

**DETERMINATION OF ORGANOPHOSPHATE PESTICIDES
USING GAS CHROMATOGRAPHY**



**A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(APPLIED ANALYTICAL AND INORGANIC CHEMISTRY)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2009**

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Thesis
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**DETERMINATION OF ORGANOPHOSPHATE PESTICIDES
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.....
Miss Orawee Deerasamee
Candidate

.....
Lect. Tinnakorn Tiensing,
Ph.D. (Environmental Science)
Major-Advisor

.....
Asst. Prof. Atitaya Siripinyanond,
Ph.D. (Analytical Chemistry)
Co-Advisor

.....
Prof. Banchong Mahaisavariya,
M.D.
Dean
Faculty of Graduate Studies

.....
Asst. Prof. Duangjai Nacapricha,
Ph.D. (Analytical Chemistry)
Chair
Master of Science Program in Applied
Analytical and Inorganic Chemistry
Faculty of Science

Thesis
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was submitted to the Faculty of Graduate Studies, Mahidol University
for the Degree of Master of Science
(Applied Analytical and Inorganic Chemistry)

on
January 15, 2009

.....
Miss Orawee Deerasamee
Candidate

.....
Lect. Sukjit Kungwankunakorn,
Ph.D. (Analytical Chemistry)
Chair

.....
Lect. Tinnakorn Tiensing,
Ph.D. (Environmental Science)
Member

.....
Asst. Prof. Atitaya Siripinyanond,
Ph.D. (Analytical Chemistry)
Member

.....
Prof. Banchong Mahaisavariya,
M.D.
Dean
Faculty of Graduate Studies
Mahidol University

.....
Prof. Skorn Mongkolsuk,
Ph.D. (Biological Science)
Dean
Faculty of Science
Mahidol University

ACKNOWLEDGEMENTS

I am extremely thankful to my advisor, Dr. Tinnakorn Tiensing, for his valuable suggestion, criticism, assistance and encouragement throughout my M.Sc. research. Sincere appreciation is extended to Asst. Prof. Dr. Atitaya Siripinyanond, my co-advisor and Dr. Sukjit Kungwankunakorn, the external examiner for their comments and suggestion in completion of my thesis.

I would like to thank the financial support provided by the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education and also appreciate the financial support for my teaching assistantship from the Department of Chemistry, Faculty of Science, Mahidol University.

Special thanks to all teachers and members of Applied Analytical and Inorganic Chemistry Program (AAICP), Philosophy in Inorganic Chemistry (PIC), and Analytical Chemistry Program (ACP) especially my lovely friends in chromatographic laboratory and TTS group for their great friendship, assistance and helpful, supportive, valuable discussion during my work.

Finally, my deepest thankfulness is given to my lovely parents for their endless support, being understanding and care throughout my life. I can not put into words how much you all mean to me.

Orawee Deerasamee

DETERMINATION OF ORGANOPHOSPHATE PESTICIDES USING GAS CHROMATOGRAPHY

ORAWEE DEERASAMEE 4836433 SCAI/M

M.Sc. (APPLIED ANALYTICAL AND INORGANIC CHEMISTRY)

THESIS ADVISORS: TINNAKORN TIENSING, Ph.D. (ENVIRONMENTAL SCIENCE), ATITAYA SIRIPINYANOND, Ph.D. (ANALYTICAL CHEMISTRY)

ABSTRACT

Nowadays, organophosphorus pesticides (OPPs) are one class of pesticides widely used as insecticides in the agricultural field for pest control during the whole growth process of plants. They have been used to replace organochlorine pesticides due to their high effectiveness, relatively low price and especially low environmental persistence. However, these pesticides present a high toxicity on humans because they act as an acetylcholinesterase inhibitor, leading to the interruption of the nervous system. Acute toxicity of the inhibition of acetyl cholinesterase causes respiratory, myocardial, and neuromuscular transmission impairment. In some cases, they may result in acute pneumonary edema and be fatal.

The main OPPs which are diazinon, fenitrothion, malathion, chlorpyrifos and triazophos, were focused on in this study. An analytical method was developed for determining OPPs in vegetable samples (cabbage, cucumber and tomato) and also for investigating the degradation of OPPs in water using gas chromatography with flame photometric detection (GC-FPD). Then, the analytes was successfully separated within 10 minute on an HP-5 (5% methyl phenyl silicone) column. Detection limits of all analytes were in the range of 0.04 - 0.5 $\mu\text{g mL}^{-1}$. The identity of the pesticides was confirmed by GC-MS using selected ion monitoring mode. The preparation of cabbage samples included extraction with the mixture of ethyl acetate and hexane (1:1 v/v) and subsequently cleaned up by silica gel-solid phase extraction (silica gel-SPE). This analytical method was applied to determine pesticide contamination in the other vegetable samples, i.e., cucumber and tomato. The method was validated by spiking OPP standard in vegetable samples at the concentration of 0.02 - 1.00 mg kg^{-1} . The average recoveries of all analytes were in the range of 70 - 95%, 72 - 98% and 68 - 107% for cabbage, cucumber and tomato, respectively. The degradation of OPPs in water found that hydrolysis was the major process and there was no effect of sunlight on the hydrolytic degradation of OPPs in water. In this study, octadecyl-solid phase extraction (C_{18} -SPE) was used for extraction and preconcentration the OPP analytes from a water sample. Breakthrough volume of C_{18} -SPE was 250 mL, after which the water loading should be less than this value. The average recoveries of all analytes were in the range of 83 - 132%.

KEY WORDS: ORGANOPHOSPHATE PESTICIDES/SPE/GC/FPD

109 pp.

การวิเคราะห์หาปริมาณสารเคมีกำจัดศัตรูพืชประเภทออร์กาโนฟอสเฟตด้วยเทคนิคแก๊สโครมาโทกราฟี
(DETERMINATION OF ORGANOPHOSPHATE PESTICIDES USING GAS CHROMATOGRAPHY)

อรวี ศิริศมี 4836433 SCAI/M

วท.ม. (เคมีวิเคราะห์และเคมีอินทรีย์ประยุกต์)

คณะกรรมการควบคุมวิทยานิพนธ์; ทินกร เตียนสิงห์, Ph.D. (Environmental Science),

อติศยา ศิริภิญญานนท์, Ph.D. (Analytical Chemistry)

บทคัดย่อ

ปัจจุบันนี้สารเคมีกำจัดศัตรูพืชประเภทออร์กาโนฟอสเฟตจัดว่าเป็นสารเคมีกำจัดศัตรูพืชประเภทหนึ่งที่นิยมใช้เป็นยาฆ่าแมลงกันอย่างแพร่หลายในการเกษตรกรรมเพื่อควบคุมแมลงระหว่างกระบวนการเจริญเติบโตของพืช สารเคมีกำจัดศัตรูพืชกลุ่มนี้ถูกใช้แทนสารเคมีกำจัดศัตรูพืชประเภทออร์กาโนคลอรีน เนื่องจากมีฤทธิ์ในการกำจัดแมลงสูง ราคาถูก โดยเฉพาะอย่างยิ่งการตกค้างในสิ่งแวดล้อมต่ำกว่า อย่างไรก็ตามสารเคมีกำจัดศัตรูพืชกลุ่มนี้ยังมีพิษมากต่อมนุษย์ เนื่องจากจะออกฤทธิ์เป็นตัวยับยั้งการทำงานของเอนไซม์อะซิติลโคลีนเอสเตอเรสทำให้ระบบประสาทเกิดการรบกวน พิษเฉียบพลันของการยับยั้งการทำงานของเอนไซม์นี้ยังทำให้เกิดการทำลายระบบการสื่อสารประสาทในกระบวนการหายใจ กล้ามเนื้อหัวใจ และกล้ามเนื้อสมอง ในบางกรณีอาจทำให้เกิดอาการปวดบวมเฉียบพลันและเสียชีวิตได้

งานวิจัยนี้ได้ศึกษาสารเคมีกำจัดศัตรูพืชประเภทออร์กาโนฟอสเฟต ได้แก่ ไดอะซินอน เฟนิโตรโทออน มาลาโทออน คลอไพริฟอส และไครอะโซฟอส วิธีการวิเคราะห์หาปริมาณสารเคมีกำจัดศัตรูพืชเหล่านี้ในตัวอย่างพืชชนิดต่างๆ ได้แก่ กะหล่ำปลี แตงกวา และมะเขือเทศ และการติดตามการสลายตัวของสารเคมีกำจัดศัตรูพืชกลุ่มนี้ในตัวอย่างน้ำด้วยเทคนิคแก๊สโครมาโทกราฟีที่ตรวจวัดด้วยเฟรมโฟโตเมตริกดีเทกชัน ได้ถูกพัฒนาขึ้น สารเป้าหมายถูกแยกภายใน 10 นาทีด้วยคอลัมน์ชนิดเอชพี-5 และมีขีดจำกัดต่ำสุดของการตรวจวัดอยู่ในช่วง 0.04 - 0.50 ไมโครกรัมต่อมิลลิลิตร เทคนิคแก๊สโครมาโทกราฟีที่ตรวจวัดด้วยแมสสเปกโตรเมตริกถูกใช้ในการพิสูจน์เอกลักษณ์ของสารเคมีกำจัดศัตรูพืชแต่ละชนิด การเตรียมตัวอย่างกะหล่ำปลีประกอบด้วยการสกัดตัวอย่างด้วยตัวทำละลายผสมของเอทิลอะซิเตทและเฮกเซนในอัตราส่วน 1:1 โดยปริมาตร และกำจัดสิ่งปนเปื้อนด้วยการสกัดด้วยเฟสของแข็งโดยมีซิลิกาเจลเป็นตัวดูดซับ วิธีการวิเคราะห์นี้ได้นำไปประยุกต์ใช้ในการหาปริมาณสารเคมีกำจัดศัตรูพืชที่ตกค้างอยู่ในตัวอย่างผักชนิดอื่นๆ ได้แก่ แตงกวาและมะเขือเทศ ค่าการสกัดกลับคืนเฉลี่ยของสารเป้าหมายที่ความเข้มข้นในช่วง 0.02 - 1.00 มิลลิกรัมต่อกิโลกรัมถูกใช้ในการสร้างความน่าเชื่อถือให้กับวิธีการสกัดซึ่งอยู่ในช่วง 70 - 95, 72 - 98 และ 68 - 107 เปอร์เซ็นต์สำหรับตัวอย่างกะหล่ำปลี แตงกวา และมะเขือเทศตามลำดับ ในการศึกษาการสลายตัวของสารเป้าหมายในตัวอย่างน้ำพบว่ากระบวนการไฮโดรไลซิสเป็นกระบวนการหลักสำหรับการสลายตัวของสารเคมีกำจัดศัตรูพืชประเภทออร์กาโนฟอสเฟตและแสงแดดไม่มีผลต่อการสลายตัวของสารกลุ่มนี้ในตัวอย่างน้ำ การสกัดด้วยเฟสของแข็งโดยมีออกตะเดซิลเป็นตัวดูดซับถูกใช้สำหรับการสกัดสารเป้าหมายออกจากตัวอย่างน้ำและเพิ่มความเข้มข้นของตัวอย่าง ปริมาตรของตัวอย่างน้ำที่ใช้ควรมีน้อยกว่า 250 มิลลิลิตร เนื่องจากไม่ทำให้ตัวดูดซับเสียสภาพ ค่าการสกัดกลับคืนเฉลี่ยของสารเป้าหมายที่ความเข้มข้นในช่วง 0.5 - 2.0 มิลลิกรัมต่อลิตรอยู่ในช่วง 83 - 132 เปอร์เซ็นต์

