การเตรียมตัวอย่างโดยดิสเพอร์ซีฟลิควิด-ลิควิดไมโครเอกซ์แทร็กชัน สำหรับการตรวจวัดสารกำจัดศัตรูพืชตกค้างในขิง

นางพรศิริ สายะพันธ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2555

SAMPLE PREPARATION BY DISPERSIVE LIQUID-LIQUID MICROEXTRACTION FOR DETERMINATION OF PESTICIDE RESIDUES IN GINGER

Ms. Phonsiri Sayaphan

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	SAMPLE PREPARATION BY DISPERSIVE LIQUID-LIQUID MICROEXTRACTION FOR DETERMINATION OF PESTICIDE RESIDUES IN GINGER
By	Ms. Phonsiri Sayaphan
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.)

พรศิริ สายะพันธ์ : การเตรียมตัวอย่างโดยดิสเพอร์ซีฟลิควิด-ลิควิดไมโครเอกซ์แทร็กชันสำหรับการตรวจวัดสารกำจัดศัตรูพืชตกค้างในขิง (SAMPLE PREPARATION BY DISPERSIVE LIQUID-LIQUID MICROEXTRACTION FOR DETERMINATION OF PESTICIDE RESIDUES IN GINGER) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : อาจารย์ ดร. พุทธรักษา วรานุศุภากุล 71 หน้า.

การสกัดสารกำจัดศัตรูพืชกลุ่มออร์แกโนฟอสเฟต ออร์แกโนคลอรีนและไพรีทรอยด์จากขิงโดยใช้เทคนิคการสกัดตัวอย่างด้วยตัวทำละลายโดยใช้อะซีโตไนไตรล์และร่วมด้วยเทคนิคการเตรียมตัวอย่างแบบดิสเพอร์ซีฟลิควิด-ลิควิดไมโครเอกซ์แทร็กชัน มีการศึกษาพารามิเตอร์ต่างๆ ที่มีผลต่อประสิทธิภาพการสกัดด้วยเทคนิคการเตรียมตัวอย่างแบบดิสเพอร์ซีฟลิควิด-ลิควิดไมโครเอกซ์แทร็กชัน เช่น ชนิดและปริมาณตัวทำละลายอินทรีย์ที่เป็นตัวสกัด, ชนิดและปริมาณตัวทำละลายอินทรีย์ที่ใช้ในการกระจายตัว, ระยะเวลาการสกัด, การเติมเกลือโซเดียมคลอไรด์, การปรับสภาพความเป็นกรด ด่าง, และระยะเวลาในการปั่นเหวี่ยงความเร็วสูง สภาวะที่เหมาะสมสำหรับการเตรียมตัวอย่างคือใช้ เตตระคลอโรเอทิลีนเป็นตัวทำละลายอินทรีย์ที่มีขั้วสูงเป็นตัวสกัด 50 ไมโครลิตร, สารละลายอะซีโตไนไตรล์ เป็นตัวทำละลายอินทรีย์ที่ใช้ในการกระจายตัว 1 มิลลิลิตร, ระยะเวลาการสกัด 2 นาที โดยเริ่มจากนำตัวทำละลายอินทรีย์ที่มีขั้วสูงผสมใส่เข้าไปในสารละลายอะซีโตไนไตรล์ที่มีสารกำจัดศัตรูพืชที่ต้องการวิเคราะห์แล้วเขย่าด้วยมือ, ระยะเวลาในการปั่นเหวี่ยงความเร็วสูง 5 นาทีและเติม 4 % โซเดียมคลอไรด์ ศึกษาคุณลักษณะเฉพาะของวิธีโดยการหาค่าความแม่นให้ค่าการนำกลับคืนมาทุกสารกำจัดศัตรูพืชที่ความเข้มข้นระดับ 5.0, 15.0, และ 100.0 นาโนกรัมต่อกรัม มีค่าอยู่ในช่วง 60 - 105 %, ขีดจำกัดของการตรวจพบมีค่าอยู่ในช่วง 2.2 -3.1 นาโนกรัมต่อกรัม, ขีดจำกัดของการหาเชิงปริมาณมีค่าอยู่ในช่วง 7.2-10.2 นาโนกรัมต่อกรัม และความเที่ยงโดยใช้ HORRAT ประเมินค่าได้ 0.13 – 1.86 ถ้าเปรียบเทียบวิธีนี้กับวิธีการเตรียมตัวอย่างทั่วไปพบว่าวิธีที่เสนอนี้มีความได้เปรียบของการเป็นวิธีการสกัดที่รวดเร็ว และง่ายต่อการทำงานและมีการใช้ตัวทำละลายอินทรีย์ปริมาณน้อยกว่าทั่วไป

ภาควิชา เคมี ลายมือชื่อนิสิต.....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา 2555

5272445023 : MAJOR CHEMISTRY
KEYWORDS : DISPERSIVE LIQUID-LIQUID MICROEXTRACTION / DLLME / GINGER / PESTICIDE RESIDUES / GAS CHROMATOGRAPH-MASS SPECTROMETRY

PHONSIRI SAYAPHAN : SAMPLE PREPARATION BY DISPERSIVE LIQUID-LIQUID MICROEXTRACTION FOR DETERMINATION OF PESTICIDE RESIDUES IN GINGER. ADVISOR : PUTTARUKSA VARANUSUPAKUL, Ph.D., 71 pp.

In this thesis, the multiresidues of organophosphates (OPPs), organo-chlorines (OCLs) and pyrethoids (PYs) pesticides in ginger were determined. The OPPs, OCLs and PYs pesticides in ginger sample were extracted by liquid-liquid extraction with acetonitrile combined with dispersive liquid-liquid microextraction (DLLME) and analyzed by gas chromatography–mass spectrometry. Various parameters that affected the extraction efficiency of DLLME including type and volume of extraction and disperser solvents, extraction time, centrifugation time, salt addition, and pH were evaluated. The optimum conditions were using 50 μL of tetrachloroethylene as the extraction solvent, 1 mL of acetonitrile as the disperser solvent, extraction time of 2 min, centrifugation time of 5 min, and the addition of 4% of sodium chloride. Recovery tests were performed at concentrations levels of 5.0, 15.0 and 100.0 ng/g; recoveries for each target were in the range between 60 to 105%. Limits of detection and limits of quantitation of this method were ranging from 2.2 to 3.1 ng/g and 7.2 to 10.2 ng/g, respectively. The repeatability of the proposed method, expressed as HORRAT, was in range from 0.13 to 1.86. Compared with the conventional sample preparation method, the proposed method has the advantage of being quick and easy of operation, and low consumption of organic solvent.

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

ACKNOWLEDGEMENTS

Firstly, I would like to express my gratitude to my advisor, Dr. Puttaruksa Varanusupakul for continuously providing important guidance and always giving the great opportunity throughout my three and a half years of M.Sc. study at Chulalongkorn University. I truly appreciate and thank her for unwavering encouragement during the writing of this thesis. I also would like to thank members of the thesis examination committee, Assistant Professor Dr. Warinthorn Chavasiri, and Dr. Luxsana Dubas, who give helpful comments and advice in this thesis. My sincere appreciation is also extended to the external committee member, Dr. Apinya Navakhun, for her suggestions.

I especially want to thank director, office of research and development region 2, and head, development of inspection for crops and production resources group laboratory, department of agriculture who provide advice, support chemicals, and equipment throughout this research and all those others whom I can not mention here.

Finally, I am affectionately thankful to my Sisukdee and Sayaphan Family for their heartful unlimited support, belief, and encouragement throughout my education.

CONTENTS

PAGE

PAGE

PAGE

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

FIGURE **PAGE**

FIGURE **PAGE**

LIST OF ABBREVIATIONS

µg/kg	microgram per kilogram
µg/L	microgram per liter
µg/mL	microgram per milliliter
[Bmim][PF_6]	1-Butyl-3-methylimidazolium hexafluorophosphate
$CHCl_3$	chloroform
$C_2H_2Cl_4$	tetrachloroethane
CH_3CCl_3	methyl chloroform
C_2Cl_4	tetrachloroethylene
CO_2	carbon dioxide
[C_6MIM][PF_6]	1-hexyl-3-methylimidazolium hexafluorophosphate
[C_8MIM][PF_6]	1-octyl-3-methylimidazolium hexafluorophosphate
DAD	diode Array detector
DCM	dichloromethane
DLLME	liquid–liquid microextraction
DSPE	dispersive solid-phase extraction
EC	european commision
ECD	electron-capture detection
EFs	enrichment factor
EtAc	ethyl acetate
FID	flame Ionization Detector
FLD	fluorescence detector
FPD	flame-photometric detection
GC	gas chromatography
GC-MS	chromatography–mass spectrometry
HCOOH	formic acid
HFLPME	hollow fiber liquid phase microextraction
HPLC	high-performance liquid chromatography
H_2SO_4	sulfuric acid

L	liter
LC	liquid chromatography
LLE	liquid liquid extraction
LPME	liquid phase microextraction
LOQ	limit of quantification
LOD	limits of detection
M	molar
mL	milliliter
MAE	microwave-assisted extraction
MeCN	acetonitrile
$MgSO_4$	magnesium sulfate
MRLs	maximum residue limits
MRM	multiclass multiresidue methods
MSD	mass spectrometry detection
MSPD	matrix solid phase dispersion
ng/g	nanogram per gram
NaCl	sodium chloride
NPD	nitrogen-phosphorus detection
OCLs	organochlorine
OPPs	organophosphate
pg/g	picogram per gram
PAHs	aromatic hydrocarbons
ppm	parts per million
PSA	primary secondary amine
PYs	pyrethroids
QuEChERS	Quick Easy Cheap Effective Rugged Safe
R^2	coefficient of determination
RTILs	room temperature ionic liquids
s	second
SD	standard deviation
SDME	single drop microextraction
SE	solvent extraction

SIM	selected ion monitoring
SPE	solid-phase extraction
SPME	solid-phase microextraction
S/N	signal-to-noise ratio
v/v	volume by volume
w/v	weight by volume

CHAPTER I

INTRODUCTION

1.1 Introduction

Nowadays, pesticides are applied worldwide to a broad variety of crops both in field and post-harvest for killing or controlling pests such as rodents, insects, fungi, and weeds. Also, it may be used on animal farms to control insect pests. Pesticides can be grouped into chemical family. Prominent insecticide family includes organochlorines, organophosphates, pyrethroids and carbamates. Residues of these pesticides are sometimes found in food grown on contaminated soil, or in the fish that live in contaminated waters. Pesticide residues have an effect on human health: acute neurologic toxicity, chronic neuro, and cancer. In recent years, public concern about possible health risks from pesticide residues in the diet is increased. Food quality and safety issue was widespread concerned and led to strict a regulation of maximum residue limits (MRLs) and total dietary intakes of pesticide residues in food commodity. MRLs are established to protect consumers from the harmful health effects of over exposure. There are various organizations that set the MRLs, such as European Commision (EC), Codex Alimentarius or national governments in Australia, Canada, Japan, etc. Due to varying and stringent regulations across various borders, the accurate detection and quantification of contaminants in food has become a necessity.

Ginger is the plant underground stem, or rhizome, of *Zingiber officinale* [1]. The fresh ginger rhizome can be yellow, white or red in color, depending upon the variety and covers with a brownish skin as shown in Figure 1.1 [2]. Ginger has been used as a medicine since ancient times to help digestion, treat stomach upset, and nausea. Ginger has also been used to treat arthritis, colic, diarrhea, and heart conditions [3]. In addition, ginger is used throughout the world as an important cooking spice. Ginger is low in calories and contains no cholesterol, but contains a

very rich source of nutrients, vitamins and minerals such as vitamin C, B-6, B-5, folate, potassium, manganese, copper, calcium, iron, phosphorous, zinc and magnesium [2-3]. In 2008, India was the leader in ginger export, with over 30% of the global share, followed by China (20.5%), Indonesia (12.7%), Nepal (11.5%) and Thailand (10%). In Thailand, original grown is located in the north of Thailand and the most ginger production is at Khao Kho, Phetchabun province. Moreover, most ginger from the lower northern region is exported to Europe and Japan.

Figure 1.1 The ginger harvested in November.

Many groups of pesticides are applied in fruit and vegetables. Therefore, the methods for multiresidue determination of pesticides have been developed as the most cost-effective approach to residue analysis. However, multiresidue analysis is difficult due to the fact that compounds of different polarities, solubilities, volatilities and p*K*a values have to be simultaneously extracted and analyzed. Several multiresidue methods for determination of organophosphate, organochlorine and organonitrogen pesticides in crops were reported [4-5] using gas chromatography (GC) with selective and sensitive detectors such as electron-capture detection (ECD), nitrogen-phosphorus detection (NPD), flame-photometric detection (FPD) and mass spectrometry (MS). Amongst, mass spectrometry is good for both multiresidue determination and trace-level identification of a wide range of pesticides.

At present, the determination of pesticide residues mostly analyzes by GC and GC–MS, hence, sample preparation step becomes important to obtain accurate and sensitive results. Methods have been established for separation and preconcentration of pesticide residues, such as liquid–liquid extraction (LLE), and solid-phase extraction (SPE) [5]. However, LLE always uses large amount of toxic organic solvents, time-consuming, and emulsion formation problem [6]. SPE is an alternative and already a well-established and routine technique. Although SPE uses much less solvent than LLE and can be automated, this entails complexity [7]. Microextraction techniques, such as solid-phase microextraction (SPME), liquid-phase microextraction (LPME) and single drop microextraction (SDME) have been widely applied for the preconcentration and quantification of pesticides [8-11]. These techniques are miniaturization and reduction of organic solvent consumption and improve the selectivity of the extraction. SPME are extracted target analytes of low or medium polarity from aqueous or gaseous samples onto a solid polymeric fiber. Extraction occurs by passive diffusion and the extraction yield is essentially determined by the fiber to sample partition coefficient. However, the coated fibers have limited lifetimes [12-14]. LPME is an alternative miniaturized sample-preparation approach using a microliter volume of the solvent to extract analytes from the aqueous samples [15-20]. It overcomes many disadvantages of LLE as well as some of those of SPME (e.g. independence of a commercial supplier and sample

carryover or cross-contamination). Single drop microextraction (SDME) was developed as a solvent-minimized sample pretreatment procedure. It is inexpensive, and since very little solvent is used, there is minimal exposure to toxic organic solvents [21-25]. However, some disadvantages of this method are as follows: fast stirring would tend to break up the organic drop; air bubble formation; extraction is time-consuming and equilibrium could not be attained after a long time in most cases.

Dispersive liquid-liquid microextraction (DLLME) is an alternative method of sample preparation based on the use of a ternary component solvent system: extraction solvent, disperser solvent and water. The extraction solvent and disperser solvent are rapidly injected into the aqueous sample. The mixture is then gently shaken and a cloudy solution is formed. After centrifugation, the fine particles of extraction solvent are sediment at the bottom of the test tube. Mostly, this technique applies for the analysis of the pesticide residues in liquid samples such as rainwater, groundwater, and river water [26-28], or in the high water content vegetable such as watermelon, cucumber and apple [29-30]. Even though chlorinated solvent, which is a toxic organic solvent are used as the extraction solvent, the small volume (less than 100 microliter) was used [31-32].

In this research, a simple and rapid method for multiresidue analysis of pesticide residues in ginger was developed. DLLME technique was applied for a low water content solid sample and analyzed by GC-MS. Several factors such as extraction solvent type and its volume, disperser solvent type and its volume, extraction time, pH of the matrix, and ion strength were optimized.

1.2 Objectives of the research

Sample preparation for determination of organophosphates, organochlorines, and pyrithriods in ginger by dispersive liquid-liquid microextraction (DLLME) was studied prior to analyze by gas chromatography– mass spectrometry (GC-MS).

CHAPTER II

THEORY AND LITERATURE

2.1 Pesticides residues in foods

Residues of pesticides are sometimes found in food grown on contaminated soil, or in the fish that live in contaminated waters. Many pesticides can be grouped into chemical family. Prominent pesticide residues in foods are organophosphates (OPPs), organochlorines (OCLs), and pyrethroids (PYs).

2.1.1 Organophosphate

Organophosphate (OPPs) pesticides are used extensively worldwide. These compounds are harmful and cause a serious public health problem, particularly in developing nations. The toxicity of OPPs poisoning vary not only with the route and extent of exposure, but also the chemical structure of the agent [33]. General structure of OPPs is the esters of phosphoric acid shown in Figure 2.1. There are the basis of many insecticides, herbicides, and nerve gases. Phosphates are probably the most pervasive OPPs and its irreversibly inactivate acetylcholinesterase, which is essential to nerve function in insects, humans, and many other animals [34]. Commonly used OPPs are parathion, parathion methyl, malathion, chlorpyrifos,

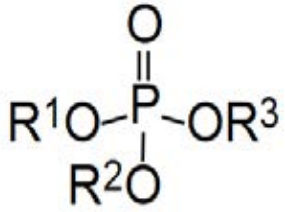


Figure 2.1 General chemical structure of an organophosphate

diazinon, dichlorvos, fenitrothion and azinphos methyl. OPPs pesticides have a higher acute toxicity than organochlorines, but they have the advantage of being rapidly degraded in the environment [34].

