination of dichlorodiphenyltrichloroethane and its main metabolites in environmental water samples with dispersive liquid-liquid microextraction in combination with high performance liquid chromatography and ultraviolet detector. Journal of Chromatography A 25 (2009): 6680-4.

[36] Brereton, R. G. Applied chemometrics for scientists. Chichester, UK: John Wiley & Sons, 2007.

[37] Hamilton, D. J., et al. REGULATORY LIMITS FOR PESTICIDE RESIDUES IN WATER (IUPAC Technical Report). Pure and Applied Chemistry 75 (2003): 1123-1155.

[38] Zhou, Q.; Pang, L. and Xiao, J. Trace determination of dichlorodiphenyltrichloroethane and its main metabolites in environmental water samples with dispersive liquid-liquid microextraction in combination with high performance liquid chromatography and ultraviolet detector. Journal of Chromatography A 25 (2009): 6680-6684.

VITAE

Miss Panida Khamnoon was born on the 1st of May 1987 in Bangkok, Thailand. She received her Bachelor's degree of Science (Chemitry) from Chulalongkorn University in 2008. Then, she has continued the graduate study in the Department of Chemistry, Faculty of Science, Chulalongkorn University in 2010 and has become a member of Chromatography and Separation Research Unit (ChSRU). She finished her Master's degree of Science in May 2013.

Poster presentation and proceeding

"Development of dispersive liquid-liquid microextraction based on adjustment of solvent mixture density for determination of Organophosphorus residues in water" Panida Khamnoon, Puttaruksa Varanusupakul. Poster presentation and proceeding, *Pure and Applied Chemistry International Conference (PACCON 2013)*, The Tide Resort, Chonburi, Thailand, 23-25 January, 2013.