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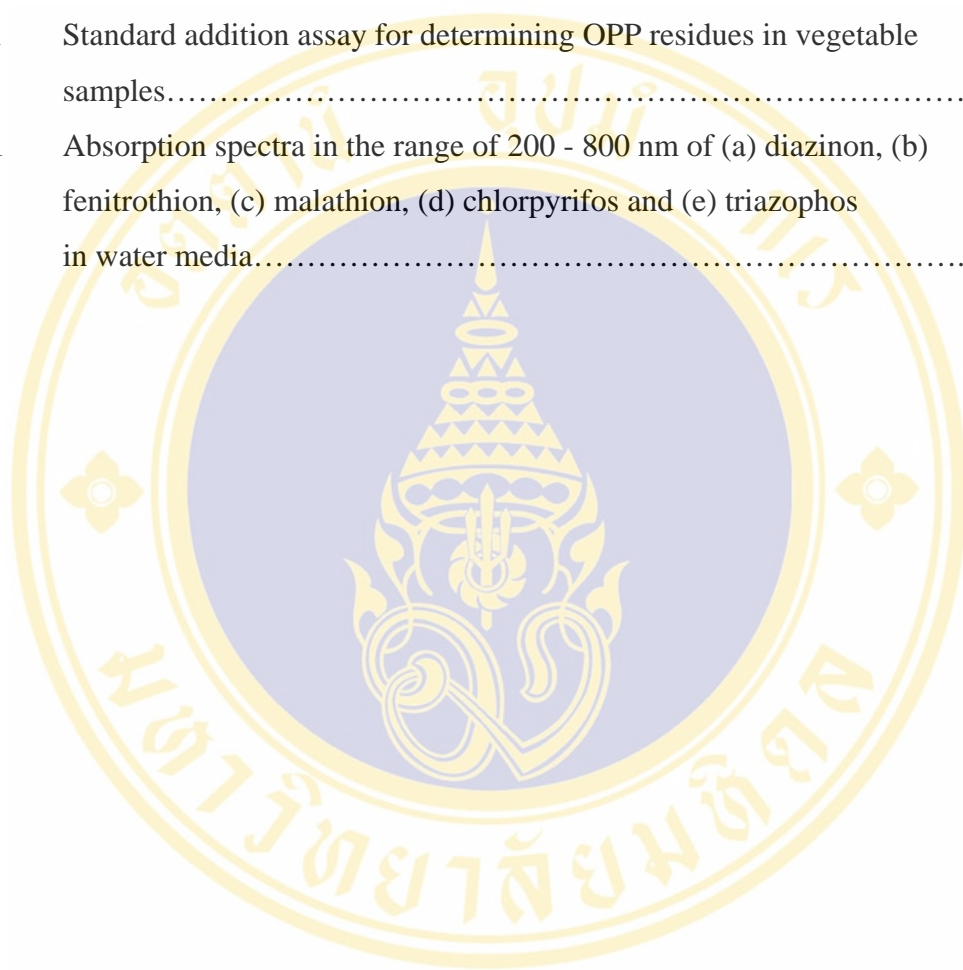
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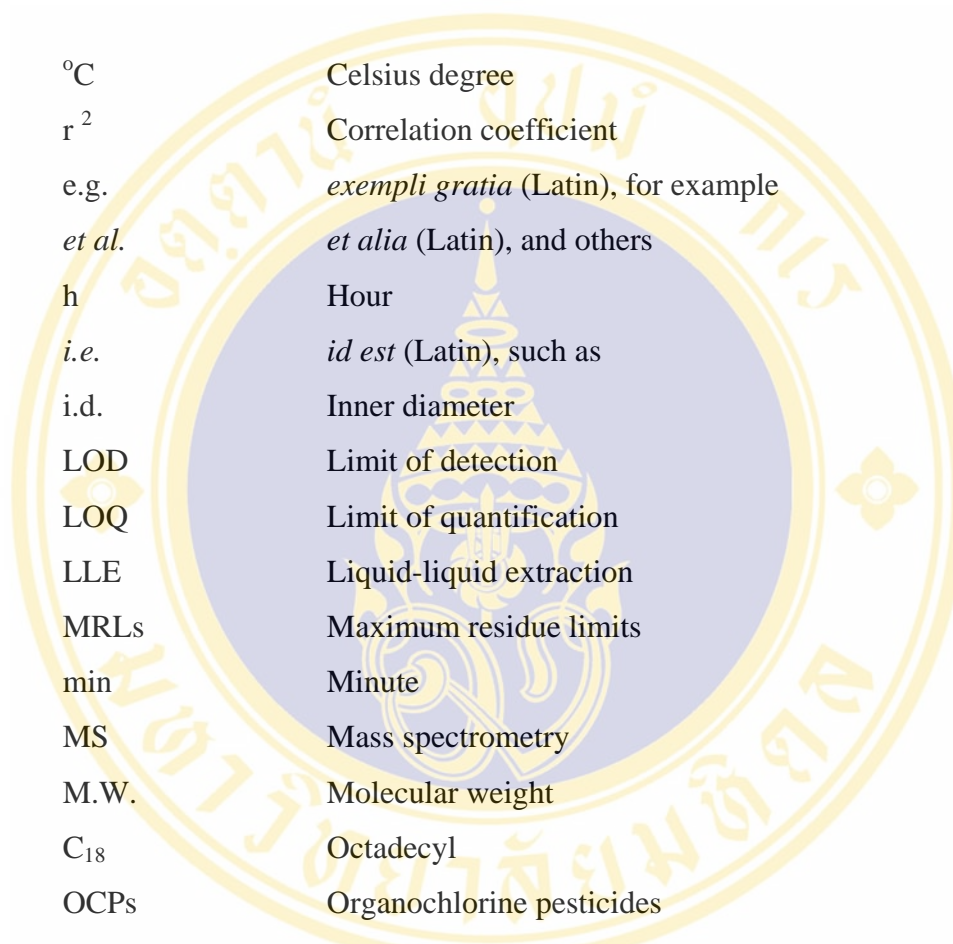
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LIST OF ABBREVIATIONS



°C	Celsius degree
r^2	Correlation coefficient
e.g.	<i>exempli gratia</i> (Latin), for example
<i>et al.</i>	<i>et alia</i> (Latin), and others
h	Hour
<i>i.e.</i>	<i>id est</i> (Latin), such as
i.d.	Inner diameter
LOD	Limit of detection
LOQ	Limit of quantification
LLE	Liquid-liquid extraction
MRLs	Maximum residue limits
min	Minute
MS	Mass spectrometry
M.W.	Molecular weight
C ₁₈	Octadecyl
OCPs	Organochlorine pesticides
OPPs	Organophosphorus pesticides
PFB-Br	2,3,4,5,6-pentafluorobenzyl bromide
R.S.D.	Relative standard deviation
S/N	Signal to noise
SPE	Solid phase extraction
SD	Standard deviation

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

Thailand is one of the leader importer and exporter of agricultural products i.e., vegetables and fruits. So, organophosphorus pesticides are widely used as insecticides in the agricultural field to destroy pests during the whole growth process of plant. To ensure that these agricultural products are safe for consumer's health, the maximum residue limits (MRLs) for these pesticides in these agricultural products have been established. This study developed an analytical method for determination of OPPs i.e., diazinon, fenitrothion, malathion, chlorpyrifos and triazophos in vegetable samples and also investigation of OPP degradation in water sample using GC-FPD. The residues of these pesticides were determined in vegetable samples i.e., cabbage, cucumber and tomato and the residue values were below the level of MRL. Therefore, the developed analytical method can be applied for determination of OPP residues in the agricultural products. In addition, the improved analytical method can be applied for investigation of OPP degradation in water samples which is a useful information to evaluate the toxicity of water for drinking water and aquatic organism.

CHAPTER I

INTRODUCTION

Pesticides are widely used in the agriculture and household in many countries to prevent and destroy any pests including animals (insects, mollusks, birds, fishes or nematodes), unwanted plant (plant pathogens or weeds), and microorganism, during the growth process of plants [U.S Environmental Protection Agency, U.S. EPA; Wikipedia encyclopedia]. Organophosphorus pesticides (OPPs) are one class of pesticides which have been enormously used today. They were used to replace organochlorine pesticides (OCPs) which were the most commonly used as insecticides in previous time [Kuet and Seng, 2004].