2.1.2 Organochlorines

Organochlorine (OCLs) pesticides are synthetic organic chemicals. There are widely used around the world. This class comprises a variety of compounds containing carbon, hydrogen, and chlorine. These compounds can be highly toxic to humans and other animals and highly toxic to most aquatic life. They can have serious short-term and long-term impacts at low concentrations. And some agents, such as DDT, have been banned in Thailand because of their unacceptably slow degradation and subsequent bioaccumulation. Commonly used OCLs are α- and β-endosulfan (Figure 2.2). OCLs pesticides have a lower acute toxicity than OPPs.

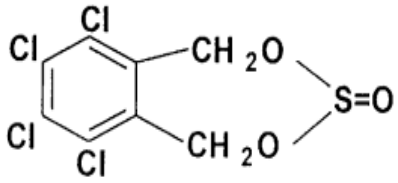


Figure 2.2 General chemical structure of an organochlorine-α, and β-endosulfan

2.1.3 Pyrethroids

Pyrethroids (PYs) are synthesized derivative of pyrethrins, and were developed in order to maintain the effective insecticidal activity of the pyrethrins while increasing stability to light and residence time in the environment [35]. There are two types that differ in chemical structure and symptoms of exposure. Type I pyrethroids include allethrin, tetramethrin, resmethrin, d-phenothrin, bioresmethrin, and permethrin (1, 2). Some examples of type II pyrethroids are cypermethrin, cyfluthrin, deltamethrin, cyphenothrin, fenvalerate, and fluvalinate (1, 2). Both type I

and type II pyrethroids inhibit the nervous system of insects [34-35]. This occurs at the sodium ion channels in the nerve cell membrane [35].

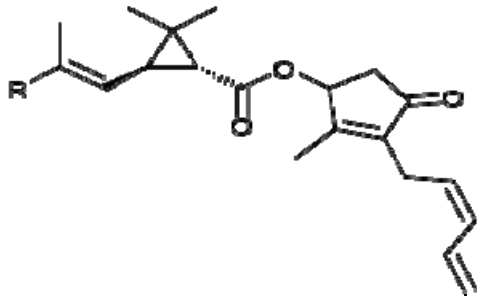


Figure 2.3 General chemical structure of a pyrethrins ; Pyrethrin I, R = CH_3 ; Pyrethrin II, R = CO_2CH_3

2.2 Method of sample preparation for analysis of pesticide residues

According to the status list of active substances available commercially in the EU, more than 1,100 pesticides are currently registered. Increasing public concern in recent years about the possible health risk of pesticide residues in the diet has profoundly modified crop-protection strategies, with emphasis on food quality and safety, and widespread concern for the health of society has led to the strict regulation of maximum residue limits (MRLs) of pesticide residues in food [36-38]. The European Commission has specified the MRLs of 10 µg/kg for pesticide residues in foods [38]. This has led to the development of many multiresidues analytical methods, which allow the simultaneous determination of a several number of pesticides in food at very low concentration in response to the legislation in many countries. In most instances, capillary gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been the techniques selected for the analysis of pesticide residues in vegetables [39-40]. The most pesticide analysis involves multiclass multiresidue methods (MRM) to detect a wide variety of potential pesticides in the sample. Because of the wide range of chemical properties of pesticides (including acidic, basic and neutral) and the wide variety of matrices (polar, nonpolar, fatty, and waxy), the sample must initially be cleaned up using a compatible

sample preparation technique before injection into the chromatographic system. Typical strategies for the GC determination in liquid, gaseous and solid samples are summarized in Figure 2.4.

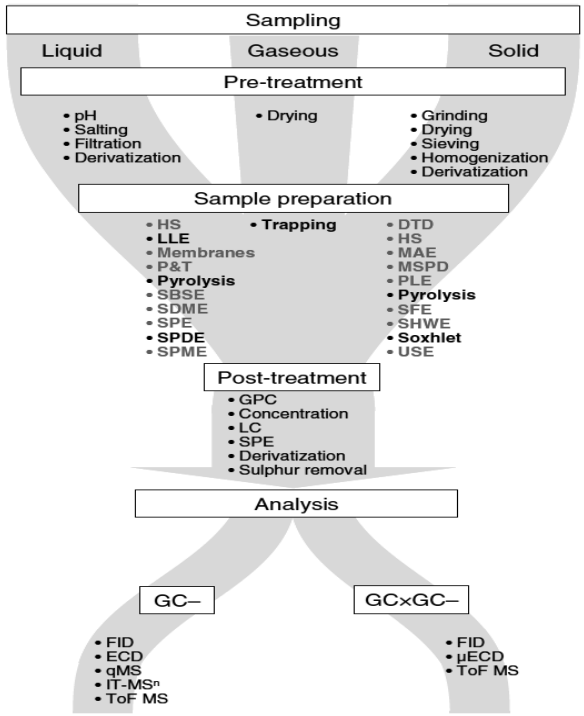


Figure 2.4 Typical strategies for the GC determination in liquid, gaseous and solid samples [41].

Sample preparation and chromatographic analysis should take a consideration of the limit of quantification (LOQ) and selectivity. In multiresidue pesticides analysis, several chromatographic runs are usually necessary for qualitative and quantitative analysis to monitor the presence of pesticide residues at MRLs. Positive samples exceeding the MRLs require subsequent confirmation. In GC analysis, the need for positive identification and more flexible methods that enable analysis of a wide variety of samples in one system is the trend. Mass spectrometric detection (MSD) is clearly served the need, because it enables structural for analyte identification. Quadrupole instruments have been most widely used with capillary GC. MSD in

selected ion monitoring (SIM) mode is mostly used to obtain the low limit of detection (LOD) and limit of quantification (LOQ) required for regulation purposes.

Analysis of pesticides in food matrices is a difficult task, because of the complexity and the low concentrations. Moreover, the determination methods of multiresidue pesticides in foods and agricultural products involved many use of organic solvents to extract, usually acetone followed by water dilution and partitioning into a nonpolar solvent such as methylene chloride and petroleum ether. This approach worked fine for nonpolar pesticides but certain polar compounds such as organophosphorus insecticides and several modern pesticides were partially lost. The development of highly efficient analytical instrumentation for determination is concerned and sample pretreatment is also an important part to obtain accurate quantitative results. Nevertheless, an enrichment step in sample preparation is usually needed when the concentration levels are low.

2.2.1 Liquid-liquid extraction (LLE)

Analytes in solutions or liquid samples can be extracted by direct partitioning with an immiscible solvent. Liquid-liquid extraction (LLE) is based on the relative solubility of an analyte in two immiscible phases and is governed by the equilibrium distribution/partition coefficient. Extraction of an analyte is achieved by the differences in the polarity of the two immiscible liquid phases.

LLE is traditionally one of the most common methods of extraction, particularly for organic compounds from aqueous matrices. Typically a separating funnel is used and the two immiscible phases are mixed by shaking and then allowed to separate. To avoid emulsions, in some cases, a salt may be added and centrifugation can be used if necessary. Alternatively a matrix solid-phase dispersion (MSPD) approach can be used to avoid emulsions. Both layers can be collected for further analysis. To ensure the complete extraction of an analyte into the required phase, multiple extractions may be necessary. Due to the limited selectivity, particularly for trace level analysis, there is a need for cleanup or analyte enrichment and concentration steps prior to instrumental analysis. In the case of multiresidue

methods, the extracting solvent has to be suitable for the extraction of compounds within a wide polarity range from a variety of matrices containing different amounts of water, fats, sugars and other substances. The usual way for extracting pesticide residues from the sample is by thorough disintegration of the matrix in a high speed homogenizer in the presence of the solvent or solvent mixture (Figure 2.5). In this way, the original methods were extracted the pesticides with acetonitrile, followed by liquid-liquid partitioning with petroleum ether/dichloromethane and a laborious florisil column cleanup. Later, the use of acetone instead of acetonitrile is applied [42]. Acetone extraction is usually preferred since it is suitable for both non-polar and polar pesticides, as has been demonstrated in many comparative studies performed by GC and HPLC. In addition, acetone has low toxicity, easy to purify, evaporate and filter as well as inexpensive. Fruit and vegetable extracts in acetone are usually cleaner than those obtained with other solvents of similar polarity.

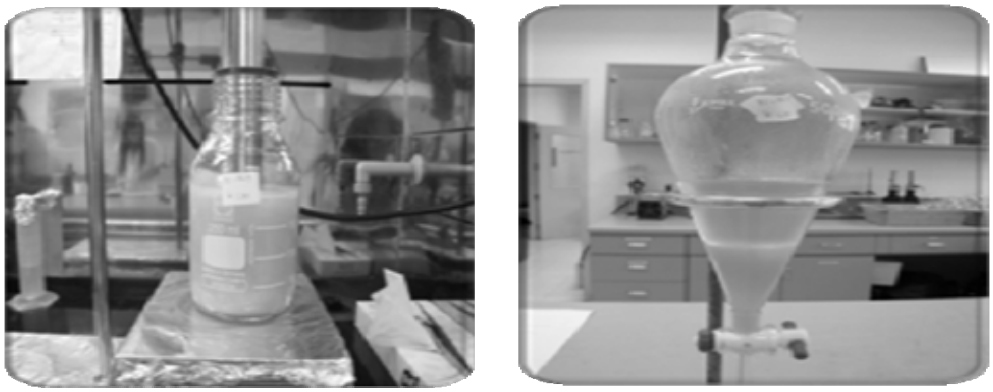


Figure 2.5 Sample preparation by liquid-liquid extraction for analysis of pesticide residues in ginger.

2.2.2 Solid-phase extraction (SPE)

Solid-phase extraction (SPE) is a separation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties. Analytical laboratories use solid phase extraction to concentrate and purify samples

for analysis. SPE is more efficient than liquid/liquid extraction, yields quantitative extractions that are easy to perform, is rapid, and can be automated. Solvent use and lab time are reduced. SPE also enables avoidance of the emulsion formation often encountered in LLE. SPE is used most often to prepare liquid samples and extract semivolatile or nonvolatile analytes, but also can be used with solids that are pre-extracted into solvents. They are available in a wide variety of chemistries, adsorbents, and sizes. Selecting the most suitable product for each application and sample is important. Stajnbaher and Zupancic-Kralj [43] used solid-phase extraction on a highly cross-linked polystryrene divinylbenzene column for the simultaneous isolation of 90 pesticides from fruits and vegetables and pre-concentration of the pesticides from the water-diluted acetone extract. Hernandez et al. [44] determination of 52 pesticides residues in fruits and vegetables by SPE 20 g of homogenized sample was mixed in 60 mL of methanol:water (80:20, v/v) 0.1% HCOOH. The recoveries ranged from 70 to 110% with satisfactory precision.

2.2.3 QuEChERS

QuEChERS [45-48] stands for a quick, easy, cheap, effective, rugged, and safe method. It is the most recent method for analysis of pesticide residues in food. QuEChERS has advantages on a minimum number of steps and low consumption of solvent and glassware. The original procedure is as the diagram in Figure 2.6, which a homogenized sample is extracted by mixing it with an extraction solvent, normally use acetonitrile. Anhydrous magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) are added to the sample to drive partitioning of the analytes between the aqueous residue and the solvent. Then, cleanup and removal of residual water is performed simultaneously by the use of dispersive solid-phase extraction (DSPE), in which a primary secondary amine (PSA) adsorbent and anhydrous $MgSO_4$ are added to the sample extract. The mixture is finally centrifuged and the supernatant can be taken for analysis directly or subjected to a concentration and solvent exchange step if necessary. There have been several modifications of the technique depending on analytes, matrices, instrumentation and analyst preferences. The QuEChERS method has the advantages of high recovery, accurate results, high sample throughput,

low consumption of solvent and glassware, less labor, lower reagent costs, and ruggedness.

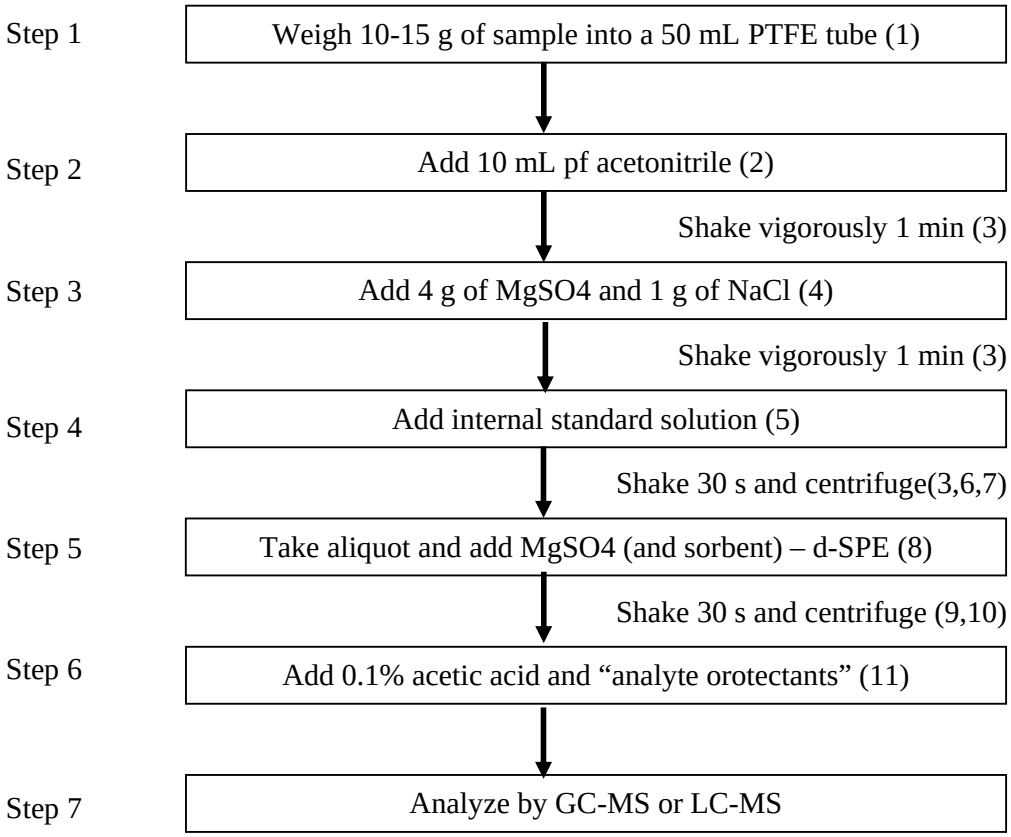


Figure 2.6 Flow diagram of QuEChERS procedure

2.2.4 Dispersive liquid-liquid microextraction (DLLME)

Dispersive liquid–liquid microextraction (DLLME) is a simple and rapid preconcentration and microextraction method developed by Assadi and co-workers [49]. This technique was initially applied for the determination of polycyclic aromatic hydrocarbons (PAHs) in water samples. This method is based on the use of a ternary component solvent system (extraction solvent, disperser solvent and water). The extraction solvent and disperser solvent were rapidly injected into the aqueous sample by syringe. The mixture was then gently shaken and a cloudy solution was formed. After being centrifuged, the fine droplets of extraction solvent were sediment at the bottom. The resultant sediment phase was taken using microsyringe and injected into

GC or LC for analysis [31-32]. The extraction steps of DLLME are illustrated in Figure 2.7.

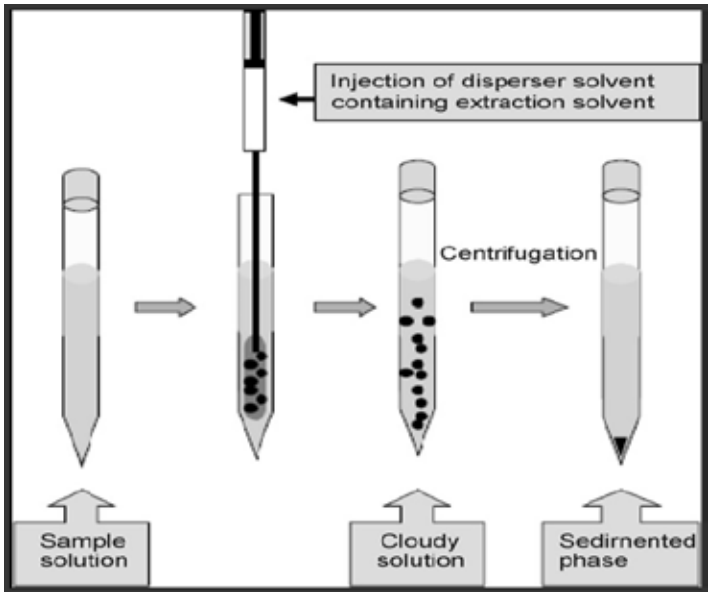


Figure 2.7 Dispersive liquid-liquid microextraction procedure. [50]

The extraction efficiency for the target analyte by DLLME is influenced by many factors, such as the kind of extraction and disperser solvent, and their volume, the extraction time, and salt addition [31-32, 50].

2.2.4.1 Factors in DLLME

1) Extraction solvent

The selection of an extraction solvent is an important parameter for DLLME process. The extraction solvent should have a capability to extract the compounds of interest, good chromatographic behavior, and low solubility in water. A density higher than that of water is required to attain a sediment organic solvent phase separated from aqueous phase after centrifugation. Halogenated hydrocarbon, such as

chlorobenzene, chloroform, carbon tetrachloride, and tetrachloroethylene are usually selected as extraction solvents because of their promise properties.

The volume of extraction solvent has great effects on the enrichment factor. The organic phase obtained by centrifugation is increased with the increase of the extraction solvent volume, resulting in a decrease of the concentration of the target analyte in organic phase. Although the extraction recovery keeps almost constant, the enrichment factor will be decreased, leading to a decrease of the sensitivity for trace analysis. Therefore, the optimal extraction solvent volume should ensure both the high enrichment factors and the enough volume for the subsequent determination by instrument.