In recent years, OPPs are the most employed pesticides worldwide because of being soluble in water, high efficiency for all insect elimination, easily decomposition and relatively low price. Major function of OPPs, acts as acetylcholinesterase inhibitors, is very toxic after absorbed by inhalation, ingestion, and dermal absorption because of acetylcholinesterase de-activation. The decreasing of the cholinesterase enzyme results in the accumulation of acetylcholine at the nerve synapses, leading to the interruption of the nervous working. Acute toxicity of the inhibition of acetyl cholinesterase causes respiratory, myocardial, and neuromuscular transmission impairment and may results in acute pneumonary edema and fatal. [Costa, 2006; Hemakanthi De Alwis *et al.*, 2006; Tsoukali *et al.*, 2005; Tarbah *et al.*, 2001; International Programme on Chemical Safety, IPCS].

From the hazardous of pesticides, governments in many countries are interested in the pesticide residues on the agricultural products i.e., fruits and vegetables. So, the international organization about health and environmental protection i.e., Food and Agriculture Organization of the United Nations World Health Organization (FAO/WHO), Pesticide Action Network (PAN), European Union (EU),

Environmental Protection Agency (EPA) and governments in many countries as well as Thailand have established values of maximum residue limits (MRLs) for pesticides in agricultural products to ensure that the consumer will get a good agricultural product which is safe for their health. In addition, the residues of OPPs in agriculture can be contaminated into canal water by watering or rinsing of rain and polluted to the consumer as well as the aquatic organism. Therefore, the understanding of organophosphorus degradation after their release into water is extremely important for development of purification and removal of organophosphorus compounds from polluted water and evaluation of the toxicity of their degradation products.

Several methodologies have been developed for determining OPP residues in agricultural products such as spectrophotometry, biosensor, immunoassay and chromatography. However, these techniques are not directly analysed the low OPP concentration and co-matrix in vegetable samples including lipid, pigment and a variety of polar and non-polar substances which interfere with the analyte peak and accumulate in the injector and the front end of the capillary GC column [Lal *et al.*, 2008; Hammarstrand, 1976]. Therefore, sample preparation is essentially performed prior to GC analysis. Solid-phase extraction (SPE) has been widely used as an alternative method for sample preparation due to its simplicity, economy in terms of time and solvent and relatively low cost. This technique was applied to this work for clean-up and preconcentration steps.

The aim of this study was to improve an analytical technique for determination of main OPPs i.e., diazinon, fenitrothion, malathion, chlorpyrifos and triazophos in cabbage, cucumber and tomato and also investigation of the OPPs degradation in water sample using gas chromatography with flame photometric detection (GC-FPD). Separation of OPPs was carried out within 10 min and limit of detections (LODs) were in the range of 0.04 - 0.50 $\mu\text{g mL}^{-1}$. OPP residues in three vegetable samples were extracted with a mixture of ethyl acetate and hexane (1:1 v/v) and then were passed through a silica gel column for clean-up. Recoveries of all OPPs in three vegetables at five fortification levels (0.02, 0.10, 0.20, 0.50, 1.00 mg kg^{-1}) were in the range of 68 - 107% with relative standard deviation (R.S.D) < 10 %. In this work, the performed

method can be used to determine pesticide residues in cabbage, cucumber and tomato samples at below registered level of the MRLs of the pesticides for these samples. For water analysis, an octadecyl-solid phase extraction (C₁₈-SPE) was used to extract and preconcentration OPP residues from water sample. This study found that hydrolysis was the major process of the OPP degradation and there was no effect of sunlight on the hydrolytic degradation of OPPs in water.

In addition, derivatization of OPPs was performed to enhance the sensitivity of the OPP analysis. This study found that only malathion occurred its derivative when 1-chloro-3-iodopropane was used as derivatizing agents. However, its derivative could not increase the sensitivity of the analysis because of lower peak area than the underivatized malathion. The derivatization step was not necessary for these analytes when detected by FPD. Phosphorus and sulfur atoms in their molecules are sensitive to this detector which can determine the concentration of the analytes in the ppb level.

This method should be further applied for determining OPPs in biological samples, foodstuff and other environmental samples. Development of an efficient analytical method to detect such contaminants is an important topic for food safety and health protection. This study expects that the human will be realized the risk of these pesticides and help to reduce the amount of pesticide usage in the agricultural products.

CHAPTER II

OBJECTIVES

The aim of this thesis was to improve an analytical method for determination of five OPPs, i.e., diazinon, fenitrothion, malathion, chlorpyrifos and triazophos in vegetable samples and to investigate the OPP degradation in water samples using gas chromatography with flame photometric detection. Solid-phase extraction was used to preconcentrate and to clean-up the samples. The derivatization step was also studied for increasing the sensitivity of the analysis. The purposes of this study can be summarized as following:

1. To study the optimum condition of GC-FPD.
2. To optimize the derivatization procedures using PFB-Br and 1-chloro-3-iodopropane as derivatizing agents.
3. To optimize the solid-phase extraction with C₁₈, florisil and silica gel sorbents to reduce matrix and preconcentrate sample.
4. To optimize the extraction procedure for determination of OPPs in cabbage sample and then the method was applied to determine OPPs in other vegetable samples, such as cucumber and tomato.
5. To investigate the degradation of OPPs in water samples.

CHAPTER III

LITERATURE REVIEWS

In the chapter is divided into subsection of organophosphorus pesticides, regulation of usages and analytical techniques for determining these compounds. This work focuses on organophosphorus pesticides because these compounds have been applied for crop protection in the agricultural plants. Residues of these compounds in consuming vegetable plants are more concerned because of their toxicity to nervous system by inhibiting cholinesterase. The content of these compounds in consuming plant is required to observe the used in the field and effect on human health which may lead to set the regulation for controlling the used of pesticides and consuming vegetable.

Pesticides can be defined as a chemical compound which uses to protect the crop for preventing, destroying, repelling, mitigating any pests. It can be classified by two broad categories following as [Department of Agriculture, Thailand]:

1. Divided by chemical properties for the elimination of each pest e.g., insecticide, fungicide and rodenticide.

2. Divided by chemical composition or chemical structure such as inorganic compounds which have metal elements (arsenic, mercury, copper) and organic compounds consisting of carbon, nitrogen and oxygen or sulfur. This category can be divided into four sub-categories including organochlorine, organophosphorus, carbamate and pyrethroid.

3.1 Organophosphorus pesticides

Organophosphorus pesticides (OPPs) are chemical substances originally synthesized by the reaction of phosphoric acid and alcohols to replace organochlorine pesticides (OCPs) because of more easily decomposition and lower polluting effect. In 1800s, a number of organic phosphorus compounds were synthesized and used as insecticides in the late 1930s and early 1940s. Until in the 1944, Gerhard Schrader discovered the general chemical structure of anticholinesterase organic phosphorus compounds and produced the first commercial OPPs, namely, bladon (containing tetraethyl pyrophosphate, TEPP) and parathion. Afterwards, the organic phosphorus compounds have been continuously developed and widely commercialized in various formulations, is currently known as organophosphorus pesticides [Costa, 2006].

3.1.1 Chemical properties

OPPs are normally esters, amides, or thiol derivatives of phosphoric, phosphonic, phosphorothioic, or phosphonothioic acid, are illustrated in Table 3.1. Most OPPs are slightly soluble in water and have high oil-to-water partition coefficient and low vapour pressure.

A variety of OPP chemistry leads to their classification in several subclasses. These compounds consist of main elements i.e., carbon, nitrogen, phosphorus and oxygen or sulfur. The general chemical structure of OPPs is shown in Figure 3.1.

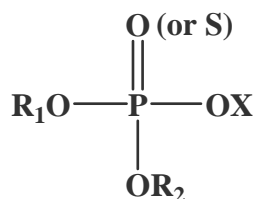


Figure 3.1 General chemical structure of OPP compound.

A terminal oxygen or sulfur atom bond by a double bond to the phosphorus which can be called phosphates (P=O) or phosphorothioates (P=S). The toxicity of these compounds in form of terminal sulfur atom is much less than in oxygen atom because of an inactive acetylcholinesterase inhibitor of P=S compounds. R_1 and R_2 are usually simple alkyl or aryl groups and X is a leaving group, can be any one of a wide variety of substituted and branched aliphatic, aromatic or heterocyclic groups [Costa, 2006; Wikipedia encyclopedia].

3.1.2 Classification of OPPs

All OPPs can be classified by chemical structure together with the common names which are divided into six main groups such as phosphate, phosphorothioate, phosphoramidate, phosphorothioamidate, phosphonate, and phosphonothioate, as shown in Table 3.1 [IPCSINTOXdatabank].

Table 3.1 Variations in the chemical structure of OPPs.

Phosphorus group	General structure	Common name
Phosphate	$\begin{array}{c} \text{O} \\ \parallel \\ \text{RO}-\text{P}-\text{OX} \\ \\ \text{OR} \end{array}$	chlorfenvinphos, crotoxyphos, dichlorvos, dicrotophos, heptenphos, mevinphos, monocrotophos, naled, phosphamidon, TEPP, tetrachlorvinphos
<i>O</i> -alkyl phosphorothioate	$\begin{array}{c} \text{O} \\ \parallel \\ \text{RO}-\text{P}-\text{SX} \\ \\ \text{OR} \end{array}$	amiton, demeton-s-methyl, omethoate, oxydemeton-methyl, vamidothion
	$\begin{array}{c} \text{S} \\ \parallel \\ \text{RO}-\text{P}-\text{OX} \\ \\ \text{OR} \end{array}$	azothoate, bromophos, bromophos-ethyl, chlorpyriphos, chlorpyriphos-methyl, coumaphos, diazinon, dichlofenthion, fenchlorphos, fenitrothion, fenthion, fensulfothion, iodofenphos, parathion, parathion-methyl, phoxim, pyrimiphos-ethyl, pyrimiphos-methyl, pyrazophos, sulfotep, temephos, thionazin,
<i>S</i> -alkyl phosphorothioate	$\begin{array}{c} \text{O} \\ \parallel \\ \text{RS}-\text{P}-\text{OX} \\ \\ \text{OR} \end{array}$	profenofos, trifenofos, prothiofos
Phosphorodithioate	$\begin{array}{c} \text{S} \\ \parallel \\ \text{RO}-\text{P}-\text{SX} \\ \\ \text{OR} \end{array}$	amidithion, azinophos-ethyl, azinophos-methyl, dimethoate, dioxathion, disulfoton, ethion, formothion, malathion, mecarbam, menazon, methidathion, morphothion, phenthoate, phorate, phosalone, phosmet, phothoate

Table 3.1 (Continue) Variations in the chemical structure of OPPs.

Phosphorus group	General structure	Common name
S-alkyl phosphorodithioate	$\begin{array}{c} \text{S} \\ \\ \text{RS}-\text{P}-\text{OX} \\ \\ \text{OR} \end{array}$	sulprofos
Phosphoroamidate	$\begin{array}{c} \text{O} \\ \\ \text{RO}-\text{P}-\text{NR}_2 \\ \\ \text{OR} \end{array}$	cruformate, fenamiphos, fosthistan, mephosfolan, phosfolan
Phosphorotriamidate	$\begin{array}{c} \text{O} \\ \\ \text{R}_2\text{N}-\text{P}-\text{NR}_2 \\ \\ \text{NR}_2 \end{array}$	triamiphos
Phosphorothioamidate	$\begin{array}{c} \text{O} \\ \\ \text{RO}-\text{P}-\text{NR}_2 \\ \\ \text{SR} \end{array}$	methamidophos, acephate
	$\begin{array}{c} \text{S} \\ \\ \text{RO}-\text{P}-\text{NR}_2 \\ \\ \text{OR} \end{array}$	isofenphos
Phosphonate	$\begin{array}{c} \text{O} \\ \\ \text{RO}-\text{P}-\text{OX} \\ \\ \text{R} \end{array}$	butonate, trichlorfon
Phosphonothioate	$\begin{array}{c} \text{S} \\ \\ \text{RO}-\text{P}-\text{OX} \\ \\ \text{R} \end{array}$	cyanofenphos, EPN, leptophos, trichlornat
Phosphonodithioate	$\begin{array}{c} \text{S} \\ \\ \text{RO}-\text{P}-\text{SX} \\ \\ \text{R} \end{array}$	fonofos

3.1.3 Toxicity of OPPs

OPP can be absorbed by many routes such as oral, inhalation, ingestion, and dermal absorption. The absorbed OPP can distribute over organs via lipid especially in nervous system and liver and then act as an acetylcholinesterase inhibitor. Normally, acetylcholine (ACh) that is a neurotransmitter, can be broken down to choline and acetic acid, and inactivated in milliseconds by acetylcholinesterase enzyme (AChE), as presented in Figure 3.2.

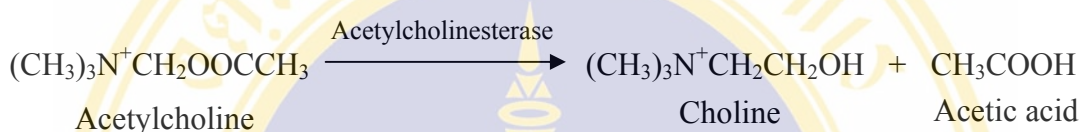


Figure 3.2 The reaction of acetylcholine decomposition by acetylcholinesterase enzyme [Pophan and Maketon, 1999].

After the absorption of OPPs, metabolism of OPPs occurs by oxidation and hydrolysis pathways with glutathione or by esterases. Majority of OPPs that containing a P=S bond are not directly toxic because they are not active as an acetylcholinesterase inhibitor. They need to be converted into their oxygen metabolites (P=O bond) by liver Cytochrome P450 microsomal enzyme. Their oxygen metabolites are more able to act as an acetylcholinesterase inhibitor. The loss of available AChE leads to an excess of ACh in nerve ending which initially over-stimulates in the nerve synapses and effects to the interruption with nerve impulse-transmission. The symptoms of patient can be classified by period of symptom occurrence [PAN; IPCSINTOX databank; Bunchet et al., 1997; Hierons and Johnson, 1978]:

3.1.3.1 Acute toxicity

Generally, OPPs are acutely toxic occurring within 2 - 3 days after exposure. Acute poisoning depends on amount of OPP dosing. It is characterized by widespread muscarinic and nicotinic effects which are caused by inhibition of acetylcholinesterase at peripheral nerve endings, in ganglia, and in the

brain. The earliest muscarinic symptoms, in eyes, are miosis, blurred vision and eye pain, in digestion system, are hypersalivation, nausea, vomiting, abdominal cramps, diarrhea and tenesmus, in inhalation system, are cough, expectoration of frothy secretions, pulmonary oedema, chest tightness and wheeze, especially secretory effects (salivation, bronchorrhoea) are often seen. The nicotinic effects of poisoning include fasciculation, progressive flaccidity, and weakness of proximal muscle groups, in particular the neck flexors but later extra-ocular muscles and muscles of respiration. In serious cases, respiratory failure and death can occur.

3.1.3.2 Chronic toxicity

The symptom of chronic effect occurs after exposure at a low concentration of OPPs for a long time which is known to cause a delayed neuropathy. The first symptoms are often sensory with tingling and burning sensations followed by weakness of arms and legs, cuff pain and paresthesia of tiphands and tiptoe, wasting of hand muscles. In serious cases, extensive flaccid paralysis can occur for several days.

However, the hazard of pesticides can be classified based primarily on the acute oral and dermal toxicity to the rat [WHO, 2004]. The classification distinguishes between the more and the less hazardous forms of each pesticide in that it is based on the toxicity of the technical compound and on its formulations. In particular, allowance is made for the lesser hazards from solids as compared with liquids.

Table 3.2 The classification of pesticide toxicity published by WHO.

Class of pesticide toxicity		LD ₅₀ for the rat (mg/kg body weight)*			
		Oral		Dermal	
		Solid	Liquid	Solid	Liquid
Ia	Extremely hazardous	5 or less	20 or less	10 or less	40 or less
Ib	Highly hazardous	5 – 50	20 – 200	10 – 100	40 – 400
II	Moderately hazardous	50 – 500	200 – 2000	100 – 1000	400 – 4000
III	Slightly hazardous	over 500	over 2000	over 1000	over 4000

* The LD₅₀ value is a statistical estimate of the number of mg of toxicant per kg of body weight required to kill 50% of a large population of test animals.

From this classification, the toxicity of diazinon, fenitrothion and chlorpyrifos are classified in class II, malathion is in class III, while triazophos which has the highest toxicity is in class Ib. So, the usage of these pesticides for a long time can extremely effect to the human health.

3.2 Regulatory limits for organophosphorus pesticides

As OPPs can exert significant adverse effects in human, the international organization, i.e., FAO/WHO, Pesticide Action Network (PAN), European Union (EU), Environmental Protection Agency (EPA) and governments in many countries as well as Thailand are interested in the pesticide residues on agricultural products and has been strictly regulated the maximum residue limits (MRLs) in the agricultural products for health protection.

The MRLs of pesticides in agricultural products which established by Ministry of Agriculture and Cooperative of Thailand [2006] found that the OPPs i.e., diazinon, fenitrothion, malathion, chlorpyrifos and triazophos were controlled in their agricultural products. In this work, the residues of these pesticides were determined in vegetable samples i.e., cabbage, cucumber and tomato. The examples of MRLs of

these pesticides for the investigated vegetables established by various organization and governments in many countries are shown in Table 3.3.

Most MRLs of pesticides for vegetables in many countries often cite to CODEX alimentarius and/or depend on the commercial agreement of each organization or country. However, we notice that their MRLs of pesticide for all agricultural products are not lower than 0.01 mgkg^{-1} . So, the purpose of this study was to develop an analytical method for determination of these OPPs at the concentration below the permitted level of MRLs.

3.3 Analytical techniques for determination of OPPs in vegetable samples

Several analytical techniques have been continuously developed for the determination of OPPs in agricultural samples such as spectrophotometry, biosensor, immunoassay and chromatography.

The reviews of determining OPP residues in vegetable samples using various techniques are shown in Table 3.4.

Table 3.3 The maximum residue limits (MRLs) of OPP residues in cabbage, cucumber and tomato issued by international organization and governments in many countries.

Regulation	MRLs (mgkg ⁻¹)													
	Cabbage				Cucumber				Tomato					
	Diazinon	Fenitrothion	Malathion	Chlorpyrifos	Diazinon	Fenitrothion	Malathion	Chlorpyrifos	Triazophos	Diazinon	Fenitrothion	Malathion	Chlorpyrifos	Triazophos
CODEX ^I	0.5	-	-	1	0.1	-	0.2	-	-	0.5	-	0.5	0.5	-
EU ^{II}	0.5	0.5	3	1	0.01	0.5	3	0.05	0.01	0.05	0.5	3	-	0.01
EPA ^{III}	0.7	-	8	2.0	0.75	-	8	0.05	-	0.75	-	8	0.5	-
KOREA ^{IV}	0.1	0.5	0.5	0.5	-	-	-	0.01	-	0.3	0.2	0.5	0.5	-
JAPAN ^V	0.1	0.5	2.0	0.05	0.1	0.2	0.5	0.05	-	0.1	0.2	0.5	0.5	-
THAILAND ^{VI}	0.5	-	8	-	0.1	-	-	-	-	0.5	-	3	0.5	-

I: http://www.codexalimentarius.net/mrls/pestides/jsp/pest_q-e.jsp; Last update: 9 April 2008

II: http://ec.europa.eu/food/plant/protection/pesticides/legislation_en.htm

III: <http://www.epa.gov/pesticides/food/viewtols.htm#database>; Last update: 1 July 2005

IV: The report of Food Sanitation Act of Korea Food and Drug Administration 2005

V: The report of Food Sanitation Law of Japan External Trade Organization 2006

VI: The report of the Thai Agricultural Commodity and Food Standard 9002 – 2006

Table 3.4 The literature reviews of determining OPP residues in vegetable samples using various techniques.