2) Disperser solvent

The selection of disperser solvent is based on its solubility and immiscibility in both extraction solvent and water. Thus, an extraction solvent is enable dispersed as fine droplets in aqueous phase to form a cloudy solution (water/disperser solvent/extraction solvent). In such a case, the surface area between extraction solvent and aqueous phase (sample) can be infinitely large leading to an increase of the extraction efficiency. The commonly used disperser solvents include methanol, ethanol, acetonitrile, acetone, and tetrahydrofuran.

The disperser solvent volume directly affects a formation of the fine droplets in aqueous phase, a degree of the dispersion of the extraction solvent in aqueous phase, and subsequently, extraction efficiency. The optimal volume of disperser solvent should ensure both the high enrichment factors and extraction efficiency.

3) Extraction time

Extraction time in DLLME is defined as the interval between injecting the mixture of disperser solvent and extraction solvent into aqueous sample and

centrifugation. The extraction time is occasionally having slight effect on the extraction efficiency because of the fact that the extraction solvent can be evenly and easily dispersed after injected into the aqueous solution. The transition of the analyte from aqueous phase (sample) to extraction phase can be very fast, and the equilibrium state can be subsequently achieved very quickly. As a result, a very short extraction time is needed for equilibrium. This is a remarkable advantage of the DLLME technique.

4) Effect of salt addition

The solubilities of the target analyte and organic extraction solvent in aqueous phase are usually decreased with the increase of ionic strength, which is favorable for reaching a high recovery. Adding a salt in the aqueous can increase this process. However, at the same time, the obtained volume of organic phase is increased, resulting in a decrease of both the target analyte concentration and the enrichment factor.

2.2.4.2 Application of DLLME

DLLME can be coupled with GC, and HPLC for application. It has been widely applied to the analyses of pesticide residues. The typical applications are shown in Table 2.1 and 2.2

Assadi et al. [49] were first developed DLLME for extraction and determination of polycyclic aromatic hydrocarbons (PAHs) in water samples, 8.0 µL of C_2Cl_4 as extracting solvent containing 1.0 mL of acetone were rapidly injected into a 5.0 mL of the sample solution , and the mixture was gently shaken. Then, the mixture was centrifuged and 2.0 µL of the sedimented phase was injected into the GC-FID for analysis. Under the optimum conditions, the enrichment factor (EFs) was 603–1113 and the detection limits (LODs) was 0.007–0.030 µg/L for most of the analytes. In time, they developed another new method for the extraction of organophosphorus pesticides (OPPs) from water samples by DLLME-GC-FPD [26].

In this method, a mixture of 12.0 µL chlorobenzene and 1.0 mL acetone was rapidly injected into the 5.0 mL water sample by syringe. After centrifugation, 0.5µL of sedimented phase was injected into the GC. Under the optimum conditions, the EFs and LODs were obtained as 789–1070 and 0.0003-0.02 µg/L. The comparison of the new method with others such as SPME and SDME demonstrated that DLLME was very fast, simple, accurate and inexpensive. They developed another new DLLME method to the analyses of trichloromethane, chlorobenzenes, polychlorinated biphenyls ,etc. in environmental samples.

Feo et al. [27] has been successfully applied to the determination of pyrethroid pesticide residues in real water sample by DLLME-GC-ECD. Under the optimum conditions, the EFs and LODs were obtained as 708–1087 and 0.04–0.10 µg/L. Besides, DLLME has also been applied to the determination of triazine herbicides, phthalate esters, chlorophenols, and amide herbicides, etc. in environmental water samples. Farina et al.[51] proposed a new method for the analysis of volatile phenols in the aroma of red wine by combining DLLME with GC-MS. Fu et al. [28] developed DLLME method for the analysis of carbamate (carbaryl) and organophosphorus (triazophos) pesticide residues in water and fruit juice samples coupled with LC-FLD. Methanol was first used as extraction solvent for the extraction of pesticides from the soil samples and then as dispersive solvent in the DLLME procedure. Under the optimum extraction conditions, the linearity was obtained in the concentration range of 0.1–1,000 ng/g for carbaryl and 1–5,000 ng/g for triazophos, respectively.The limits of detection (LODs), based on signal-to-noise ratio (S/N) of 3, ranged from 14 to 110 pg/g etc.

Dispersive liquid-liquid microextraction is more suitable for the treatment of the target compounds with simple matrix, resulting in its wide application in the analysis of water samples. Zhao et al.[29] developed the pretreatment of samples with complex matrix by DLLME for the determination of OPPs in cucumber and watermelon by DLLME-GC-FPD. Acetonitrile (MeCN) was used as extraction solvent for the extraction of OPPs from plant samples and as

dispersive solvent in step of DLLME. under the optimum conditions, the EFs and LODs were obtained as 41-50 and 0.010 to 0.190 μg/kg for the target pesticides.

Zang et al. [30] Analysis of captan, folpet, and captafol in apples by DLLME combined with GC–ECD. Under the optimum conditions, high enrichment factors for the compounds were achieved ranging from 824 to 912. The recoveries of fungicides in apples at spiking levels of 20.0 μg/kg and 70.0 μg/kg were 93.0–109.5% and 95.4–107.7%, respectively. Used chlorobenzen and acetone as extraction solvent and dispersive solvent, respectively

Moinfar et al. [52] determination of organophosphorus pesticides (phorate; diazinon; disolfotan; methyl parathion; sumithion; malathion; fenthion; profenphose; ethion; phosalone) in tea was developed by using DLLME and GC-FPD. A mixture of acetonitrile and n-hexanewas used as an extraction solvent for the extraction of OPPs from tea samples. When the extraction process was finished, the mixture of solvents was rapidly dispersed in water; target analyte was extracted to a small volume of n-hexane, using DLLME. Recovery tests were performed for concentration 5.0 μg/kg. The recovery for each target analyte was in the range between 83.3 and 117.4%. The detection limit of the method for tea was found ranging from 0.030 to 1.0 μg/kg for all the target pesticides.

Ravelo-Perez et al. [53] describes DLLME procedure using room temperature ionic liquids (RTILs) coupled to HPLC-DAD capable of quantifying trace amounts of eight pesticides (i.e. thiophanate-methyl, carbofuran, carbaryl, tebuconazole, iprodione, oxyfluorfen, hexythiazox and fenazaquin) in bananas. DLLME procedure using 1-hexyl-3-methylimidazolium hexafluorophosphate ($[C_6MIM][PF_6]$) as extraction solvent was used. Mean recovery values of the extraction of the pesticides from banana samples were in the range of 69–97% except for thiophanate-methyl and carbofuran, which were 53–63% with a relative standard deviation lower than 8.7% in all cases. Limits of detection achieved (0.320–4.66 μg/kg).

Xiong et al. [54] developed a new method for the analysis of organosulfur pesticides (malathion, chlorpyrifos, buprofezin, triazophos, carbosulfan, and pyridaben) in environmental and beverage samples by GC-FPD via coupling DLLME with HF-LPME. In our previous work, a novel method was developed for the determination of chlorothalonil, captan, and folpet in grape samples by DLLME coupled with GC-ECD. Under the optimum conditions, the enrichment factor ranged between 788 and 876, and the detection limit was between 6.0 and 8.0 μg/kg. Practical samples were successfully analyzed by the proposed method with satisfactory results. There is also research of Wu et al. [55] developed DLLME method for the analysis of carbendazim and thiabendazole in water and soil samples also. The further development of this novel DLLME technique, the analysis of samples with complex matrix by DLLME will be more and more widely applied. The applications of DLLME in solid sample shown in Table 2.2

Table 2.1. Application of dispersive liquid- liquid microextraction in liquid sample by GC and HPLC.

Years	Analyte	Matrix	Extraction solvent	Disperser solvent	Detection	LOD (µg/ L)	Enrichment factor	Refs.
2006	PAHs	Water	Tetrachloroethylene	Acetone	GC-FID	0.007–0.030	603–1113	49
2006	Organophosphorus	Water	Chlorobenzene	Acetone	GC-FPD	0.0003-0.02	789–1070	26
2008	Amide herbicides	Environmental Water	Carbon tetrachloride	Acetone	GC-MS	0.003–0.02	-	56
2008	Organophosphorus	Environmental samples	$[C_6MIM][PF_6]$	-	HPLC	0.17–0.29	-	57
2008	Pyrethroid	Water	Chlorobenzene	Acetone	GC-ECD	0.10-0.04	708-1087	27
2009	Organochlorine	Water	Tetrachloroethylene	Tert-butyl methyl ethers	GC-MS	0.0008–0.0025	1885–2648	-
2009	Organochlorine	Water	Tetrachloroethylene	Acetone	GC-MS	1–25	46–316	-
2009	Carbamate	Water	Chloroform	Acetone	HPLC-DAD	0.4–1.0	-	58
2009	Carbendazim and thiabendazole	Water	Chloroform	Tetrahydrofuran	HPLC-FLD	0.5–1.0	-	28

Table 2.1. Application of dispersive liquid- liquid microextraction in liquid sample by GC and HPLC (continue).

Years	Analyte	Matrix	Extraction solvent	Disperser solvent	Detection	LOD (µg/L)	Enrichment factor	Ref.
2009	Organochlorine	Water	Hexadecane	Acetonitrile	GC-ECD	0.011 and 0.11	-	59
2009	24 residual pesticides	Apple juice	Carbon tetrachloride	Acetone	MD-GC/MS	0.06 to 2.20	-	60
2009	Organophosphorus	Water	$[C_8MIM][PF_6]$	Methanol	HPLC	0.1–5	>200	61
2010	Cypermethrin and permethrin	Pear juice	C_2Cl_4	Methanol	GC-FID	2.2-3.1	-	62
2010	Carbamate	Water	Toluene	Acetonitrile	GC/MSMS	0.001−0.50	-	63

Table 2.2. Application of dispersive liquid- liquid microextraction in solid sample by GC and HPLC.

Years	Analyte	Matrix	Extraction solvent	Disperser solvent	Detection	LOD (µg / kg)	Enrichment factor	Ref.
2007	Organophosphorus	Watermelon,cucumber	Chlorobenzene	Acetonitrile	GC-FPD	0.010-0.190	41–50	29
2008	Captan, folpet and captafol	Apples	Chlorobenzen	Acetone	GC-ECD	3.0–8.0	824–912	30
2009	OPPs	Tea	n-Hexane	Acetonitrile	GC-FPD	0.03-1.0	-	52
2009	Eight pesticides	Bananas	$[C_6MIM][PF_6]$	Methanol	HPLC-DAD	0.320–4.66	-	53
2009	Carbamate and organophosphorus	Soil	$C_2H_2Cl_4$	Methanol	HPLC-FLD	14 -110 pg/g	-	54
2009	Carbendazim and thiabendazole	Soil	Chloroform	Tetrahydrofuran	HPLC-FLD	1.0–1.6	-	55
2009	Eight pesticides	Table grapes and plums	$[C_6MIM][PF_6]$	Acetonitrile	HPLC-DAD	0.651–5.44, 0.902–6.33	-	64

Therefore, new extraction techniques were devised for solid samples, including supercritical fluid extraction, microwave-assisted extraction, solid-phase microextraction, matrix solid-phase dispersion and pressurized fluid extraction–accelerated solvent extraction. Although most of these techniques used less organic solvent than conventional extraction, some were slow and most of the instrumental techniques, were of high cost and required specialists to develop and troubleshoot methods, and sample sizes were limited, an important consideration for trace analysis. Some of these methods involved considerable clean up of glassware and extraction vessels before the next use.

The need for a simple, rapid, inexpensive, multiclass multiresidue method that provided high quality results with a minimal number of steps, with reduced reagent use and required little glassware. In this research, to develop a new method for the sample preparation of pesticide residues in vegetables.

CHAPTER III

EXPERIMENTAL

Reagents, materials, chemicals, instruments set-up, and method modification are explained thoroughly in this chapter.

3.1 Apparatus and Instrumentation

3.1.1 Food chopper— s-blade vertical cutter *e.g.* Robot coupe- R201 Ultra (USA)

3.1.2 Balance—Capable of accurately measuring weight from 0.05 to 100 g within ±0.01 g *e.g.* Mettler toledo, PB-1502-S (Switzerland)

3.1.3 Vortex mixer— *e.g.* Labnet-VX100

3.1.4 Centrifuge— *e.g.* Napco millenium-2028R

3.1.5 GC/MS instrumentation—equipped with EI source and appropriate columns, see details in 3.4 *e.g.* Agilent technologies, GC-6890N (G1530N, China), MS-5973 inert (G2579A, USA)

3.1.6 15 mL, 50 mL centrifuge tubes with screw caps

3.1.7 Automatic pipettes— suitable for handling volumes of 10 to 100 µL, 200 to 1,000 µL and 1 to 10 mL

3.1.8 10 mL solvent-dispenser

3.1.9 10 µL syringe—zero dead volume, Hamilton

3.1.10 Autosampler vials—suitable for GC auto-sampler

3.1.11 Volumetric flask with stoppers—for the preparation of stock and working solutions. *e.g.* 5 mL, 25 mL, 50 mL, 100 mL glass flasks

3.2 Chemicals

All reagents, materials, and chemicals for this work are listed in Table 3.1 and 3.2.

Table 3.1 List of pesticide standards[a]

Chemicals	%Purity
Diazinon	99.5
Pirimiphos-methyl	98.7
Chlorpyrifos	97.5
Ethion	91.8
EPN	95.5
Alpha-endosulfan	94.5
Beta-endosulfan	99.5
Endosulfan sulfate	98.8
Lamda-cyhalothrin	95.6
Permethrin	98.5

[a] all pesticides were supplied from Dr.Ehrenstorfer (Germany)

Table 3.2 List of chemicals

	Chemicals	Suppliers
a)	Acetonitrile (MeCN)	J.T.Baker (USA)
b)	Acetone	J.T.Baker (USA)
c)	Sodium chloride (NaCl)	J.T.Baker (USA)
d)	Sodium hydroxide (NaOH)	Merch (Germany)
e)	Primary secondary amine (PSA) sorbent 40 mm	Macherey nagel
f)	Purified water (HPLC grade)	J.T.Baker (USA)
g)	Tetrachloroethylene (C_2Cl_4)	J.T.Baker (USA)
h)	Carbon tetrachloride (CCl_4)	J.T.Baker (USA)
i)	Chloroform ($CHCl_3$)	J.T.Baker (USA)
j)	Magnesium sulfate anhydrous ($MgSO_4$) , granular 12–60 Mesh	Merch (Germany)
k)	Sulfuric acid 95%, (H_2SO_4)	Sigma (USA)

Table 3.3 Properties and EU regulation for pesticides.

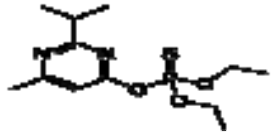
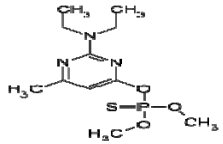
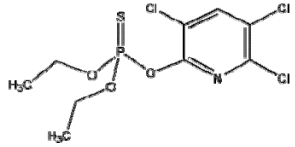
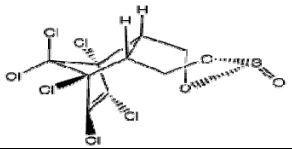
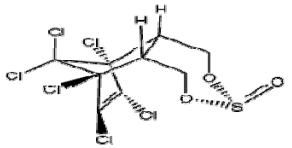
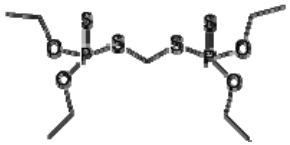
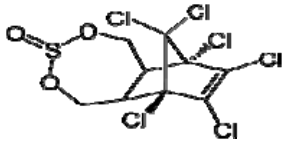
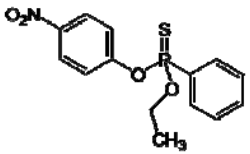
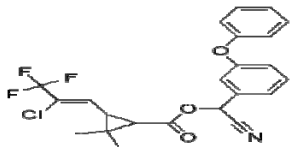
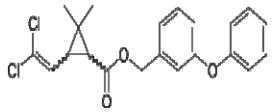
Name	Category	Log P	pKa	Structure	MRLs of EU in ginger (ng/g)
Diazinon	Organophosphate	3.81	2.60		500
Pirimiphos-methyl	Organophosphate	4.20	4.30		50
Chlorpyrifos	Organophosphate	4.70	-		1000
Alpha-endosulfan	Organochlorine	4.74	-		500
Beta-endosulfan	Organochlorine	4.79	-		500

Table 3.3 Properties and EU regulation for pesticides. (Continued)

Name	**Category**	**Log P**	**pKa**	**Structure**	**MRLs of EU in ginger (ng/g)**
Ethion	Organophosphate	5.02	-		300
Endosulfan sulfate	Organochlorine	3.13	-		500
EPN	Organophosphate	5.02	-		Not list*
Cyhalothrin	Pyrethroid	7.00	9.00		Not list*
Permethrin	Pyrethroid	6.10	-		100

* A value of pesticide residue is not list for a product. A positive list sets a default MRL of 10 ng/g.