Technique	Sample	Sample preparation	Detection	LOD	Ref.
Spectrophotometry	Cauliflower, Cabbage, Spanish	The samples were extracted with ethanol and derivatized with <i>N</i> -bromosuccinimide (NBS). The unconsumed NBS was reacted with rhodamine B for detection.	Visible (550 nm)	-	Mathew <i>et al.</i> , 2007
	Cabbage, Cauliflower, Cucumber, Greengrocery, Tomato, Spinach	The samples were extracted with Milli-Q water. The analytes were degraded to phosphate with liquid core waveguide light intensity technique (UV light source, K ₂ S ₂ O ₈ oxidant, nanosized TiO ₂ catalyst) and formed by bismuth phosphormolybdenum blue method for detection.	Visible (690 nm)	6.7 x 10 ⁻¹² gmL ⁻¹	Cheng <i>et al.</i> , 2007
Biosensor	Celery, Grape, Lettuce, Melon, Orange, Pear	The samples were extracted with ethyl acetate and then dried and dissolved with phosphate buffer. The extracts were mixed with AChE and added with acetylthiocholine. The solution was dropped on a screen-printed carbon working electrode deposited by tetracyanoquinodimethane.	Differential pulse voltammetry	-	Mascini and Palchetti, 2001
	Iceberg, Lettuce, Onion, Salad	The samples were treated with acetone and then dried and dissolved with phosphate buffer. The extracts were flowed into an enzyme immobilized glass bead column and then flowed with acetylthiocholine iodide-DTNB solution.	Thermal lens spectrometer (480 nm at 120 mW)	-	Pogačnik and Franko, 2003
Immunoassay	Grape, Peach, Pear, Tomato	OPP haptans were conjugated with bovine serum albumin to produce antibodies by injecting into white rabbit and conjugated with ovalbumin used as coating antigen. The samples were dissolved in methanol. A portion of samples and antibodies were added on to the plate and then peroxidase-labeled goat anti-rabbit immunoglobulin was added. This immobilized-enzyme was reacted with tetramethyl-benzidine in H ₂ O ₂ for appearing color.	Visible (450 nm)	0.01 x 10 ⁻⁹ gmL ⁻¹	Zhang <i>et al.</i> , 2008
Thin-layer chromatography	Apple, Cabbage, Carrot, Lettuce	The samples were extracted with acetonitrile/water-chloroform/chloroform. The extracts were spotted on a silica gel plate and eluted with methylene chloride.	Sprayed with a rat liver solution, a saturated bromine solution and the mixture of 2-naphthyl acetate and fast blue B solution	0.0005 mgkg ⁻¹	Sandroni and Schlitt, 1971

Table 3.4 (continue) Literature reviews of determining OPP residues in vegetable samples using various techniques.

Technique	Sample	Sample preparation	Detection	LOD	Ref.
Thin-layer chromatography (continue)	Tomato	The sample was extracted with ethyl acetate and cleaned by a bio-beads SX-3 GPC eluting with ethyl acetate-cyclohexane (1:1 v/v). The extract was spotted on a silica gel plate and separated with ethyl acetate or CH ₂ Cl ₂ .	Sprayed with <i>o</i> -toluidine plus potassium iodide	0.067 - 0.333 mgkg ⁻¹	Moraes <i>et al.</i> , 2003
	Pistachio nut	The sample was blended with C ₁₈ adsorbent and packed on the lanthanum silicate ion exchanger column which was washed with hexane and eluted with CH ₂ Cl ₂ -ethyl acetate. The eluent was spotted on TLC plate and eluted by CH ₃ OH-10%NH ₃ (9.5:0.5 v/v) for lanthanum silicate ion exchanger and by CH ₃ OH-CH ₂ Cl ₂ -10%NH ₃ (6.5:3.1:0.4 v/v/v) for lanthanum tungstate ion exchanger.	Sprayed with palladium chloride reagent	-	Husain <i>et al.</i> , 2003
Capillary electrophoresis	Potato, Rice, Wheat	The samples were extracted with methanol and then injected to a fully automated styrene-divinylbenzene polymer SPE system conjugating with MEKC for clean-up and separation of the analytes. Micelles were formed by adding sodium dodecyl sulfate to phosphate buffer.	UV (210 nm)	0.03 mgkg ⁻¹	Perez-Ruiz <i>et al.</i> , 2005
High-performance liquid chromatography	Green bean	The sample was extracted with acetone and then partitioned with dichloromethane. The extract was cleaned by C ₁₈ -SPE following by florisil-SPE column.	Photodiode-array (200 - 350 nm)	<0.1 mgkg ⁻¹	Parrill and Vidal, 1996
	Potato, Pepper, Tomato, Wheat, Rice	The samples were extracted with methanol and separated by HPLC column. The isolated OPPs were degraded to orthophosphate by a low-pressure mercury lamp (254 nm in peroxydisulfate). The resultant orthophosphate was reacted with molybdate to form molybdoorthophosphoric acid and subsequently reacted with thiamine to generate thiochrome.	Fluorescence (440 nm with excitation at 375 nm)	0.001 mgkg ⁻¹	Perez-Ruiz <i>et al.</i> , 2005
	Apple, Cabbage, Carrot, Tomato	The samples were extracted with acetonitrile. The extracts were cleaned with dispersive solid phase extraction (DSPE) by centrifuging with primary secondary amine sorbent.	MS	0.005 - 0.035 mgkg ⁻¹	Min <i>et al.</i> , 2006

Most spectrophotometric method involves with the determination of OPPs by total phosphorus measurement. It can not determine the speciated OPPs in the samples. Biological methods, such as enzyme-linked immunoassay and biosensor technique are highly specific for the OPP analysis. Nevertheless, these methods are suitable for single use or require multiple washing steps. Chromatographic techniques e.g., thin-layer chromatography (TLC), capillary electrophoresis (CE), high performance liquid chromatography (HPLC) and gas chromatography (GC) are the effective techniques for separating and determining each OPP residue in the samples. However, TLC is relatively lower separation efficiency and higher detection limit. CE and HPLC have low sensitivity of the analysis because they lack the selective detectors such as FPD or NPD.

GC is still the first choice of the method used for the separation and determination of OPP residues because of high separation efficiency, high sensitivity and high resolution. In addition, detection limit of this technique is low due to detecting with the selective detectors. An analytical procedure for determining OPPs residues in vegetable samples by gas chromatography consists of two main parts including sample preparation and GC analysis.

3.3.1 Sample preparation

Sample preparation for determining OPP residues in vegetable sample consists of sample extraction and clean-up procedures.

The extraction efficiency depends on the polarity of the pesticide as well as on the type of sample matrix [Lal *et al.*, 2008]. A variety of solvent extraction systems has been used as multiresidue screening procedures of OPP compounds. Especially, acetonitrile, acetone and ethyl acetate are preferred extractants because they are suitable for both non-polar and polar pesticides. The advantage of acetonitrile can not extract the lipophilic plant materials such as fat and waxes, so that it is suitable for fatty plant. Acetone is usually selected as extraction solvent because of its merits over the other solvent (lower toxicity, easy to purify and evaporate, and relatively low

price) [Tekel and Hatrík, 1996; Parrilla and Vidal, 1996]. While, the usage of ethyl acetate avoided the need for a subsequent partition with immiscible solvent from water in fresh samples [Dorea *et al.*, 1996]. Consequently, the mixture of various solvent has been used to obtain the suitable polarity of the extraction solvent for the OPP extraction [Lal *et al.*, 2008; Shuling *et al.*, 2007; Maštovská *et al.*, 2004].

The plant i.e., root and bulb vegetables, low chlorophyll and oil fruit and vegetables, high chlorophyll plants and crops should use acetone as extracting solvent. An acetone-water mixture was optimal for dried fruits of high sugar content and dichlorometane was used for dry crops of low fat (oil) content [Tekel and Hatrík, 1996].

Normally, in sample preparation, the vegetable samples were extracted with organic solvent by stirring bar. However, many analytical methods were developed to increase the extraction efficiency, for example, ultrasonic solvent extraction (USE). It was performed in an ultrasonic cleaning bath at 25°C with working frequency of 28 kHz and power of 300 W. [Pan *et al.*, 2007]

In addition, a pressurized liquid extraction (PLE) has been subsequently developed for the extraction procedure, also known as accelerated solvent extraction (ASE). The method combines between conventional organic solvent extraction and a high-speed homogenizer under elevated pressure and temperature (pressure: 5 - 200 atm and temperature: 25 - 200 °C for a short time (5 – 10 min)) [Ahmed, 2001]. Fernández Moreno *et al.* [2006] used this extraction method for extracting avocado samples by mean of the mixture of ethyl acetate and cyclohexane (1:1 v/v) as an extraction solvent.

The other matrices in vegetable samples which are amino acids and carbohydrates, particular lipids, pigments and a variety of polar and non-polar substances can be extracted into the organic solvent. These matrix compounds will interfere in the GC analysis [Hammarstrand, 1976]. Therefore, sample clean-up is an essential stage in the analytical process for matrix removal. Tekel and Hatrík [1996]

reported on the many multiresidue procedures employing different clean-up methods for pesticide residue analysis in plant materials by chromatographic techniques including liquid-liquid extraction (LLE), solid-phase extraction (SPE), gel permeation chromatography (GPC), matrix solid-phase dispersion extraction (MSPD), sweep co-distillation and supercritical fluid extraction (SFE).

3.3.2 GC analysis

The highest sensitivity and separation efficiency also depend on GC instrumentation and detector efficiency. Nowadays, fused-silica capillary columns have almost replaced the packed columns because of high efficiency for separating a large number of pesticides with similar physico-chemical properties. The selection of column depends on the nature of the pesticides. The columns coated with various materials, for example, phenylmethylsilicone [Shuling *et al.*, 2007; Martínez Salvador *et al.*, 2006], dimethylsiloxane [Dorea *et al.*, 1996], phenylmethylsiloxane [Kuet and Seng, 2004; Maria Kristenson *et al.*, 2001; Sáenz Barrio *et al.*, 1994; Hernández Hernández *et al.*, 1990], cyanophenylmethylpolysiloxane [Dugo *et al.*, 2005; Agüera and Contreras, 1993], cyanopropylphenyldimethylsiloxane [Aybar Muñoz *et al.*, 2003] were widely used for OPP analysis.

The OPP determinations have been determined by GC with an appropriate detector. A variety of detectors such as alkali flame ionization detector (AFID), electron capture detector (ECD), atomic emission detector (AED), electrolytic conductivity detector (ELCD), flame photometric detector (FPD) and nitrogen-phosphorus detector (NPD) is widely used. In addition mass spectrometry has become the standard confirmatory technique for the OPP analysis. A review of detectors used for the pesticide analysis using GC was reported by Ahmed and Tekel [Ahmed, 2001; Tekel and Hatrík, 1996].

The literature reviews for determining OPPs in vegetable samples including sample preparation procedure and limit of detection are shown in Table 3.5.

Table 3.5 The literature reviews of determining OPPs in vegetable samples by GC.

Sample	Extraction solvent	Clean-up	Detector	LOD (mgkg ⁻¹)	Ref.
Green pepper, Cucumber, Bean	Ethyl acetate	-	FPD	0.05 - 0.02	Agüera <i>et al.</i> , 1993
Cauliflower, Cabbage, Pea, Potato, Carrot, Mushroom	Ethyl acetate	-	FPD	0.01- 0.10 (LOQ)	Cai <i>et al.</i> , 1995
Sweet pepper	Ethyl acetate	-	FPD	0.01	Patel <i>et al.</i> , 2004
Cucumber	Ethyl acetate	-	Pulsed- FPD	< 0.05	Aybar Muñoz <i>et al.</i> , 2003
Green bean, Cucumber, Melon, Tomato, Eggplant, Zucchini, Pepper, Watermelon	Dichloromethane	-	Pulsed- FPD	<0.01	Salvador <i>et al.</i> , 2006
Cucumber, Spring onion	Acetone:ethyl acetate:hexane (10:80:10 v/v/v)	C ₁₈ -SPE	ECD	0.005 - 0.010	Lal <i>et al.</i> , 2008
Carrot, Cucumber, Green mustard	Acetone, Dichloromethane	Carrot: C ₁₈ -SPE; Cucumber and Green mustard: C ₁₈ -SPE following silica gel-SPE	FPD	0.02	Kuet and Seng, 2004
Pear, Apple, Tomato, Pepper	Acetone, Petroleum ether, Dichloromethane	Florisil-SPE	MS	0.01 - 0.10	Araoud <i>et al.</i> , 2007
Avocado	PLE (ethyl acetate: cyclohexane (1:1 v/v))	GPC	MS/MS	0.00001 - 0.00250	Fernández Moreno <i>et al.</i> , 2006

Table 3.5 (Continued) The literature reviews of determining OPPs in vegetable samples by GC.

Sample	Extraction solvent	Clean-up	Detector	LOD (mgkg ⁻¹)	Ref.
Leek	Acetone: dichloromethane (4:3 v/v)	GPC followed by Graphitized carbon-SPE	MS	0.01 (LOQ)	Shuling <i>et al.</i> , 2007
Lettuce	Ethyl acetate: cyclohexane (1:1 v/v)	High-performance GPC	MS	0.001 - 0.020	Maštovská <i>et al.</i> , 2004
Watermelon, Cucumber	Acetonitrile	DLLME	FPD	0.010 - 0.190	Zhao <i>et al.</i> , 2007
<i>Chrysanthemum coronarium</i>	Deionized water	SPME (100 µm poly- dimethylsiloxane fiber)	FPD	0.0047 - 0.0750	Chen <i>et al.</i> , 1998

3.4 The degradation of organophosphorus pesticides in water

OPP residues in agriculture can be contaminated into canal water by watering or rinsing of rain. The regulatory limits and guideline values for pesticides in drinking water have been legislated in many countries. According to the Regulatory Limits for Pesticide Residues in Water by the IUPAC Technical report [D.J. Hamilton *et al.*, 2003], the limits and guideline values can be derived by applying a safety factor to a no-effect-level, or from the detection limit of an analytical method, or from levels occurring when good practices are followed and also passing a safety assessment, or directly by legislative decision. The drinking water may be environmental waters, irrigation waters, effluent waters and livestock drinking waters. Most generally, the standards of drinking have been applied to ground water. The limit and guideline values for four pesticides including diazinon, fenitrothion, malathion and chlopyrifos

issued by Australia (AUS), the United States (USA), New Zealand (NZ), Japan and Canada are presented in Table 3.6.

Table 3.6 The standard and guideline values (μgL^{-1}) for pesticide residues in drinking water.

Pesticide	USA Health Advisory, lifetime	NZ MAV	Japan Std.	Aus GV	Aus HV	Cannada MAC
Diazinon	0.6	10	5	1	3	20
Fenitrothion	-	-	3	-	10	-
Malathion	100	-	10	-	50	190
Chlorpyrifos	20	70	30	-	-	90

MAV: maximum acceptable value; GV: guideline value; HV: health value; MAC: maximum acceptable concentration.

Furthermore, the EU allows a maximum concentration of $0.1 \mu\text{gL}^{-1}$ of each individual pesticide and $0.5 \mu\text{gL}^{-1}$ of the sum of pesticides in drinking water [Berijani *et al.*, 2006].

A variety of analytical methods was applied for extracting OPPs from water and preconcentration prior to GC analysis. Liquid-liquid extraction (LLE) [Sankararamkrishnan *et al.*, 2004; Bavcon *et al.*, 2003; Rani *et al.*, 2001; Freed *et al.*, 1979; Blanchet, 1979] and C_{18} -SPE [Montó *et al.*, 1991; Mañes Vinuesa *et al.*, 1989] were usually used for the extraction, preconcentration and clean-up step in the water analysis. The SPME was used to compare with C_{18} -SPE. The study found that SPE needs liter amounts of sample, whereas SPME need only a few milliliter volume of the sample [Eiserta *et al.*; 1994]. In addition, dispersive liquid-liquid microextraction (DLLME) is a new microextraction technique. The advantages of DLLME are fast,

easy to operate, relatively low solvent and sample consumption, inexpensive, high recovery and enrichment factor [Berijani *et al.*, 2006].

The understanding of organophosphorus degradation after their release into water is extremely important for development of purification and removal of organophosphorus compounds from polluted water and also evaluation of the toxicity of their degradation products. OPPs can be decomposed by various pathways in environmental condition.

Hydrolysis is the major degradation pathway of OPPs which can occur at several reactive centres, are a weak linkage of phosphate ester bond, in a given OPP molecule. It can occur by homogeneous and heterogeneous mechanism, where H_2O and OH^- act as nucleophiles. However, the rate of hydrolysis can be enhanced by dissolved metal ions, for instance, the kinetics of the hydrolysis reaction of chlorpyrifos-methyl and chlorpyrifos undergoes as fast as the concentration of the dissolved copper(II) ion increased [Blanchet and St-George, 2006]. Other effect on hydrolysis process depends on the pH values because some OPPs can susceptible to both base- and acid-catalyzed hydrolysis. For example, diazinon was rapidly hydrolyzed at acidic and basic condition, with half-life of 0.5, 171, and 6 days at pH 3.1, 7.3, and 10.4, respectively, while malathion and chlorpyrifos were slowly hydrolyzed at acidic condition, with half-life 102 days at pH 6.1 [Shemer and Linden, 2006; Zhang and Pehkonen, 1999]. Moreover, the influence of temperature also effect on the degradation rate of OPPs by hydrolysis [Freed *et al.*, 1979].

Photolytic degradation of OPPs can be occurred by absorption of light. Their mechanism can occur by direct photolysis, since the pesticides absorb in UV region and then react with substances in the environment or decompose by itself, or indirect photolysis which is caused by oxygen, hydroxy or peroxy radicals producing by photolysis in humic or inorganic substances [Bavcon *et al.*, 2003]. Chemical oxidation can occur by photo-assisted Fenton reaction ($Fe^{3+}/H_2O_2/UV$ light) [Badawy *et al.*, 2006; Pignatello and Sun, 1995], gamma radiation [Basfar *et al.*, 2007], or X-ray irradiation [Trebše and Arčon, 2003] as well as chemical oxidation of OPPs through

chlorination [Acero *et al.*, 2008] and oxonization [Meijers *et al.*, 1995]. In addition, aqueous suspension of titanium dioxide in combination with UV irradiation for diazinon and fenitrothion [Trebše and Arčon, 2003; Topalov *et al.*, 2003] or combination with solar radiation for triazophos [Aungpradit *et al.*, 2007] were improved the photodegradaton.

Furthermore, the degradation of OPPs can be occurred by biodegradation process because organophosphorus compounds are totally mineralized by the microorganisms which are capable of catalyzing similar metabolic reactions as mammals and plants. This process provides a suitable way for removal of OPP contaminants because microbial enzyme in microorganism can convert the toxic compounds into less toxic compounds [Bhagobaty *et al.*, 2007].

In our work, we monitor the degradation of the OPPs in water via hydrolysis and photolysis process. The degradation products of these pesticides were not observed in this study. However, the major degradation products occurring from hydrolysis and photolysis process of the investigated OPPs in water following as literature data are shown in Table 3.7.

Table 3.7 The major degradation products occurring from hydrolysis and photolysis process of the investigated OPPs in water samples.