3.3 Preparation of pesticides standard solutions

The preparation of the mixed standard pesticides and calibration spiking standards are described as follows:

3.3.1 Pesticide stock solutions, 1,000 µg/mL

The stock standard solution of 1,000 µg/mL of each standard was prepared by dissolved 25 mg of standard pesticide in 25 mL of acetonitrile. Store the solution at -20^{o}C in the freezer. The concentration of the standards calculation as

$$\text{The concentration of the standard } (\mu g/mL) = \frac{\text{Standard weight (mg)} \times \text{Purity (\%)} \times 10^3}{\text{Volume of the preparation (mL)} \times 100} \quad (3.1)$$

3.3.2 Intermediate mixed standard solution 10 µg/mL

Intermediate mixed standard solution of 10 µg/mL was prepared by pipetted 1 mL of 1,000 µg/mL stock standard solution of each compound into a 100 mL volumetric flask, and diluted to the mark with acetonitrile.

3.3.3 Pesticide working solutions / mixtures

Mixed standard solutions in the concentration range of 0.1 – 3.0 µg/mL were prepared for a calibration by diluting 10 µg/mL intermediate mixed standard solution in 50 mL volumetric flask with acetonitrile.

3.4 Analysis of pesticides by gas chromatography-mass spectrometry

Chromatographic analysis was performed with Agilent 6890 series gas chromatography equipped with split/splitless injector and Agilent 5973N mass spectrometer (Agilent Technologies, USA). A HP-5MS fused silica capillary column (30m×0.25mm I.D., 0.25μm film thickness) was used for separation. The column oven was programmed as initially held at 90°C for 0.50 min, 90 to 150°C at 15°C/min, 150 to 195°C at 5°C/min, 195 to 200°C at 0.5°C/min, 200 to 250°C at 5°C/min and held at 250°C for 17 min. The carrier gas was helium (purity 99.9995%) at a flow rate of 1.5 mL/min. The injection was operated at 220°C with in-pulse splitless mode. The MS transfer line temperature was held at 280°C. Mass spectrometric parameters were set as follows: electron impact ionization (EI) with 70 eV energy, ion source temperature of 230°C and MS quadrupole temperature of 150°C. The selected ion monitoring (SIM) mode was used for determination of target compounds (list in Table 3.4).

Table 3.4 Ions selected in SIM mode for analysis of 10 pesticides.

No.	Pesticides	RT	SIM ions (m/z)		
1	Diazinon	13.196	179.20	152.15	137.15
2	Pirimiphos-Methyl	15.928	290.20	276.10	305.20
3	Chlorpyrifos	16.757	197.00	199.00	
4	Alpha-Endosulfan	19.751	240.95	195.05	
5	Beta-Endosulfan	24.106	195.00	207.05	
6	Ethion	26.084	231.05	153.10	
7	Endosulfan sulfate	27.656	271.85	273.85	
8	EPN	34.434	157.1	169.1	
9	Cyhalothrin	34.434	181.15	197.10	208.15
10	Permethrin	36.109	183.15	207.10	

To ensure adequate operation of the GC and MS instruments, the injection of 30 μg/L of matrix standard solution at the conditions to be used was measured. The peak

shapes of the analytes should be Gaussian and signal to noise ratio (S/N) should be achieved >2-3 using the chosen quantitation ions at the appropriate retention time (RT).

The suitability of the instruments has been shown to be acceptable, inject the extract sequences in the following order:

(1) Solvent

(2) Working mixed standard solution 0.1 μg/mL (2 injections)

(3) Solvent

(4) Working mixed standard solution for calibration curve (0.3 – 3.0 μg/mL)

(5) Solvent blank

(6) Sample blank

(7) Sample (1-10)

(8) Working mixed standard solution (0.1, 0.5 and 3.0 μg/mL)

(9) Solvent blank

(10) Sample (11-20)

3.5 Sample preparation of ginger by liquid-liquid extraction and dispersive liquid-liquid microextraction (LLE/DLLME)

3.5.1 Sample comminution

An appropriate chopper must be used to comminute large, representative sample portions, proceed as required by codex a limentarius (CAC/GL 33) guidelines.

3.5.2 Liquid-liquid extraction (LLE)

A ginger sample was first extracted by liquid-liquid extraction based on AOAC official method 2007.01 [56] as a following procedure.

3.5.2.1 Weigh 15 ± 0.1 g of the homogenate ginger sample and placed into a 50-mL centrifuge tube. For the fortified sample, standard solution was spiked.

3.5.2.2 Add 15 mL of organic solvent that designed as a disperser solvent in DLLME step, 6 g of anhydrous $MgSO_4$ and 1.5 g of anhydrous NaCl per

15 g of ginger sample were added. Seal the tubes well to ensure that powder does not get into the screw threads or rim of the tube.

3.5.2.3 Shake the tubes vigorously by hand for 1 min, ensuring that the solvent interacts well with the entire sample and that crystalline agglomerates are broken up sufficiently during shaking.

3.5.2.4 Transfer 5 mL of an upper layer of the extracts to the dispersive-SPE tubes containing 250 mg of PSA sorbent and 750 mg of anhydrous $MgSO_4$. Shake the tubes by hand and mixed again by a vortex mixer for 30 s.

3.5.2.5 Centrifuge the dispersive-SPE tubes at 4500 rpm for 1 min. Upper layer of the final extracts (so called ginger extract) was further extracted by DLLME.

3.5.3 Dispersive liquid-liquid microextraction (DLLME)

3.5.3.1 Mix 1.00 mL of the ginger extract (V_a) from 3.5.2.5 and 50 μL of the extraction solvent in a 15-mL centrifuge tube. Shake the tubes vigorously by hand for 1 min.

3.5.3.2 Add 5 mL of water and shake the tubes vigorously by hand for 1 min. The dispersion of fine droplets of extraction solvent in aqueous phase was observed.

3.5.3.3 Centrifuge the tubes at 4500 rpm for 5 min. Then, the sediment phase (an extract) was removed using a 10-μL microsyringe (V_f) and transferred to a 2-mL vial for GC-MS analysis.

To optimize the DLLME, tetrachloroethylene (C_2Cl_4), carbon tetrachloride (CCl_4), and chloroform ($CHCl_3$) were studied as the extraction solvent in step 3.5.3.1 and the volume was varied from 30 to 100 μL. Acetonitrile and acetone were studied as a disperser solvent and used to extract the ginger in step 3.5.2.2. The extraction time in step 3.5.3.1 was varied from 1 to 5 min and the centrifugation time in step 3.5.3.3 was varied from 2 to 20 min. Moreover, the addition of salt to the solution and adjustment of the solution pH in step 3.5.3.2 were studied.

3.5.4 Data Analysis

Quantitation is based on linear least squares calibration of analyte peak areas plotted versus analyte concentration. The *y*-intercept of calibration should be near zero and coefficient of determination (R^2) should be >0.995. The analyte concentrations are the matrix-matched calibration standards.

Pesticide residues in sample were calculated as equation 3.2. %Recovery and enrichment factor were calculated by equation 3.4 and 3.5, respectively.

$$C_{sam} = \frac{C_o \times V_f}{V_a} \tag{3.2}$$

$$C_o = \frac{y - b}{m} \tag{3.3}$$

$$\%\ \text{recovery} = \frac{(C_1 - C_2) \times 100}{C_3} \tag{3.4}$$

$$\text{Enrichment factor} = \frac{C_0}{C_3} \tag{3.5}$$

Where

C_{sam} = Pesticide residues in sample (mg/kg)

C_0 = Concentration from calibration curve

C_1 = Concentration determined in fortified sample

C_2 = Concentration determined in unfortified sample

C_3 = Concentration of fortification

V_a = Aliquot volume of organic phase (mL)

V_f = Final volume (mL)

y = Peak area of sample

b = y-intercept of standard calibration curve

m = Slope of standard calibration curve

3.6 Performance Characteristics

Performance characteristics of an analysis method are the function qualities such as range/linearity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ).

3.6.1 Linearity /Working range

Linearity is the ability of the method to elicit results that are directly proportional to analyte concentration within a given range. A range is the interval between the upper and lower concentration of analyte in sample for which it has been demonstrated that the analytical procedure has an acceptable level of accuracy, precision, and linearity.

Fortified sample at least 6 different concentrations within the linear range were analyzed. Three replicate measurements at each concentration were performed. The coefficient of determination of the plot between response measurements and analyte concentrations was evaluated.

3.6.2 Accuracy

Accuracy shows a degree of conformity between a measurement result and the accepted reference value. Sample blank and fortified sample of interest at a range of concentrations were analyzed 6 replicates. % Recovery which calculated by the formula in equation 3.5 was evaluated.

The acceptance criteria of recovery were followed the Codex defined for pesticide residues and veterinary drug residues in food and the AOAC manual for the peer verified methods program (1993) which shown in Table 3.5 and 3.6, respectively.

Table 3.5 Analyte recovery of pesticide residues and veterinary drug residues in food (Codex)

Concentrations of analyte	% recovery
< 1 μg/kg	50-120
>1 μg/kg ≤ 0.01 mg/kg	60-120
>0.01mg/kg≤ 0.1 mg/kg	70-120
>0.1mg/kg < 1 mg/kg	70-110
>1 mg/kg	70-110

Table 3.6 Analyte recovery at different concentrations (AOAC)

Concentrations 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

3.6.3 Precision (Repeatability)

Precision under repeatability conditions, i.e. conditions where dependent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of

time. Sample blank and fortified sample of interest at a range of concentrations were performed 6 replicates.

Precision was evaluated by comparison the observed relative standard deviation ($\%RSD_{obs}$) with the values calculated from Horwitz's equation ($\%RSD_r$) (equaiton 3.6).

$$\% \text{ RSD}_{\text{r}} = 0.66 \times 2C^{(1-0.5\log C)} = 0.66 \times 2C^{-150} \qquad (3.6)$$

Moreover, HORRAT (Horwitz ratio) values were calculated by equation 3.7 and the acceptance criteria of precision were shown in Table 3.7.

$$\text{HORRAT} = \frac{RSD_{obs}}{RSD_r} \qquad (3.7)$$

Table 3.7 The acceptance criteria of precision

Reference	Accepted HORRAT value
AOAC	< 2
Codex, EU	≤ 2

3.6.4 Limit of Detection (LOD)

Limit of detection (LOD) is defined as the smallest amount or concentration of an analyte that can be reliably distinguished from zero with a specified level of confidence.

Single measurement of 6 independent fortified sample blanks at lowest acceptable concentration was performed. Limit of detection is calculated as a three times of standard deviation (LOD = 3SD).

3.6.5 Limit of Quantitation (LOQ)

Limit of quantitation (LOQ) is defined as the lowest concentration of analyte that can be determined with an acceptable level of repeatability precision and trueness.

Single measurement of 6 independent fortified sample blanks at different concentration close to LOD was performed. Limit of quantitation is calculated as a ten times of standard deviation (LOQ = 10SD).

CHAPTER IV

RESULTS AND DISCUSSION

In this research, the multiresidue pesticides including organophosphates (OPPs), organochlorines (OCLs) and pyrethoids (PYs) in ginger were determined by a liquid-liquid extraction and a dispersive liquid-liquid microextraction (LLE/DLLME) method prior to analyze by gas chromatography-mass spectrometry (GC-MS). Ten compounds were selected as the representative of the pesticide residues, which are diazinon, pirimiphos-methyl, chlorpyrifos, ethion, and EPN for OPPs, alpha-endosulfan, beta-endosulfan and endosulfan sulfate for OCLs, cyhalothrin and permethrin for PYs.

4.1 The optimisation of dispersive liquid-liquid microextraction

There are different factors that affect the extraction process in dispersive liquid-liquid microextraction (DLLME). Some of them are selection of suitable extraction solvent, selection of suitable disperser solvent, volume of extraction solvent, volume of disperser solvent, and extraction time. It is important to optimize them in order to obtain a good recovery.

4.1.1 Selection of extraction solvent

The selection of an appropriate solvent is important for the DLLME process. Organic solvents are selected on the basis of extraction capability of interested compounds, the water immiscibility, higher density rather than water, and good gas chromatography behavior. Therefore, tetrachloroethylene (C_2Cl_4), carbon tetrachloride (CCl_4), and chloroform ($CHCl_3$) were investigated as the extraction solvent in DLLME step. A series of solutions were studied by using 1.00 mL of ginger extract as disperser solvent and 50.0 μL of the extraction solvent. As shown in Table 4.1, different volumes of sediment phase (so called recovery volume), which

were 22.2±2.4 µL of C_2Cl_4, 14.8±1.8 µL of CCl_4 and 17.6±2.7 µL of $CHCl_3$, were observed. Recovery volume of tetrachloroethylene was higher than other extraction solvents as its density is high and its solubility in water is low. Moreover, tetrachloroethylene extract gave the highest extraction efficiency (43–82%) in all pesticides (Figure 4.1) as all pesticides have high log P value (3.81-7.00), that is all pesticides like to dissolve in non-mid polar organic solvent more than polar organic solvent. Therefore, tetrachloroethylene was selected as the extraction solvent.

Table 4.1 Properties of studied extraction solvents and recovery volume of the extract obtained from DLLME.

	C_2Cl_4	**CCl_4**	**$CHCl_3$**
Density[a] (g/cm^3)	1.622	1.589	1.483
Solubility in water[a] (g/100mL, 25°C)	0.015	0.05	0.50
Solvent used (µL)	50.0	50.0	50.0
Recovery volume (µL)	22.2±2.4	14.8±1.8	17.6±2.7

[a]J.A. Dean, Lange's Handbook of Chemistry, 15th edition, McGraw-Hill, Inc., 1999.

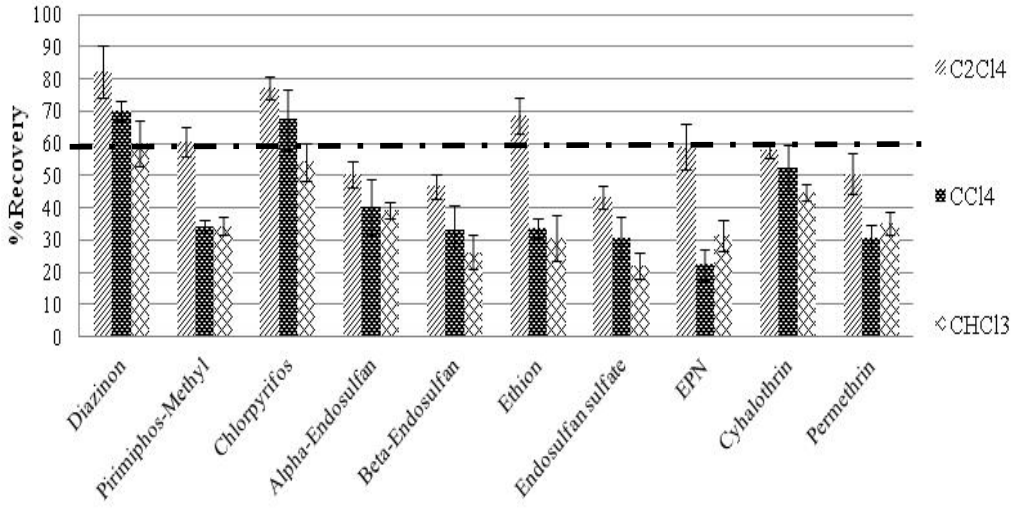


Figure 4.1 Effect of type of extraction solvent on the % recovery of pesticide residues obtained from DLLME. Extraction conditions: 1 mL of disperser solvent (ginger extract by acetonitrile) and 50.0 µL of the extraction solvent; concentration of each compound 100 ng/g.

4.1.2 Effect of extraction solvent volume

The volume of extraction solvent is one of the important parameters that affected the extraction efficiency. 1.00 mL of ginger extract containing different volumes of tetrachloroethylene (30.0, 40.0, 50.0, 60.0, 70.0, 80.0 and 100.0 µL) was evaluated. The recovery volume of extraction phase, the extraction recovery and the enrichment factor of each condition were compared in Figure 4.2–4.4. According to Figure 4.2, increasing the volume of tetrachloroethylene from 30.0 to 100.0 µL, the volume of sediment phase increased from 7.0 to 69.0 µL. It was clearly seen that the loss of extraction volume was high when used 30 µL of tetrachloroethylene (76% loss). The improvement of extraction solvent loss was observed when increasing the volume of extraction solvent used (60% loss at 50 µL and 30% loss at 100 µL).

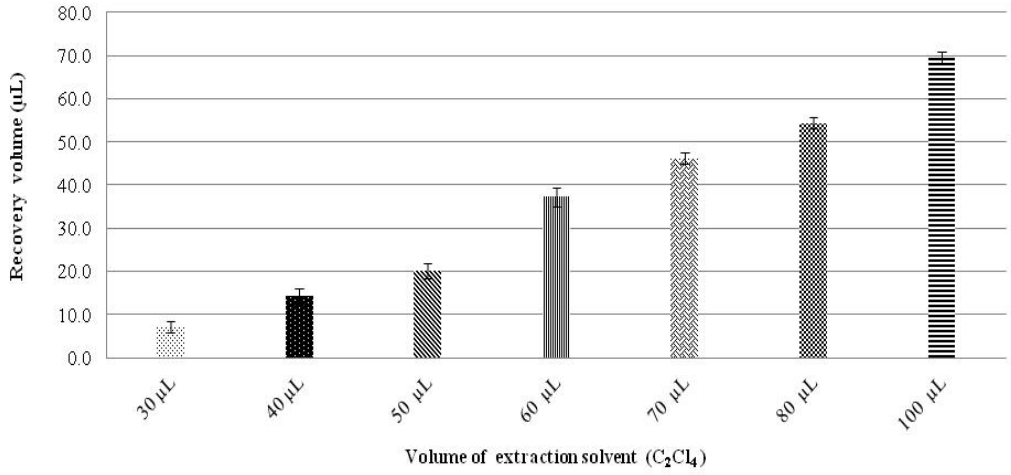


Figure 4.2 Effect of volume of extraction solvent on the recovery volume of the extract obtained from DLLME. Extraction conditions: 1 mL of disperser solvent (ginger extract with acetonitrile); concentration of each compound 100 ng/g.

According to Figure 4.3, increasing the volume of tetrachloroethylene to 50 µL or more gave acceptable extraction recoveries for most of pesticides (>60%), except beta-endosulfan, ethion, and endosulfan sulfate. However, enrichment factor decreased with increasing the volume of extraction solvent (Figure 4.4). As expected, the smaller the volume of the extraction solvent, the higher the enrichment factor.

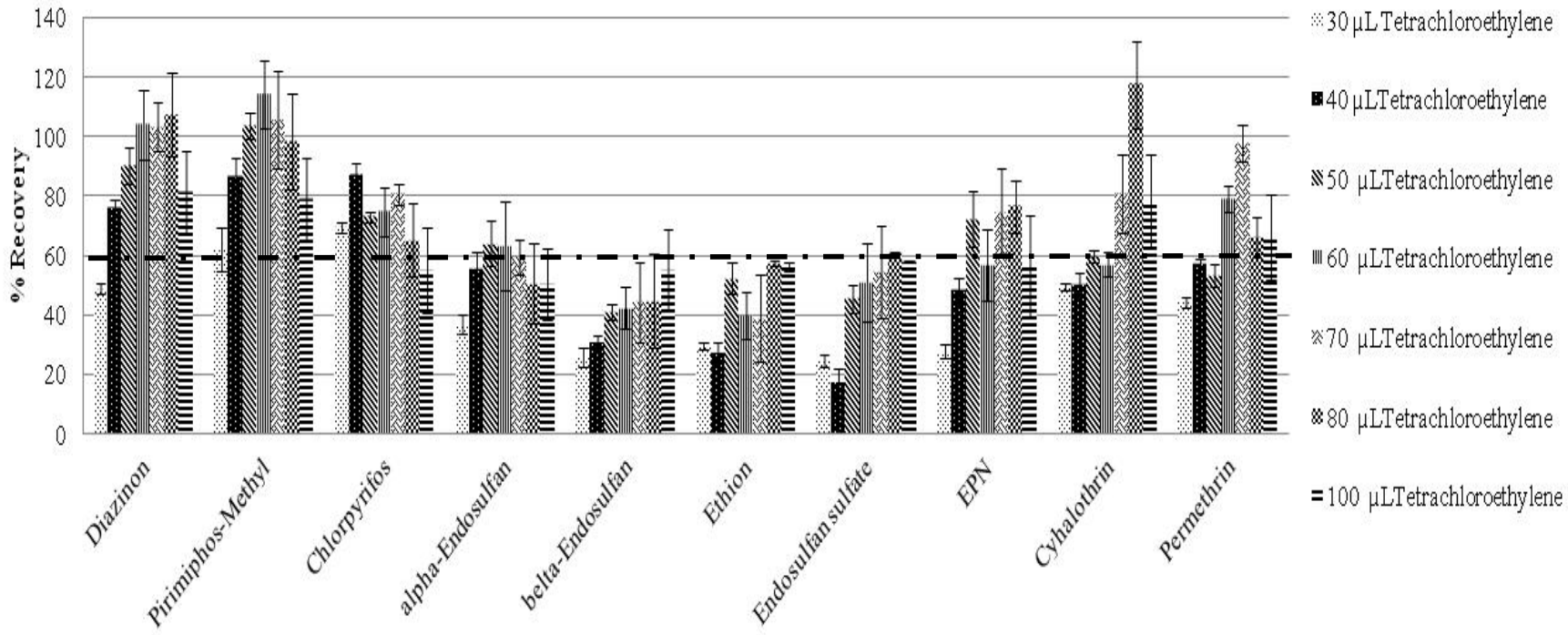


Figure 4.3 Effect of volume of extraction solvent on the % recovery of pesticide residues obtained from DLLME. Extraction conditions: 1 mL of disperser solvent (ginger extract with acetonitrile); concentration of each compound 100 ng/g.

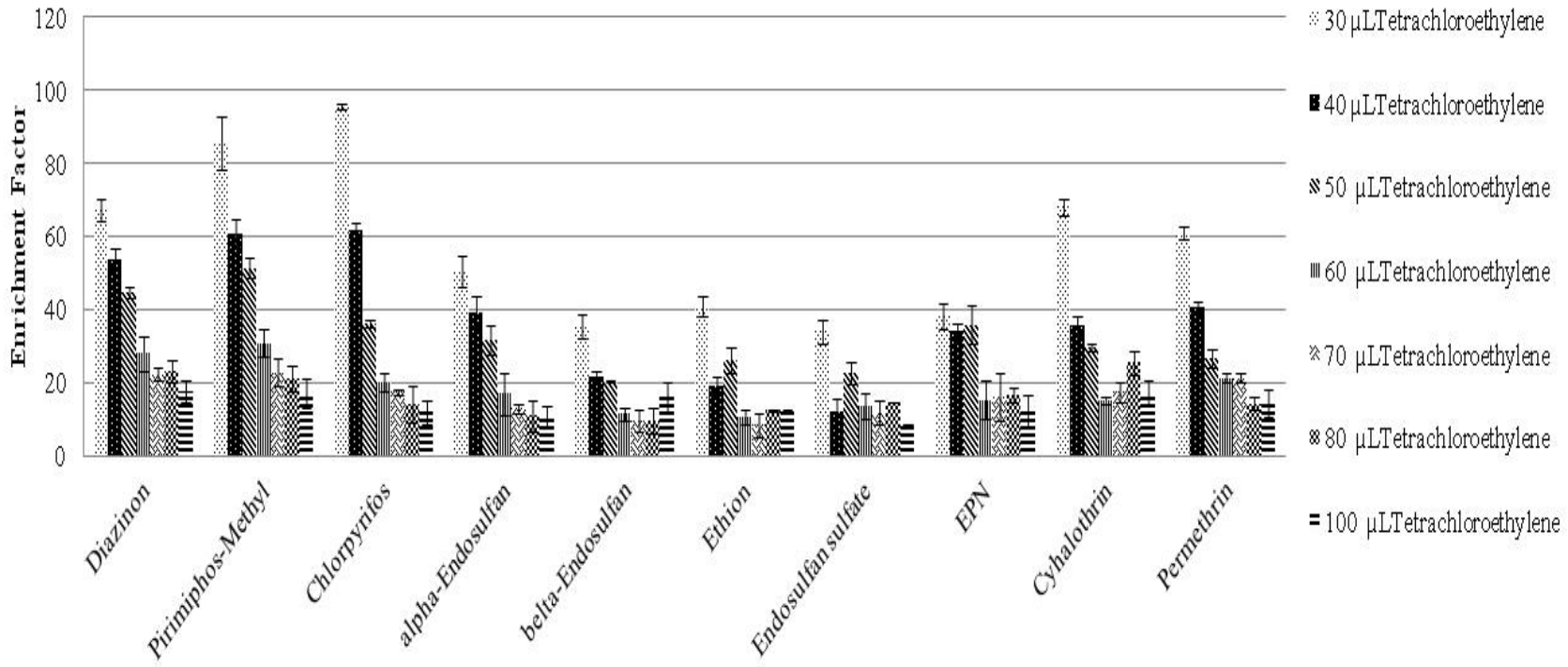


Figure 4.4 Effect of volume of extraction solvent on the enrichment factor of pesticide residues obtained from DLLME. Extraction conditions: 1 mL of disperser solvent (ginger extract with acetonitrile); concentration of each compound 100 ng/g.

Though, the volume of sediment phase should be more than 10 µL as a sufficient amount for GC analysis. Therefore, 50 µL of tetrachloroethylene was chosen which providing a good enrichment factor, acceptable extraction recovery, easy to manipulate, and minimizing toxicity to the environment.

4.1.3 Selection of type and volume of disperser solvent

The main physical property for an effective disperser solvent is the miscibility with both the organic extraction solvent and the aqueous phase. Moreover, in this research a disperser solvent in DLLME step was designed to be an extraction solvent in LLE step of sample preparation of ginger. As a consequence acetone and acetonitrile were tested as a disperser solvent. Although many reports [41-42, 57] showed a capability of acetone as an extraction solvent to extract pesticides in ginger by LLE, small volume (< 1 mL) was achieved in this research. Therefore, the acetone ginger extracts have not further extracted by DLLME. For acetonitrile, the different volumes of acetonitrile ginger extracts, (0.50, 1.00, and 2.00 mL) were extracted by DLLME. The results were summarized in Figure 4.5-4.6 and Table 4.1. According to Figure 4.5, the extraction efficiency for all pesticides increased firstly when increasing the volume of acetonitrile ginger extract from 0.50 mL to 1.00 mL and then decreased when further increasing the volume of acetonitrile ginger extract from 1.00 mL to 2.00 mL. It seems, cloudy state in solution was not formed well at a low volume of acetonitrile. Thus, the recovery is low. At higher volume of acetonitrile used, the solubility of pesticides in water might be increased; therefore, the extraction efficiency decreases because of decrease in distribution coefficient. A 1.00 mL volume of acetonitrile was then chosen as a disperser solvent.

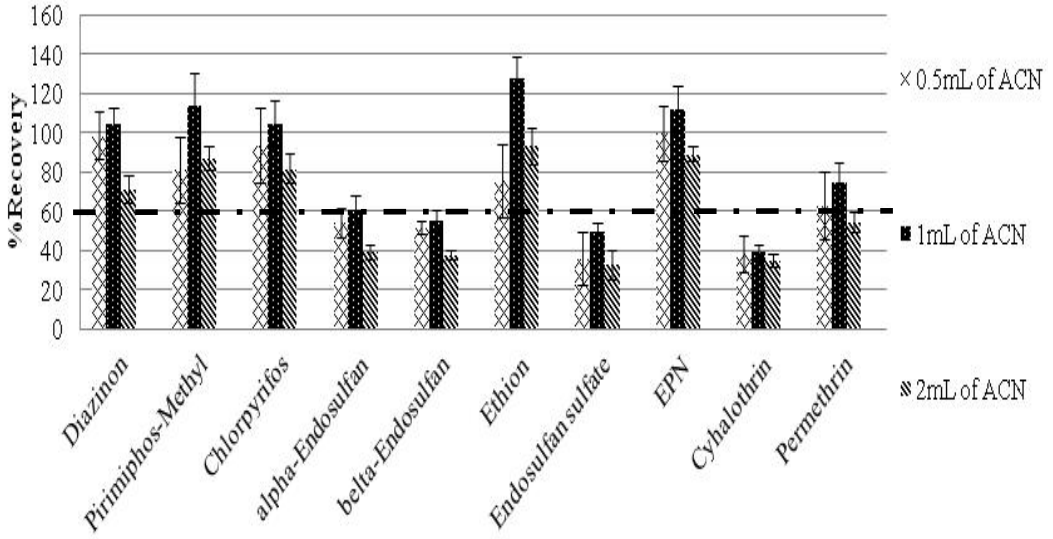


Figure 4.5 Effect of volume of disperser solvent on the % recovery of pesticide residues obtained from DLLME. Extraction conditions: 50 µL of extraction solvent, concentration of each compound 50 ng/g.

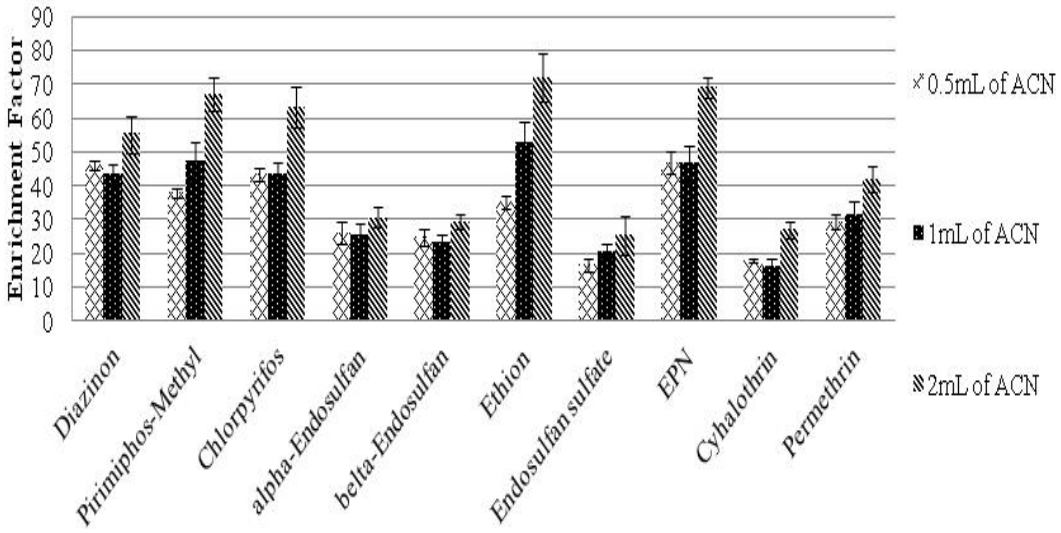


Figure 4.6 Effect of volume of disperser solvent on the enrichment factor of pesticide residues obtained from DLLME. Extraction conditions: 50 µL of extraction solvent, concentration of each compound 50 ng/g.

Table 4.2 Recovery volume of the extract obtained from DLLME with various volume of disperser solvent.

Volume of disperser solvent (mL)	Recovery volume (µL)
0.50	23.5±1.9
1.00	24.3±0.6
2.00	26.0±0.1

Note: Extraction conditions: 50 µL of extraction solvent; concentration of each compound 50 ng/g.

4.1.4 Selection of extraction time

In this method, the extraction time in DLLME is defined as an interval time of mixing an extraction solvent with a disperser solvent (ginger extract) before centrifugation. The extraction time was varied to 1, 2, 3, and 5 min at the same extraction system. The recovery volume of extract, %recovery of pesticides and the enrichment factor are shown in Table 4.3 and Figure 4.7 – 4.8. Results revealed that after 2 min, the efficiency of DLLME and the volume of sediment phase did not obviously changed. It implied that the extraction process is very fast. In the other word, a transition of analytes from a disperser solvent to extraction solvent is fast and only shaking a solution by hand was sufficient to transfer the analytes into the extraction solvent. This is the advantage of DLLME technique. Therefore, the extraction time was set to 2 min. in this study.

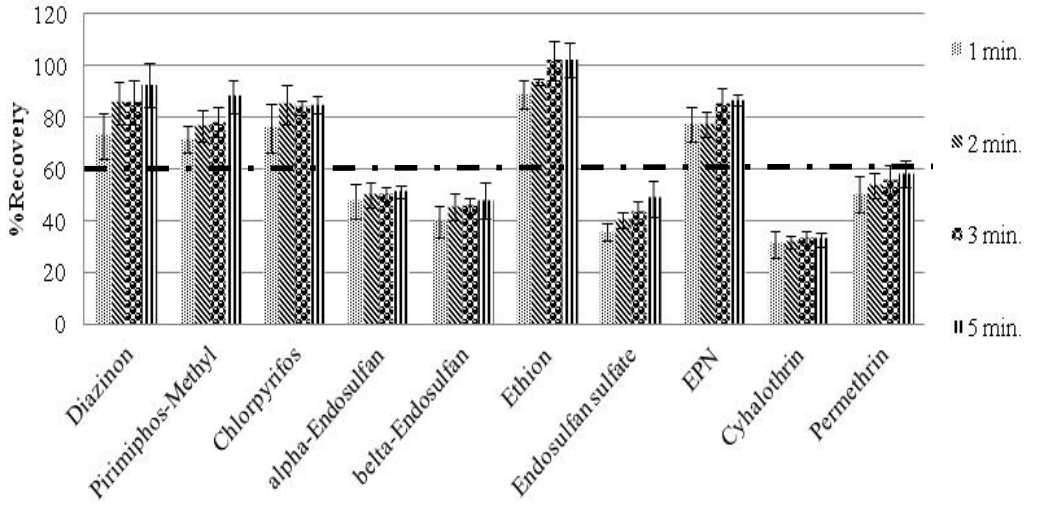


Figure 4.7 Effect of extraction time on the % recovery of pesticide residues obtained from DLLME. Extraction conditions: 50 µL of extraction solvent, 1 mL of disperser solvent (ginger extract with acetonitrile), concentration of each compound 50 ng/g.