Parent compound	Degradation product	Ref.
Diazinon	Diethylphosphate, Diethylthiophosphate, 2-Isopropyl-4-methyl-6-hydroxypyrimidine	Bavcon <i>et al.</i> , 2003; Shemer and Linden, 2006; Basfar <i>et al.</i> , 2007
Fenitrothion	Fenitroxon	Uygun <i>et al.</i> , 2007
Malathion	Malaoxon, Isomalaoxon, Phosphorothioic, Phosphorodithioic	Bavcon <i>et al.</i> , 2003; Uygun <i>et al.</i> , 2007; Bavcon kralj <i>et al.</i> , 2007
Chlorpyrifos	Chlorpyrifos-oxon, Diethyl phosphate, 3,5,6-Trichloro-2-pyridinol	Bavcon kralj <i>et al.</i> , 2007
Triazophos	<i>O,O</i> -diethyl phosphorothioic acid, momoethyl phosphorothioic acid, phosphorothioic acid, 1-phenyl-3-hydroxy-1,2,4-triazole, phenyl semicarbazide, semicarbazide, urea	Rani <i>et al.</i> , 2001; Aungpradit <i>et al.</i> , 2007

CHAPTER IV

MATERIALS AND METHODS

The method of OPP analysis consists of optimization of the instrumentation, derivatization, sample preparation, and GC analysis. The sample preparation steps involved with solvent extraction and clean-up step using various organic solvents and SPE column. The 2,3,4,5,6-pentafluorobenzyl bromide and 1-chloro-3-iodopropane were used as derivatizing agents to increase sensitivity of the analysis. In this chapter, detail of GC instruments, operating GC condition in the analytical measurements, chemicals, reagents and preparation of standard solution are presented.

4.1 Instrument and equipments

4.1.1 Gas chromatography/flame photometric detector

The OPP analysis was performed with a HP 5890 series II gas chromatograph equipped with a flame photometric detector. The detector output was recorded by HP 6890 series integrator.

4.1.2 Analytical balance

A Sartorius AG GÖTTINGEN, Germany was used to weigh samples or reagents in the preparation of all solutions.

4.1.3 Vortex mixer

A VORTEXGENIE 2TM, USA was used to mix solutions and samples.

4.1.4 Magnetic stirrer

A Heidolph MR 3001 stirrer was used to extract samples.

4.1.5 Automatic pipette

Automatic pipette, eppendorf , USA was used for the preparation of solutions.

4.1.6 Rotary vacuum evaporator

BÜCHI evaporator consisting BÜCHI rotavapor R-200, BÜCHI heating bath B-490 and the vacuum pump was used to evaporate solutions for reducing the solvent volume.

4.1.7 Blender

An OTTO blender BE-112 was used to crush and homogenize samples for the extraction.

4.1.8 Nitrogen gas for sample preparation

Nitrogen gas purity 99.9% was used to dry solvent before making up the final volume.

4.2 Chemicals and reagents

All chemicals and reagents used in this work are presented in Table 4.1.

Table 4.1 List of chemicals and reagents, formula, molecular weight and suppliers.

Chemical/Reagent	Formula	M.W. (gmol^{-1})	Supplier
Chlorpyrifos	$\text{C}_9\text{H}_{11}\text{C}_13\text{NO}_3\text{PS}$	350.59	Sigma- Aldrich (USA)
Diazinon	$\text{C}_{12}\text{H}_{21}\text{N}_2\text{O}_3\text{PS}$	304.34	
Fenitrothion solution	$\text{C}_9\text{H}_{12}\text{NO}_5\text{PS}$	277.24	
Malathion solution	$\text{C}_{10}\text{H}_{19}\text{O}_6\text{PS}_2$	330.36	
Triazophos solution	$\text{C}_{12}\text{H}_{16}\text{N}_3\text{O}_3\text{PS}$	313.32	
Octadecyl adsorbent	C_{18}	216.18	Merck (Germany)
Silica gel (70-230 mesh)	SiO_2	60.09	
Sodium chloride	NaCl	58.50	
1-Chloro-3-iodopropane	$\text{C}_3\text{H}_6\text{Cl}$	204.44	Fluka (Switzerland)
Florisol adsorbent (60-100 mesh)	MgO_3Si	100.39	
2,3,4,5,6-Pentafluorobenzyl bromide	$\text{C}_7\text{H}_2\text{BrF}_5$	261.00	
Sodium carbonate anhydrous	Na_2CO_3	105.99	BDH (England)
Acetone (HPLC grade)	CH_3COCH_3	58.08	Lab-Scan (Thailand)
Ethyl acetate (A.R. grade)	$\text{CH}_3\text{COOC}_2\text{H}_5$	88.11	
Hexane (HPLC grade and A.R. grade)	$\text{CH}_3(\text{CH}_2)_4\text{CH}_3$	86.16	
Petroleum ether (40 – 60 °C, A.R. grade)	-	-	
Toluene (A.R. grade)	$\text{C}_6\text{H}_5\text{CH}_3$	92.14	
Acetonitrile (HPLC grade)	CH_3CN	41.05	Fisher Scientific (UK)
n-Heptane (A.R. grade)	C_7H_{16}	100.21	
Methanol (HPLC grade)	CH_3OH	32.04	
Sodium sulfate anhydrous	Na_2SO_4	142.04	Riedel-de Haën (Germany)
Potassium carbonate (98 - 100%, powder)	K_2CO_3	138.21	

4.3 Preparation of standard solutions and reagents

4.3.1 Stock solutions

Stock solutions of each OPP (diazinon, fenitrothion, malathion, chlorpyrifos and triazophos) standard $500 \mu\text{g mL}^{-1}$ were prepared by dissolving 0.0500 g (for solid) or 50 μL (for liquid) of these compounds in hexane and then adjusted final volume to 100 mL in a volumetric flask. These stock solutions were kept in dark bottles and preserved at 4 °C in a refrigerator.

The working solutions of OPPs were prepared by diluting various volumes of the stock solutions with hexane.

4.3.2 Saturated NaCl

Saturated sodium chloride solution was prepared by dissolving NaCl in deionized water until it could not dissolve to give a saturated concentration and kept in a plastic bottle at room temperature.

4.3.3 Glassware cleaning

All glasswares, pipette tips, vials and other materials were carefully cleaned by washing with liquid detergent to remove the residue and then glasswares were rinsed with deionized water and left to dry in a cupboard.

4.4 Procedures and methods

4.4.1 Study of GC condition

Mixtures of OPP standard $10 \mu\text{g mL}^{-1}$ were injected into GC-FPD to optimize the separation condition of GC parameters including injector temperature and temperature program and flow rate of He carrier gas.

4.4.2 Analytical performance of gas chromatography methods

Analytical performances including retention time, linear range, detection limit were investigated in this work. All experiments were carried out in triplicate of the performance and shown in term of percentage relative standard deviation (%RSD) values.

4.4.2.1 Retention time

The repeatability of retention time defined as the average retention time ± 3 standard deviations of retention time by injecting the mixture of OPP standard $10 \mu\text{g mL}^{-1}$ 10 injections.

4.4.2.2 Linear range

Linear ranges of OPPs were studied by injecting standards of diazinon, fenitrothion, malathion, chlorpyrifos and triazophos. The mixtures of OPPs were prepared at the concentration of 0.1, 0.5, 1, 5, 10, 20, 50, 80 and $100 \mu\text{g mL}^{-1}$ in hexane. Correlation coefficient value (r^2) was used to show the reliable linear ranges.

4.4.2.3 Limit of detection

Limit of detection (LOD) was the lowest concentration at the ratio of peak area to noise at the level of 3. In this experiment, LOD was confirmed by injecting the standard solution at the concentration of 3 times for standard deviation of noise peak area.

4.4.3 Compound confirmation

Mixture of OPP standard $10 \mu\text{g mL}^{-1}$ was injected by an Agilent 7683 series injector which equipped with an Agilent 6890N network GC system and an Agilent 5973 network mass selective detector. The Agilent 19091S-433 HP-5S, 3m x 0.25 mm i.d. x 0.25 μm film thickness capillary column was used as a separation column. The flow rate of helium carrier gas was 1 mL min^{-1} (pressure 9.10 psi). The injector temperature and temperature program were used as summarized in Table 5.1. The injection volume was 1 μL in the split mode. The mass spectrometer was electron impact mode at 69.9 eV and scanning from m/z 30 - 500 units. The compound confirmation was studied by comparison between GC-FPD and GC-MS.

4.4.4 Derivatization of OPPs

Mixtures of OPP standard $10 \mu\text{g mL}^{-1}$ of 1 mL, potassium carbonate (or sodium carbonate) and 1-chloro-3-iodopropane (or PFB-Br) were added in the vial containing acetonitrile of 1 mL and mixed with vortex mixing. The mixture was maintained at 65 or 90 °C for 2 or 3 hours. After that, the mixture tubes were allowed to cool at room temperature. The top layer was carefully transferred to a test tube by pasteur pipette without disturbing the sediment at the bottom of the vial. The mixture was evaporated to dryness with nitrogen stream. Then, OPP derivatives were extracted by 1 mL of toluene (for 1-chloro-3-iodopropane) or heptane (for PFB-Br) and then injected to GC instrument. The derivatization conditions of the study were divided into nine conditions, as presented in Table 4.2.

Table 4.2 The derivatization conditions.

Condition	Catalyst		Derivatizing agent		Reaction temperature (°C)	Reaction time (hour)	
	Type	Weight (mg)	Type	Volume (μL)			
1	K ₂ CO ₃	50	-	-	90	3	
2	K ₂ CO ₃	20	1-chloro-3-iodopropane	30	65	2	
3	K ₂ CO ₃	50		30	65	2	
4	K ₂ CO ₃	20		100	65	2	
5	K ₂ CO ₃	20		30	90	2	
6	K ₂ CO ₃	50		100	65	3	
7	K ₂ CO ₃	50		100	90	3	
8	Na ₂ CO ₃	50		100	90	3	
9	K ₂ CO ₃	50		PFB-Br	100	90	3

4.4.5 Sample preparation

4.4.5.1 Study of sample extraction

Four solvents i.e., acetonitrile, acetone, ethyl acetate and hexane were used for extracting OPPs from vegetable samples. In this study, the parameters i.e., solvent mixture and solvent volume were studied to obtain the best recovery of the analytes.