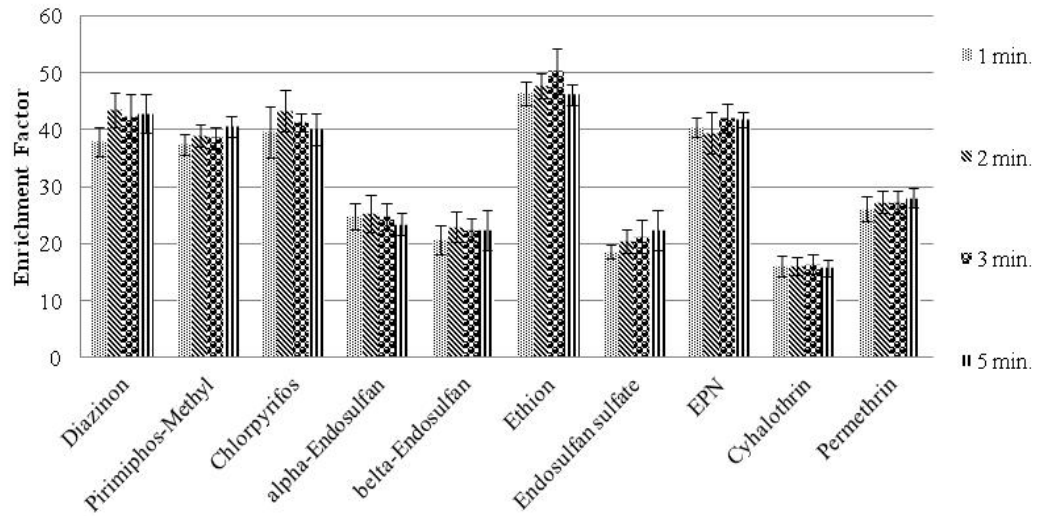


Figure 4.8 Effect of extraction time on the enrichment factor of pesticide residues obtained from DLLME. Extraction conditions: 50 µL of extraction solvent, 1 mL of disperser solvent (ginger extract with acetonitrile), concentration of each compound 50 ng/g

Table 4.3 Recovery volume of the extract obtained from DLLME with various extraction time.

Extraction time (min)	Recovery volume (µL)
1	19.2±1.0
2	19.8±0.6
3	20.3±0.8
5	20.7±0.6

Note: Extraction conditions: 50 µL of extraction solvent, 1 mL of disperser solvent (ginger extract with acetonitrile), concentration of each compound 50 ng/g.

4.1.5 Selection of centrifugation time

Centrifugation was an important procedure to the phase separation of extractant from an aqueous phase in this proposed DLLME method. The interval of centrifugation seriously affected the separation extent of the mixture and sequentially affected the extraction efficiency. In general, a higher rate of centrifugation can lead to a shorter centrifugation time and better phase separation. So 4,000 rpm of the centrifugation was used in the experiments. The centrifugation time was varied at 2, 5, 10, 15 and 20 min. The results are shown in Figure 4.9 and Table 4.4. It indicated that the centrifugation time did not obviously affect the efficiency of DLLME and the volume of sediment phase. Therefore, 5 min was selected as the optimal centrifugation time as a short analysis time with satisfactory recovery.

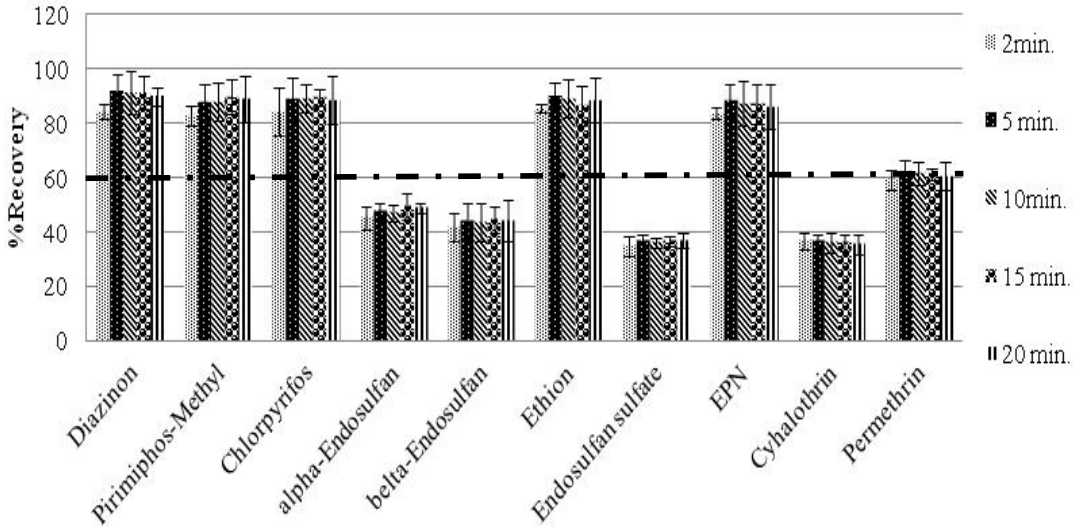


Figure 4.9 Effect of centrifugation time on the % recovery of pesticide residues obtained from DLLME. Extraction conditions: 50 µL of extraction solvent, 1 mL of disperser solvent (ginger extract with acetonitrile), concentration of each compound 50 ng/g.

Table 4.4 Recovery volume of the extract obtained from DLLME with various centrifugation times.

Centrifugation time (min)	Recovery volume (µL)
2	20.2±0.8
5	21.3±0.6
10	21.0±1.0
15	20.9±0.9
20	20.7±1.2

Note: Extraction conditions: 50 µL of extraction solvent, 1 mL of disperser solvent (ginger extract with acetonitrile), concentration of each compound 50 ng/g.

4.1.6 Effect of pH

In general, analytes are expected to be in a nonionic state for good extraction efficiency. As, pH can affect the existing forms of the pesticide compounds in solution, the pH upon of sample solution was investigated in this study from 2 to 8 by adjusting the solution pH with 0.1 M H_2SO_4 or 0.1 M NaOH. The recovery of all pesticides at 3 pH levels (pH 2-3, pH 5-6 and pH 7-8) was shown in Figure 4.10 and recovery volume of the extract was summarized in Table 4.5. The good extraction efficiency for all compounds was observed at pH 5-6. This can be explained by the pKa values of all the target compounds. Since the OPPs can be in ionized form at low pH which was not easy to extract by tetrachloroethylene, the significant decrease of extraction efficiency at pH 2-3 was observed. However, at high pH (pH 7-8), the %recovery was also decreases. This might caused by the matrix in ginger extract as the tense yellowish color was noticed when adjust the pH to 7-8. As the pH of a ginger extract was around 5, therefore, the acid or base addition was not adopted, which further simplified the extraction procedure.

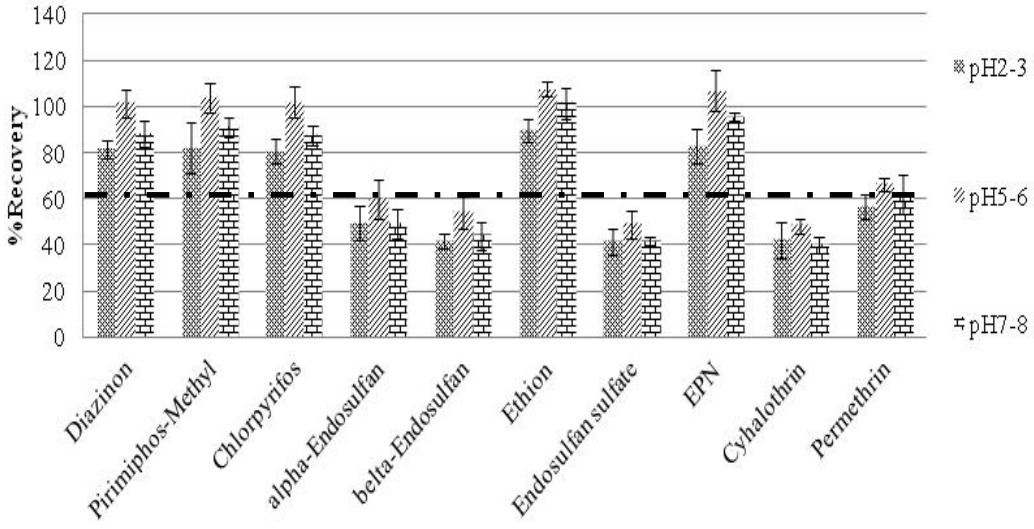


Figure 4.10 Effect of pH on the % recovery of pesticide residues obtained from DLLME. Extraction conditions: 50 µL of extraction solvent, 1 mL of disperser solvent (ginger extract with acetonitrile), concentration of each compound 50 ng/g.

Table 4.5 Recovery volume of the extract obtained from DLLME at various pH.

pH of solution	Recovery volume (µL)
2-3	20.1±1.4
5-6	22.3±1.9
7-8	19.4±1.7

Note: Extraction conditions: 50 µL of extraction solvent, 1 mL of disperser solvent (ginger extract with acetonitrile), concentration of each compound 50 ng/g.

4.1.7 Salt addition

In DLLME techniques, organic extraction solvent is broken up into tiny drops to increase the area of analyte transfer from aqueous phase to extractant. Adding the inorganic salt to the aqueous phase can poach water molecules surrounding the droplets of organic solvents to the surrounding by molecules of salt. As a result, the separation of water and the organic solvent is enhanced. Moreover, addition of salt can decrease the solubility of the analytes in water and therefore enhance the extraction efficiency because of the salting-out effect. In this study, the addition of sodium chloride (NaCl) at concentrations of 0, 2, 4, and 8 % (w/v) was evaluated. The highest recovery was obtained when 4% of NaCl was added (Figure 4.11). Further addition of sodium chloride did not result in an increase of extraction efficiency. Therefore, subsequent experiments were carried out with additional of 4% NaCl.

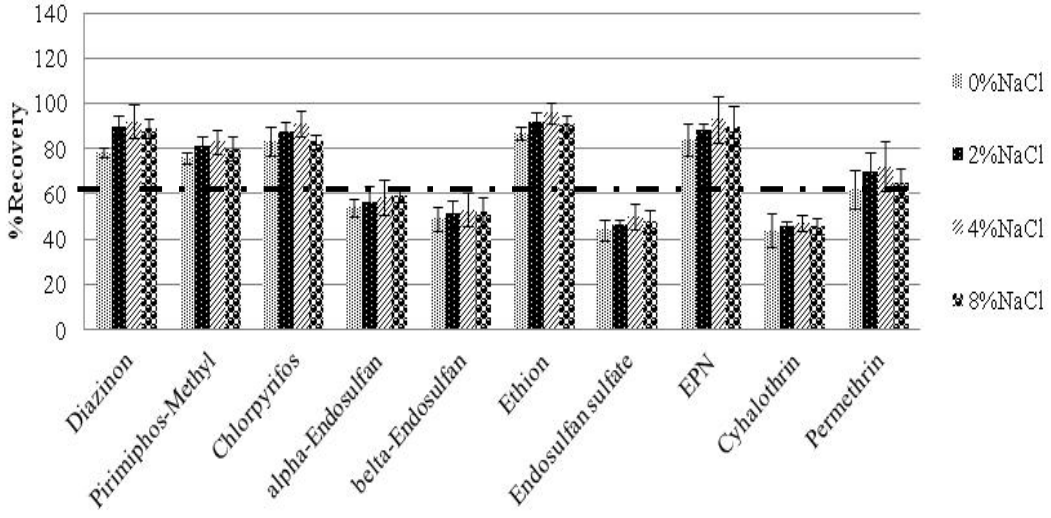


Figure 4.11 Effect of salt addition on the % recovery of pesticide residues obtained from DLLME. Extraction conditions: 50 µL of extraction solvent, 1 mL of disperser solvent (ginger extract with acetonitrile); concentration of each compound 50 ng/g.

Table 4.6 Recovery volume of the extract obtained from DLLME at various addition of salt.

% (w/v) NaCl added	Recovery volume (µL)
0	21.4±0.5
2	22.3±0.3
4	22.0±0.7
8	22.2±0.6

Note: Extraction conditions: 50 µL of extraction solvent, 1 mL of disperser solvent (ginger extract with acetonitrile), concentration of each compound 50 ng/g.

4.2 Performance Characteristics

4.2.1 Calibration Curve

The peak area of each standard pesticide solution was plotted against the concentrations of the standard solution at 6 levels as shown in Figure 4.12. The coefficients of determination (R^2) for each pesticide were above 0.995 (Table 4.7) which indicated a good linearity of calibration.

Table 4.7 A linearity of standard calibration curve .

Compounds	Range (µg/mL)	R^2
Diazinon	0.10-3.00	0.9950
Pirimiphos-methyl	0.10-3.00	0.9951
Chlorpyrifos	0.10-3.00	0.9961
Ethion	0.10-3.00	0.9970
EPN	0.10-3.00	0.9973
Alpha-endosulfan	0.10-3.30	0.9953
Beta-endosulfan	0.10-3.50	0.9992
Endosulfan sulfate	0.10-3.50	0.9954
Lamda-cyhalothrin	0.05-2.00	0.9968
Permethrin	0.05-2.00	0.9950

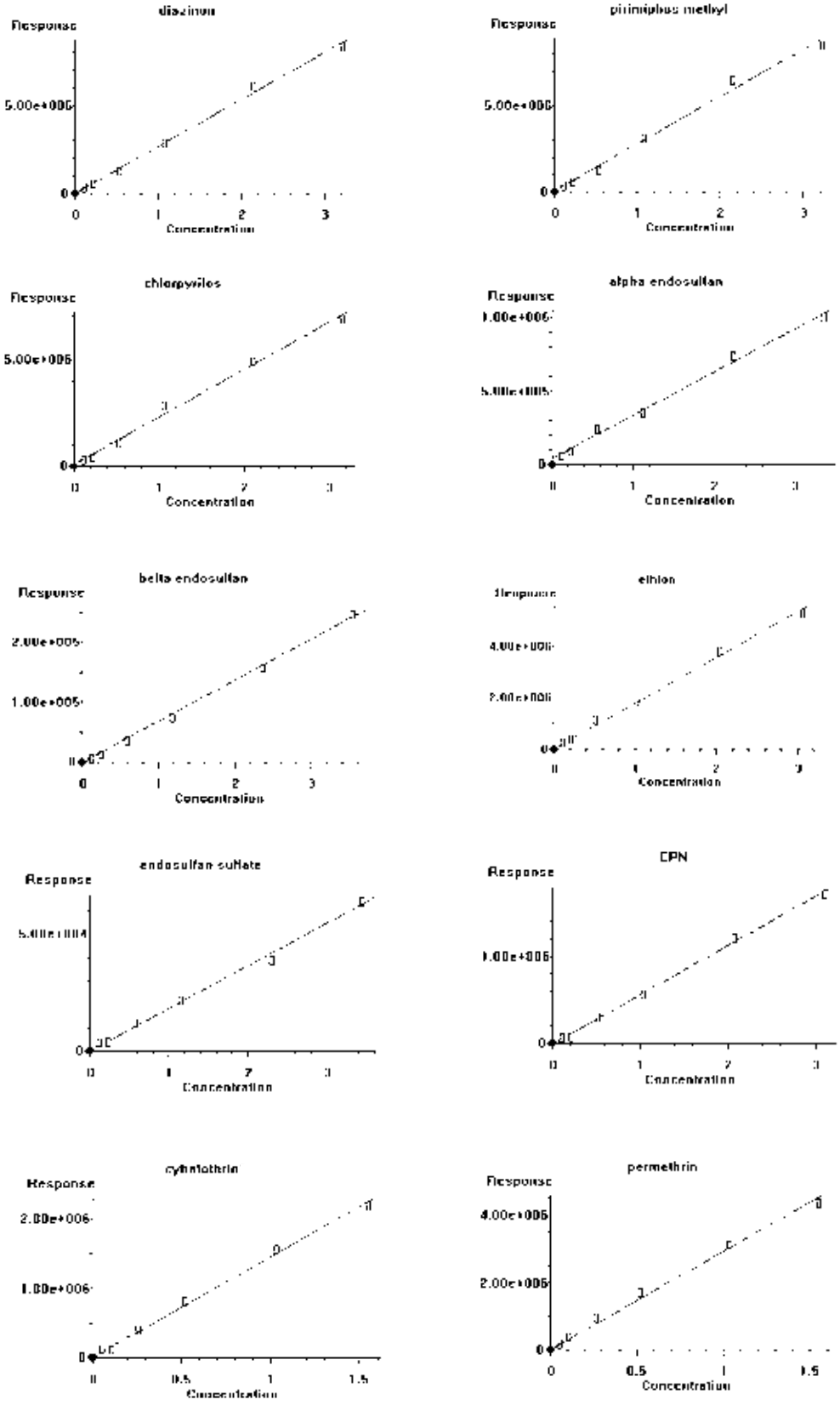


Figure 4.12 A standard calibration curve of studied pesticides.

4.2.2 Range / Linearity

The matrix calibrations were plotted by injected reagent blank and fortified sample at 6 levels of concentrations, and 3 replicates at each concentration. The results were summarized in Table 4.8. The coefficient of determination (R^2) for all pesticides was in the range 0.9950-0.9977 which indicated a good linearity of the calibration as shown in Figure 4.13.

Table 4.8 A linearity / range for extraction of pesticides by DLLME.

Compounds	Range (ng/g)	R^2
Diazinon	5-100	0.9957
Pirimiphos-methyl	5-100	0.9977
Chlorpyrifos	5-100	0.9972
Alpha-endosulfan	5-100	0.9969
Beta-endosulfan	5-100	0.9950
Ethion	5-100	0.9953
Endosulfan sulfate	5-100	0.9956
EPN	5-100	0.9951
Lamda-cyhalothrin	5-100	0.9966
Permethrin	5-100	0.9990

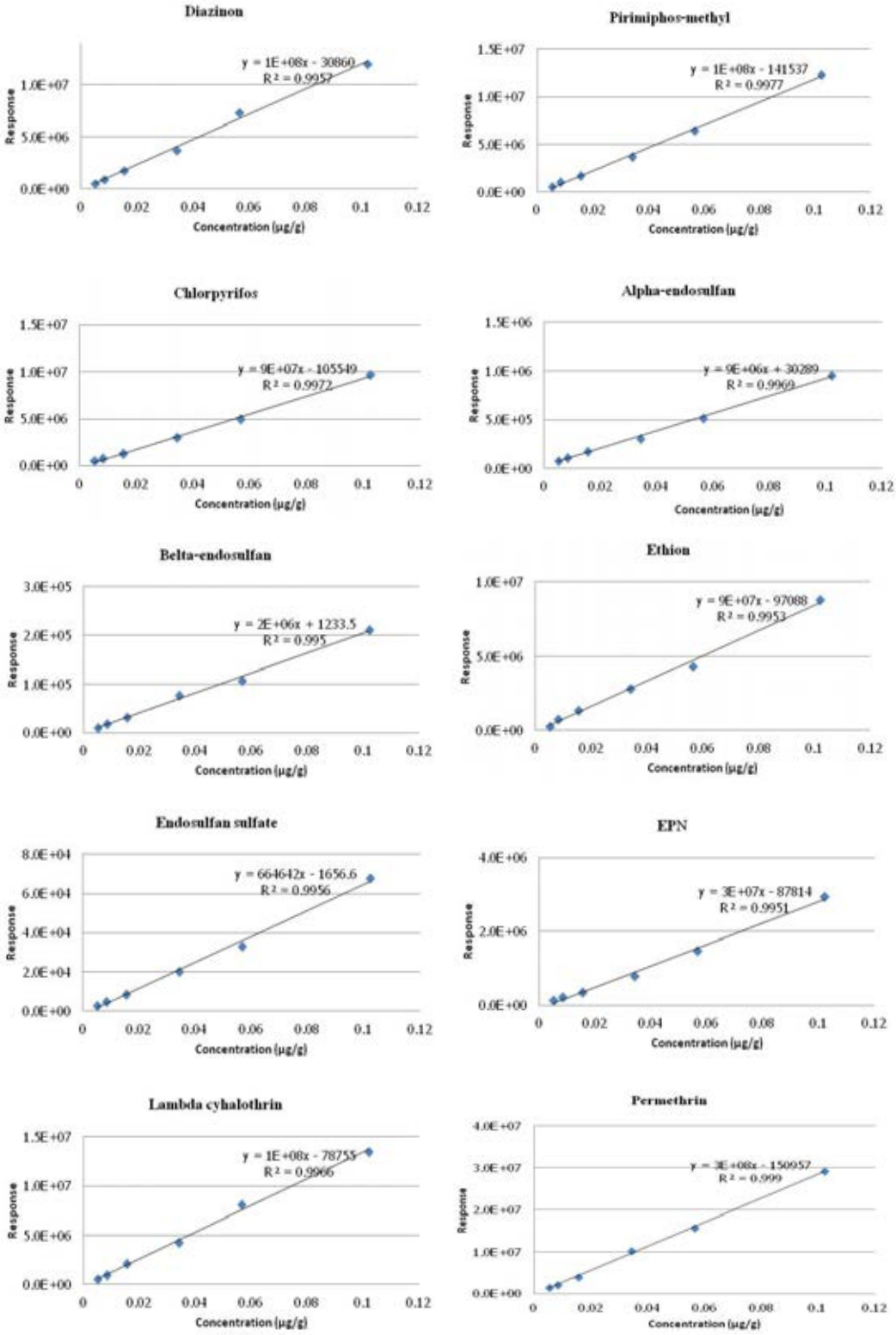


Figure 4.13 Matrix calibrations for extraction of pesticides by DLLME

4.2.3 Accuracy

Reagent blank, sample blank and fortified sample at 3 levels of concentrations (5, 15, and 100 ng/g) were analyzed and result showed in Table 4.9. %Recovery of studied pesticides were in the range of 59-92, 64-105, and 62-101 at concentration of 5, 15, and 100 ng/g, respectively. The obtained %recoveries were acceptable range at these concentration levels followed the Codex and the AOAC manual for the peer verified methods program (1993) as shown the criteria in Table 3.5 and 3.6 in chapter III.

Table 4.9 % recoveries and **%**RSD, for extraction of pesticides by DLLME.

Compounds	**Concentration (n=6)**					
	5 ng/g		**15 ng/g**		**100 ng/g**	
	%recovery	%RSD	%recovery	%RSD	%recovery	%RSD
Diazinon	92	15.3	94	11.0	92	7.7
Pirimiphos-methyl	80	18.1	87	14.0	91	13.5
Chlorpyrifos	87	21.7	73	19.3	90	27.7
Alpha-endosulfan	59	12.3	64	7.5	63	4.5
Beta-endosulfan	65	3.8	64	19.1	62	7.2
Ethion	68	3.2	105	10.9	101	16.3
Endosulfan sulfate	61	7.2	65	4.4	76	4.3
EPN	81	3.3	83	6.9	105	9.5
Lamda-cyhalothrin	70	4.3	75	3.3	89	4.3
Permethrin	84	3.8	81	3.2	90	3.8

4.2.4 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Reagent blank, sample blank and fortified sample at the low end of the range were analyzed. The results showed that LOD and LOQ of this method were in the range of 2.2-3.0 ng/g, and 7.2-10.2 ng/g, respectively (Table 4.10). The results of

LODs were less than MRLs of EU (Data from Table 3.3), and implied that the proposed method can applied for analysis of pesticide residues in real sample.

Table 4.10 Limit of Detection (LOD) and Limit of Quantitation (LOQ) for extraction of pesticides by DLLME.

Compounds	Concentration (ng/g)	
	LOD	**LOQ**
Diazinon	2.2	7.2
Pirimiphos-methyl	2.2	7.4
Chlorpyrifos	2.9	9.6
Alpha-endosulfan	2.4	8.0
Beta-endosulfan	2.3	7.5
Ethion	2.2	7.4
Endosulfan sulfate	3.0	10.0
EPN	2.7	8.9
Lamda-cyhalothrin	3.1	10.2
Permethrin	2.6	8.7

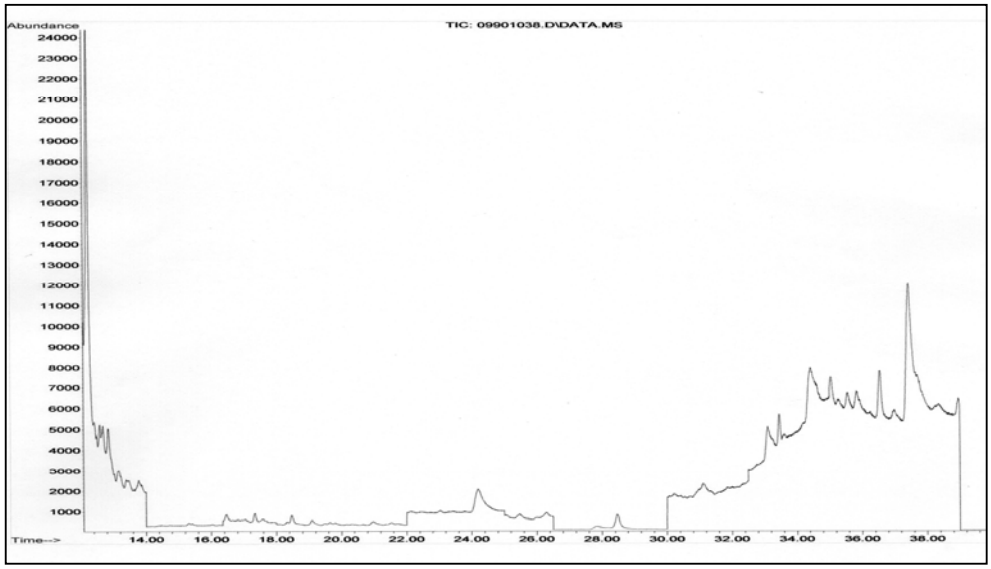
4.2.5 Precision

Reagent blank, sample blank and fortified sample at 3 levels of concentrations (8, 34, and 100 ng/g) were performed. The results in terms of % RSD_r and HORRAT values were summarized in Table 4.11. The HORRAT values of all pesticides indicated the good precision of the method (the criteria shown in Table 3.7) and ranged 0.13-0.89 at concentration of 8 ng/g, 0.13-1.72 at concentration of 34 ng/g, and 0.52-1.86 at concentration of 100 ng/g.

Table 4.11 Precision for extraction of pesticides by DLLME.

Compounds	Concentration (n=6)											
	8 ng/g				34 ng/g				102 ng/g			
	%recovery	$\%RSD_r$	Expected $\%RSD_r$	HORRAT	%recovery	$\%RSD_r$	Expected $\%RSD_r$	HORRAT	%recovery	$\%RSD_r$	Expected $\%RSD_r$	HORRAT
Diazinon	91	11.6	21.8	0.53	92	10.3	17.6	0.58	92	7.7	14.9	0.52
Pirimiphos-methyl	96	13.1	21.8	0.60	86	11.1	17.6	0.63	91	13.6	14.9	0.91
Chlorpyrifos	81	10.6	21.8	0.49	85	30.2	17.6	1.72	89	27.7	14.9	1.86
Alpha-endosulfan	62	11.9	21.8	0.55	57	11.4	17.6	0.65	63	4.5	14.9	0.30
Beta-endosulfan	70	11.7	21.8	0.54	73	8.5	17.6	0.49	62	7.2	14.9	0.48
Ethion	106	3.1	21.8	0.14	101	10.2	17.6	0.58	101	16.3	14.9	1.10
Endosulfan sulfate	68	5.0	21.8	0.23	72	6.0	17.6	0.34	76	4.3	14.9	0.29
EPN	91	2.8	21.8	0.13	90	2.2	17.6	0.13	105	9.8	14.9	0.66
Lamda-cyhalothrin	82	15.2	21.8	0.70	93	15.8	17.6	0.90	92	14.5	14.9	0.97
Permethrin	85	19.3	21.8	0.89	103	17.8	17.6	1.0	92	20.8	14.9	1.40

(a)



(b)

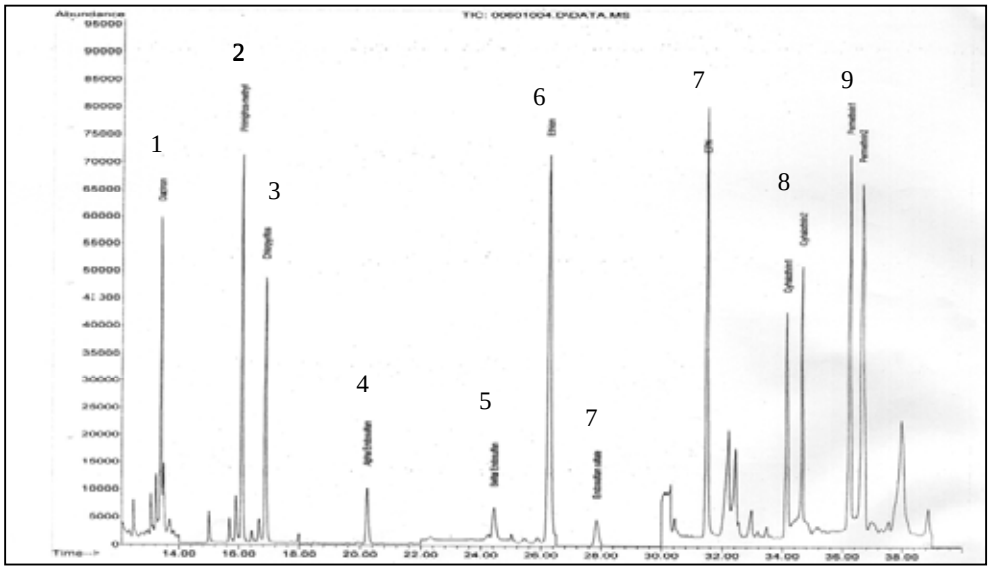


Figure 4.14 Chromatograms of pesticides in ginger (a) sample blank and (b) spiked sample at concentration level of 10.0 ng/g of each pesticide by GC-MS/SIM: (1) Diazinon, (2) Pirimiphos-Methyl, (3) Chlorpyrifos, (4) Alpha-Endosulfan, (5) Beta-Endosulfan, (6)Ethion, (7) Endosulfan sulfate, (8) EPN, (9) Cyhalothrin, and (10) Permethrin.

4.3 Comparison of DLLME and conventional method

The extraction efficiency of the presented DLLME method was compared with other methods such as the conventional extraction of pesticides based on liquid–liquid extraction [58]. The conventional extraction was performed as followed: 25 g of sample was put into laboratory bottle. A mixture of 50 mL acetone, 40 mL dichloromethane, and 7.5 g sodium chloride was added and subsequently homogenized. Wait for the solvent separation and transfer 50 mL of upper solution for concentrated by rotavaporation under reduced pressure at 40^{o}C. Subsequently, dissolve and adjust to 5mL by ethyl acetate. 2 mL of the solution was used to analyze OPPs by GC-FPD. The left solution was evaporated and adjusted to 3 mL by hexane. Then, the solution was cleanup using SPE prior to analyze OCLs and PYs by GC-ECD. From Table 4.12, the DLLME method has comparable in sample size, LOD, % recovery, and precision with conventional method. Noticeably, the organic solvent consumption and extraction time of LLE/DLLME method were lower than the conventional method as well as the improvement of enrichment factor. This is the most important advantages of DLLME.

Table 4.12 Comparison of DLLME and conventional method for the determination of multiresidue pesticides.

Properties	LLE / DLLME	Conventional method
Sample size (g)	15	25
Extraction volume	15 mL acetonitrile / 50 µL C_2Cl_4	50 mL acetone + 40 mL dichloromethane)
Extraction time (min)	10 - 15	45 - 60
LOD (ng/g)	2.0 -3.0	3.0 -9.0
Enrichment Factor	30-60	3 - 5
Recovery (%)	60-110	60 - 118
HORRAT at 100 ng/g	0.52-1.86	0.23-1.63

CHAPTER V

CONCLUSIONS AND FUTURE PERSPECTIVE

5.1 Conclusions

The analysis method for multipesticide residues in ginger was developed and validated for the rapid concentration and simultaneous determination of 10 pesticides in 3 groups; organophosphates (diazinon, pirimiphos-methyl, chlorpyrifos, ethion, EPN), organochlorines (alpha-endosulfan, beta-endosulfan, endosulfan sulfate), and pyrethoids (cyhalothrin, permethrin). Liquid-liquid extraction (LLE) combined with dispersive liquid–liquid microextraction (DLLME) procedure was applied for extraction and cleanup of multiple classes pesticide residues in ginger and analyzed by gas chromatography–mass spectrometry. Acetonitrile was used as extraction solvent in LLE and designed as the disperser solvent in DLLME. The diagram of the proposed LLE/DLLME method for extraction of multipesticide residues in ginger was summarized in Figure 5.1. In fact, acetonitrile was acted as an extraction solvent in LLE (step 1) and a disperser solvent in DLLME (step 2). These two steps were coupled together in such a good manner that makes this method a suitable procedure to extract pesticide residues from the sample without changing solvents.

Various parameters that affected the extraction efficiency in DLLME such as type and volume of extraction and disperser solvent, extraction time, centrifugation time, salt addition, and pH of solution were evaluated. The optimum conditions were using 50 μL of tetrachloroethylene as the extraction solvent, 1 mL of acetonitrile as the disperser solvent, extraction time of 2 min, centrifugation time of 5 min, and the addition of 4% of sodium chloride. Under the optimum condition, good linearity was obtained in range of 5-100 ng/g for all analytes with the coefficient of determination (R^2) > 0.995. DLLME provides good recovery, wide linearity and good repeatability within a very short time. Recovery tests were performed for concentrations between

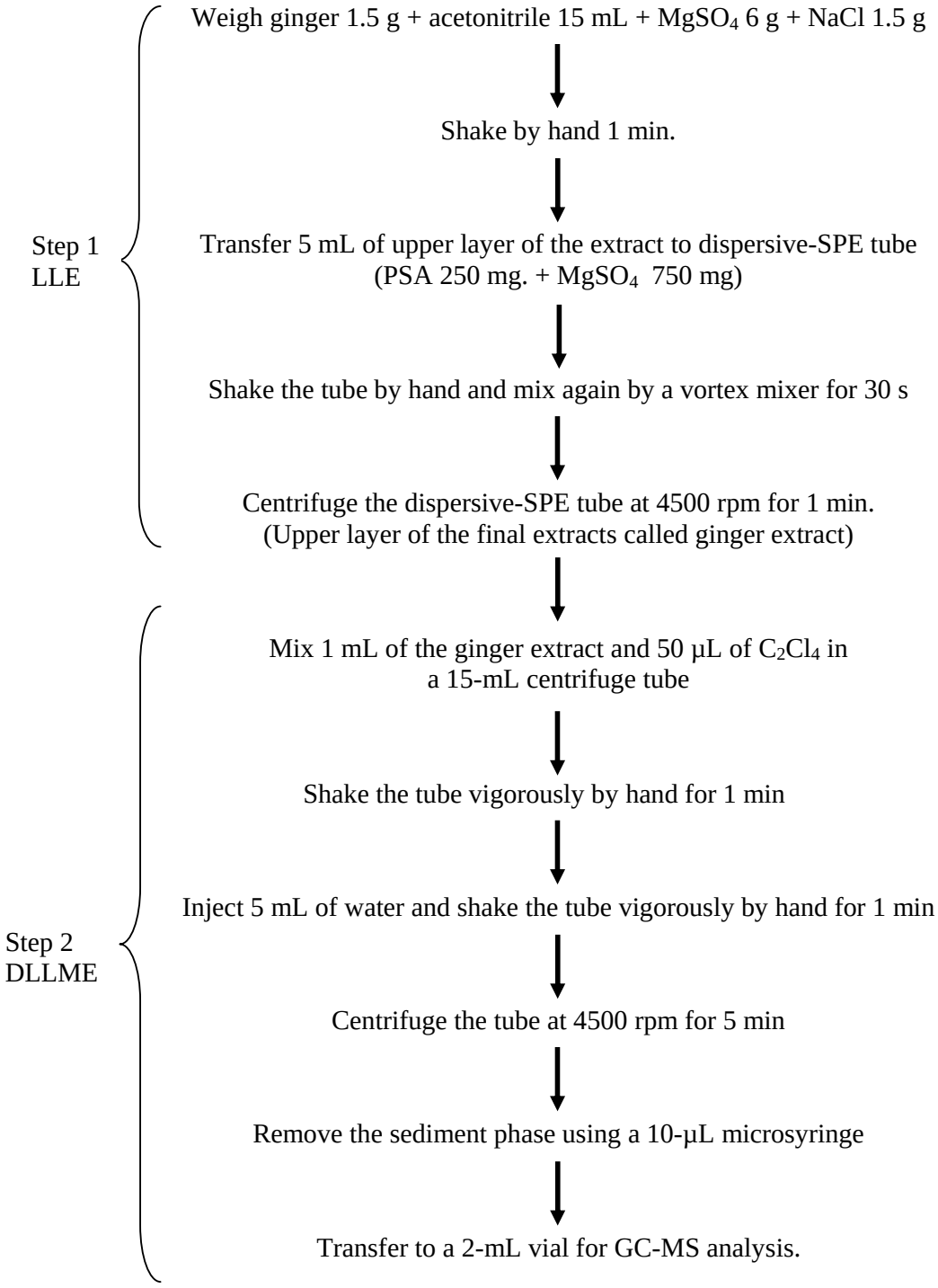


Figure 5.1 Flow diagram of the proposed LLE/DLLME method

5.0, 15.0 and 100.0 ng/g; recoveries for each target analyte were in the range between 59 to 105%. Limits of detection of this method were found ranging from 2.2 to 3.1 ng/g. Limit of quantitation were found ranging from 7.2 to 10.2 ng/g. The repeatability of the proposed method, expressed as HORRAT, were between 0.13 – 1.86.

In comparison with the conventional extraction methods (In house method based on steinwandter; H., 1985), the proposed method has the advantage of being quick and easy to operate, and low consumption of organic solvent.

5.2 Future perspective

This developed method is versatile and offers enhanced performance including the possibility of analysis many type and multiclass pesticides residues by using a variety of solvents. Therefore, it could be easily extended this method for determination of pesticide residues in other plant sample.

REFERENCES

1. University of maryland medical center. Ginger [Online]. 2011. Available from : http://www.umm.edu/altmed/articles/ginger000246.htm [2012, September 1].
2. Ali, BH.; Blunden, G.; Tanira, MO.; and Nemmar, A. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. Food and Chemical Toxicology 46 (2008): 409-20.
3. Fuhrman, B.; Rosenblat, M.; Hayek, T.; Coleman, R; and Aviram, M. Ginger extract consumption reduces plasma cholesterol, inhibits LDL oxidation, and attenuates development of atherosclerosis in atherosclerotic, apolipoprotein E-deficient mice. Journal of Nutrition 130 (2000): 1124-1131.
4. Lambropoulou , D. A.; and Albanis, T. A. Methods of sample preparation for determination of pesticide residues in food matrices by chromatography–mass spectrometry-based techniques: a review. Analytical and Bioanalytical Chemistry 389 (2007): 1663–1683.
5. Torres, C.M.; Pico, Y.; and Mafies, J. Determination of pesticide residues in fruit and vegetables: a review. Journal of Chromatography A 754 (1996): 301-331.
6. Beyer, A.; and Biziuk, M. Applications of sample preparation techniques in the analysis of pesticides and PCBs in food. Food Chemistry 108 (2008): 669–680.
7. Arrebola, F.J.; J.L., Martinez Vidal, M.J.; Gonzalez-Rodriguez, A.; and Sanchez-Morito, N. Reduction of analysis time in gas chromatography application of low-pressure gas chromatography-tandem mass spectrometry to the determination of pesticide residues in vegetables. Journal of Chromatography A 1005(2003):131 - 141.

8. Kataoka, H.*;* Lord, H.L; *and* Pawliszyn, J. Application of solid-phase microextraction in food analysis. Journal of Chromatography A 880 (2000): 35–62.

9. Lambropoulou, D.; and Albanis, T. Liquid-phase micro-extraction techniques in pesticide residue analysis. Journal of Biochemical and Biophysical Methods 70 (2007) : 195-228.

10. Baltussen, E.; Sandra, P.; David, F.; and Cramers, C. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles. Journal of Mircrocolumn Separations 11 (1999): 737-747.

11. Buldini, P.L.; Ricci, L.; and Sharma, J.L. Recent applications of sample preparation techniques in food analysis – a review. Journal of Chromatography A 975(2002): 47-70.

12. Kataoka, H.*;* Lord, H.L; and Pawliszyn, J. Application of solid-phase microextraction in food analysis. Journal of Chromatography A 880 (2000): 35–62.

13. Zhang, Z.; Yang, M. J.;and Pawliszyn, J. Solid phase microextraction: a new solvent-free alternative for sample preparation. Analytical Chemistry 66 (1994): 844A-853A.

14. Menezes-Filho, A.; Santos, F.N.; and Paula-Pereira, P.A. Development validation and application of a methodology based on solid-phase micro extraction followed by gas chromatography coupled to mass spectrometry (SPME/GC–MS) for the determination of pesticide residues in mangoes. Talanta 81(2010): 346-354.

15. Plaza Bolanos, P.; Romero-Gonzalez, R.; Garrido Frenich, A.; and Martinez Vidal, J. L. Application of hollow fibre liquid phase microextraction for the multiresidue determination of pesticides in alcoholic beverages by ultra-high pressure liquid chromatography coupled to tandem mass spectrometry. Journal of Chromatography A 1208 (2008):16–24.

16. Arthur,C. L.; and Pawliszyn , J. Solid phase microextraction with thermal desorption using fused silica optical fibers. Analytical. Chemistry 62 (1990): 2145-2148.

17. Louch, D.; Motlagh, S.; and Pawliszyn , J. Dynamics of Oorganic compound extraction from water using liquid-coated fused silica fibers. Analytical. Chemistry 64 (1992):1187-1199.

18. Berlardi, R.; and Pawliszyn, J. The application of chemically modified fused silica Fibers in the extraction of organics from water matrix samples and their rapid transfer to capillary columns. Water Pollution Research Journal of Canada 24 (1989):179.

19. Lambropoulou, D.; and Albanis, T. Application of hollow fiber liquid phase microextraction for the determination of insecticides in water. Journal of Chromatography A 1072 (2005):55–61.

20. Psillakis, E.; Mantzavinos, D.; and Kalogerakis, N. Development of a hollow fibre liquid phase microextraction method to monitor the sonochemical degradation of explosives in water. Analytica Chimica Acta 501 (2004) 3–10.

21. Lopez-Blanco, C.; Gomez-Alvarez, S.; Rey-Garrote, M.; Cancho-Grande, B.; and Simal-Gandara, J. Determination of carbamates and organophosphorus pesticides by SDME–GC in natural water. Analytical and Bioanalytical Chemistry 383 (2005): 557–561.

22. Xiao, Q.; Hu, B.; Yu, C.; Xia, L.; and Jiang, Z. Optimization of a single drop microextraction procedure for the determination of organophosphorus pesticides in water and fruit juice with gas chromatography-flame photometric detection. Talanta 69 (2006) 848-855.

23. Zhang, M.; Huang, J.; Wei, C.; Yua, B.; Yang, X.; Chen, X. Mixed liquids for single drop microextraction of organochlorine pesticides in vegetables. Talanta 74(2008) 599-604.

24. Ahmadi, F.; Assadi, Y.; Hosseini, S.M.R.M; and Rezaee, M. Detemination of organophosphorus pesticides in water samples by single drop microextraction and gas chromatography flame photometric detector. Journal of Chromatography A 1101(2006): 307-312.

25. Fiamegos, Y.C.; and Stalikas, C.D. A Single drop micro extraction and future trends. International Journal of Chem Tech Research 2 (2010): 1638-1652.

26. 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.

27. Feo, M.L.; Eljarrat, E.; and Barcel, D. A rapid and sensitive analytical method for the determination of 14 pyrethroids in water samples. Journal of Chromatography A 1217 (2010): 2248–2253.

28. Fu, L.; Liu, X.; Hu J.; Zhao, X.; Wang, H.; and Wang, X. Application of dispersive liquid–liquid microextraction for the analysis of triazophos and carbaryl pesticides in water and fruit juice samples. Analytica Chimica Acta 632 (2009): 289–295.

29. 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.

30. Zang, X.; Wang, J.; Wang, O.; Wang, M.; Ma, J.; Xi, G.; and Wang, Z. Analysis of captan, folpet, and captafol in apples by dispersive liquid–liquid microextraction combined with gas chromatography. Analytical and Bioanalytical Chemistry 392 (2008): 749–754.

31. Rezaee, M.; Yamini , Y.;and Faraji , M. Evolution of dispersive liquid–liquid microextraction method: a review. Journal of Chromatography A (2009): 1-16.

32. Xiao-Huan ,Z.; Qiu-Hua, W.; Mei-Yue, Z.; Guo-Hang, X.; and Zhi, W. Developments of dispersive liquid-liquid microextraction technique. Chinese Journal of Analytical Chemistry 37 (2009): 161–168.

33. Gosselin, R.E., Clinic Toxicological of Commercial Products, Williams and Wilkins, Baltimore, MD, USA, 1984.

34. The WHO recommended classification of pesticides by hazard. International programme on chemical safety (2004).

35. U.S. Environmental protection agency. Types of pesticides [online]. 2012. Available from: http://www.epa.gov/pesticides/about/types.htm [2012, August 20].

36. FAO/ WHO food standards codex alimentarius. Codex maximum residue limits for pesticides [online]. 2010. Available from: http://www.codexalimentarius.net/pestres/data/index.html;jsessionid=685A65C0CA8ED6256F513E7C347CDF19 [2012, September 10].

37. The Japan food chemical research foundation. The Japanese positive list system for agricultural chemical residues in foods [online]. 2012. Available from: http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/MRLS-p [2012, October 1].

38. Pesticide residues MRLs. Pesticides EU Maximum Residue Limits [online]. 2008. Available from: http://ec.europa.eu/sanco_pesticides/public/index.cfm [2010, September 10].

39. Tawabkhan, I.A.; Parveen, Z.; and Ahmed, M. Multi-residue determination of organophosphorus pesticides and synthetic pyrethroids in wheat. International Journal of Agriculture Biology. 9 (2007): 905-907.

40. Godula, M.; Hajslova, J.; Alterova, K. Pulsed splitless injection and the extent of matrix effects in the analysis of pesticides. Journal of High Resolution Chromatography 22 (1999): 395–402.

41. Koning, S. ; Janssen, H.G.; and Brinkman, Udo A. Th. Modern methods of sample preparation for GC analysis. Chromatographia Supplement 69 (2009): S33-S78.

42. Torres, C.M., Pico, Y. and Manes, J.. Determination of pesticide residues in fruit and vegetables :a review. Journal of Chromatography A 754 (1996) 301-331.

43. Stajnbaher, D. and Zupancic-Kral, L. Multiresidue method for determination of 90 pesticides in fresh fruits and vegetables using solid-phase extraction and gas chromatography-mass spectrometry. Journal of Chromatography A 1015 (2003): 185-198.

44. Hernandez, F.; Pozo, O.J.; Sancho, J.V.; Bijlsma, L.; Barreda, M.; and Pitarch E. Multiresidue liquid chromatography tandem mass spectrometry determination of 52 non gas chromatography-amenable pesticides and

metabolites in different food commodities. Journal of Chromatography A 1109 (2006): 242-252.

45. Anastassiades, M.; Lehotay, SJ., Stajnbaher, D., and Schenk, FJ. 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.
46. Schenk, FJ; and Hobbs, JE. Evaluation of the quick, easy, cheap, effective, rugged, and safe (QuEChERS) approach to pesticide residue analysis. Bulletin of Environmental contamination and Toxicology 1: (2004) : 24-30.
47. Lehotay, SJ.; de Kok, A.; Hiemstra, M.; and Van Bodegraven, P. Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. Journal of AOAC International 88(2005) :595–614.
48. Lehotay, SJ.; Mastovska, K.; and Lightfield , AR. Qualitative aspects and validation of a screening method for pesticides in vegetables and fruits based on liquid chromatography coupled to full scan high resolution (Orbitrap) mass spectrometry. Journal of AOAC International 88 (2005): 615–629.
49. Assadi, Y.; Rezaee , M.; Millani, M.R.; Aghaee, E.; Ahmadi,F.; Berijani,S. Determination of organic compounds in water using dispersive liquid–liquid microextraction. Journal of Chromatography A 1116 (2006): 1-9.
50. Ojeda, C. B.; Sanchez Rojas, F. Separation and preconcentration by dispersive liquid–liquid microextraction procedure: a review. Chromatographia 69 (2009): 1149-1159.
51. Farina, L.; Boido, E.; Carrau, F.; and Dellacassa, E. Determination of volatile phenols in red wines by dispersive liquid–liquid microextraction and gas chromatography–mass spectrometry detection. Journal of Chromatography A 1157 (2007): 46-50.
52. Moinfar, S.; Milani Hosseini, M.R. Development of dispersive liquid–liquid microextraction method for the analysis of organophosphorus pesticides in tea Journal of Hazardous Materials 169 (2009): 907–911.
53. 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 1216 (2009): 7336–7345.

54. Xiong, J; and Hu, B. A new method for the analysis of organosulfur pesticides in environmental and beverage samples by gas chromatography. Journal of Chromatography A 1193 (2008): 7–18.

55. Wu, Q.; Li, Y.; Wang, C; Liu, Z.; Zang, X.; Zhou, X.; and Wang, Z. Dispersive liquid–liquid microextraction combined with high performance liquid chromatography–fluorescence detection for the determination of carbendazim and thiabendazole in environmental samples. Analytica Chimica Acta 638 (2009): 139–145.

56. Zhao, R.S; Diao, C.P.; Wang; X.; Jiang; T. and Yuan; J.P. Rapid determination of amide herbicides in environmental water samples with dispersive liquid–liquid microextraction prior to gas chromatography–mass spectrometry. Analytical and Bioanalytical Chemistry 391 (2008): 2915–2921.

57. Zhou, Q.; Bai, H.; Xie, G.; and Xiaoc, J. Trace determination of organophosphorus pesticides in environmental samples by temperature-controlled ionic liquid dispersive liquid-phase microextraction. Journal of Chromatography A 1188 (2008): 148–153.

58. Wu, Q.; Zhou, X.; Li, Y.; Zang, X.; Wang, C.; and Wang, Z. Application of dispersive liquid–liquid microextraction combined with high-performance liquid chromatography to the determination of carbamate pesticides in water samples. Analytical and Bioanalytical Chemistry (2009) 393:1755–1761.

59. Leong, M.I.; and Huang, S.D. Dispersive liquid–liquid microextraction method based on solidification of floating organic drop for extraction of organochlorine pesticides in water samples. Journal of Chromatography A, 1216 (2009):7645–7650.

60. Cunha, S.C.; Fernandes, J.O.; and Oliveira, M.B. Fast analysis of multiple pesticide residues in apple juice using dispersive liquid–liquid microextraction and multidimensional gas chromatography–mass spectrometry. Journal of Chromatography A 1216 (2009): 8835–8844.

61. He, L.; Luo, X.; Xie, H.; Wang, C.; Jiang, X.; and Lu, K. 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.

62. Du; J., Yan; H., She; D., Liu B., and Yang; G. Simultaneous determination of cypermethrin and permethrin in pear juice by ultrasound-assisted dispersive liquid-liquid microextraction combined with gas chromatography. Talanta 391 (2010): 387–393.
63. Chen; H., and Li; S. Low-density extraction solvent-based solvent terminated dispersive liquid–liquid microextraction combined with gas chromatography-tandem mass spectrometry for the determination of carbamate pesticides in water samples. Journal of Chromatography A, 1217 (2010):1244–1248.
64. Ravelo-Perez, L.M.; Hernandez-Borges, J.; and Herrera-Herrera, V. Pesticide extraction from table grapes and plums using ionic liquid based dispersive liquid–liquid microextraction. Analytical and Bioanalytical Chemistry 395 (2009): 2387–2395.
65. Pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate. AOAC International (2007).
66. Zweig, G. Chromatographic techniques for pesticide residue analysis. Chromatographic Reviews 6 (1964): 110–128.
67. Steinwandter, H. Universal 5-min on-line method for extracting and isolating pesticide residues and industrial chemicals. Fresenius Journal of Analytical Chemistry 322 (1985): 752-754.

VITA

Name : Ms. Phonsiri Sayaphan

Date of Birth : May 14, 1977

Place of Birth: Phitsanuloke, Thailand

Gender : Female

Education :

1995-1999	Bachelor Degree of Science (Chemistry), Naresual University
2004-2012	Post graduate study leading to a master of science (Analytical Chemistry), Chulalongkorn University

Home Address : 225/28, Tambol Mouang, Amphur Mouang, Phitsanuloke 65000, Thailand