Fresh cabbage samples were chopped and blended by blender. A 50 g portion of homogenized cabbage sample was weighted into a beaker and spiked with the mixture of OPP standard 10 μg mL⁻¹ of 1 mL. The samples were allowed to stand for 1 hour and added with the organic solvent. Then, the sample was stirred for 15 minutes using a magnetic stirrer and filtered through a filter paper by mean of a Buchner funnel. For sample extraction with ethyl acetate and hexane, the filtrate was discarded water in the samples using a separation funnel and then evaporated by rotary evaporation at 45 °C to reduce the solvent volume, while sample

extraction with acetonitrile and acetone, petroleum ether of 150 mL was added to the filtrate for separating the analytes from water content in the extracted before the evaporation. The minimum volume of the extracted was transferred to a test tube and dried by nitrogen stream. The dry residue was redissolved in 0.5 mL hexane. This solution was passed through silica gel-SPE for clean-up before injection to GC system.

4.4.5.2 Study of SPE clean-up

SPE procedures consist of 4 steps which are condition step with the optimum solvent, loading sample step, washing or rinsing step and elution step. In this study, SPE cartridge was packed with the sorbent materials i.e., C₁₈, florisil and silica gel. Acetonitrile, acetone, ethyl acetate and hexane were used as elution solvent for the SPE column. Amount of sorbent materials and elution volume were optimized to obtain the highest recovery of the analytes.

First, C₁₈ cartridge was conditioned with 5.0 mL of methanol, while florisil and silica gel cartridge which were activated at 120 °C overnight were conditioned with 5.0 mL of hexane. Conditioned cartridge should not dry. Second, mixture of OPP standard 100 µg mL⁻¹ of 0.5 mL was loaded into a cartridge. Third, the C₁₈ cartridge was washed by passing distilled water of 10.0 mL through the cartridge, while florisil and silica gel cartridges did not require this step. Finally, elution step, the adsorbed OPPs were eluted by the organic solvents and evaporated to dryness by nitrogen stream. The dry residue was made up the final volume to 1.0 mL with hexane and injected to GC system.

4.4.5.3 Recovery study

Recovery values of OPP extraction from three vegetable samples were obtained from spiking the mixture of OPP standard solution at the concentration of 1, 5, 10, 25, and 50 µg mL⁻¹ of 1 mL in each homogenized vegetable sample (50g) and then followed by the extraction and the SPE clean-up. The recovery

values were calculated by comparing between the obtained concentration and the concentration of spiked standard solution.

4.4.6 Liquid-liquid extraction

Mill acetonitrile procedure was used to compare with SPE procedure. Mixture of OPP standards $100 \mu\text{g mL}^{-1}$ of 50, 100 and 250 μL were spiked in each blended cabbage sample of 50 g in a beaker. Each sample was allowed to stand for 1 hour and extracted by adding acetonitrile (35% v/v) of 200 mL. Then, the mixture was mixed by Heidolph stirrer for 30 min and filtered through a Buchner funnel with a vacuum pump. The filtrate was transferred to a separatory funnel and added with 100 mL of petroleum ether. The funnel was vigorously shaken for 2 minutes. After that, 10 mL of saturated NaCl and 600 mL of deionized water were added into the funnel. The funnel was mixed gently and stood until two phases were separated.

The bottom aqueous phase was discarded and then the upper petroleum ether phase was washed twice with 100 mL of water. Finally, the upper petroleum ether phase was dried with 15 g anhydrous Na_2SO_4 . The extracted was evaporated by the evaporator. The minimum volume of the extracted was transferred to a test tube and was dried by nitrogen stream. The dry residue was made up the final volume to 1.0 mL with hexane and then injected to GC system.

4.4.7 Determination of OPPs in vegetable samples

4.4.7.1 Preparation of spiked vegetable samples

Fresh vegetable samples i.e., cabbage, cucumber and tomato were prepared by spiking the mixture of OPP standard in the samples. Each fresh vegetable sample (2 kg) was sprayed with the mixture of OPP standard and allowed to stand over night. The amount of OPP residues in cabbage, cucumber and tomato were approximately 0.5, 0.1 and 0.1 mg kg^{-1} , respectively. These spiked

vegetable samples were homogenized using the blender before the determination by external standard and standard addition method were performed.

4.4.7.2 External standard method

The homogenized vegetable samples of 50 g were extracted and clean-up before injection to GC system. The calibration curves of OPPs were constructed by plotting between different OPP standard concentrations and peak area of the analyte. The observed peak area of each OPP in the samples was used to compare with the peak area of the OPP standard.

4.4.7.3 Standard addition method

Sample preparation of OPP determination was performed by spiking the concentrations of OPP standard in the range of 5 - 50 $\mu\text{g mL}^{-1}$ in each 50 g of homogenized cabbage sample and in the range of 2 - 10 $\mu\text{g mL}^{-1}$ in each 50 g of homogenized cucumber or tomato samples before the extraction and the clean-up step. The standard addition curves of OPPs were constructed by plotting between spiked OPP standard concentration and peak area of the analyte. The concentration of the OPP residues in vegetable samples obtained from the X-intercept of the linear graph.

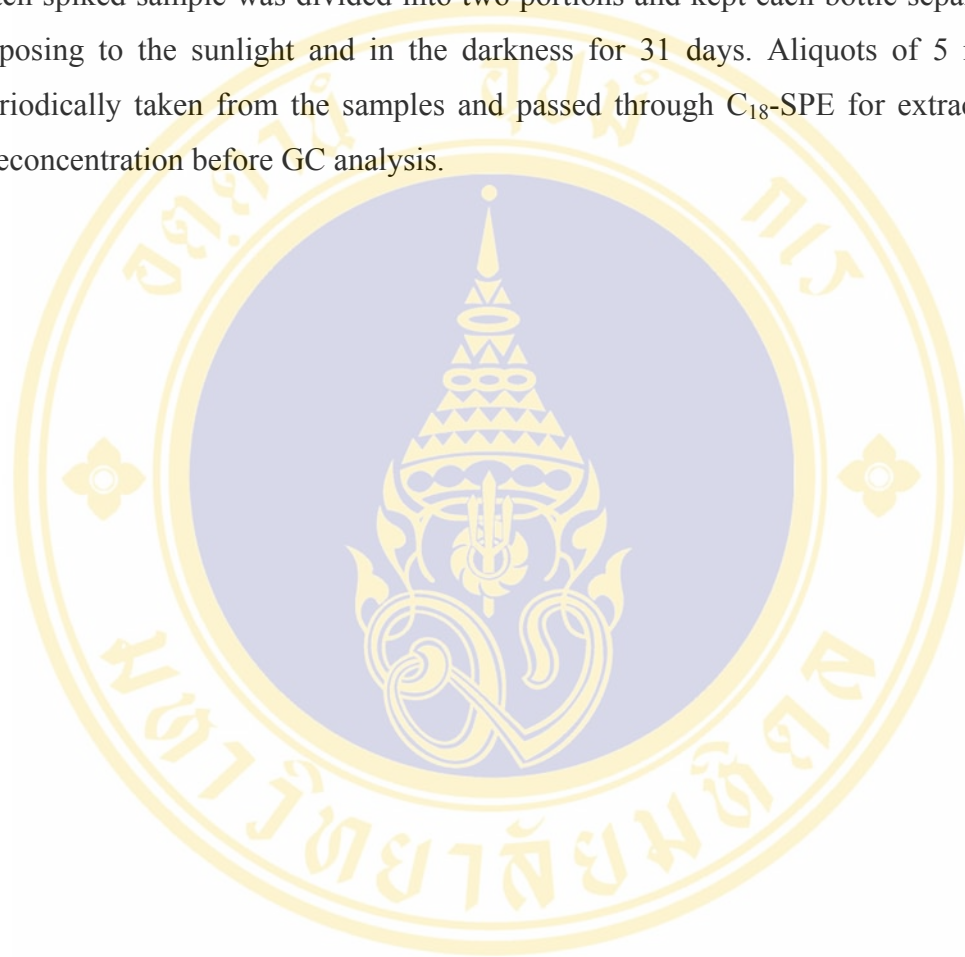
4.4.8 Application in water sample

4.4.8.1 Study of breakthrough volume

The mixture of OPP standard 100 $\mu\text{g mL}^{-1}$ of 100 μL was spiked into various volume of DI water in the range of 5 -1000 mL and loaded into each SPE cartridge. Comparing recoveries of the analytes obtained from various sample volume were evaluated for the suitable sample volume of the SPEs.

4.4.8.2 Study of OPP degradation in water samples

The water samples were prepared by spiking the mixture of OPP standard $500 \mu\text{g mL}^{-1}$ of 1, 2 and 4 mL in each deionized water of 1000 mL. Each spiked sample was divided into two portions and kept each bottle separately for exposing to the sunlight and in the darkness for 31 days. Aliquots of 5 mL were periodically taken from the samples and passed through C_{18} -SPE for extraction and preconcentration before GC analysis.



CHAPTER V

RESULTS AND DISCUSSION

This chapter presents results of the optimum GC condition, the derivatization using 2,3,4,5,6-pentafluorobenzyl bromide and 1-chloro-3-iodopropane, sample preparation (solid-phase extraction and liquid-liquid extraction method). Then, the method was applied for the determination of OPPs in cabbage, cucumber and tomato and also investigation of the OPP degradation in water sample.

5.1 GC optimization

The optimization of GC condition was adjusted for obtaining the best separation and sensitivity of the analytes by varying flow rate of the carrier gas and setting temperature parameters. Helium was used as a carrier gas with a flow rate of 36.7 mLmin^{-1} . Hydrogen and air zero were used as fuel and oxidant gases for igniting flame in the FPD. The response variables used for the optimization were considered by peak area, peak shape, peak resolution and retention time.

Method validation of these parameters were evaluated by checking analytical figures of merit i.e., retention time, linear range and detection limit. The analyte peaks were also confirmed by using GC-MS.

5.1.1 Injector temperature

Injector temperature was studied in the range of $150 - 270 \text{ }^{\circ}\text{C}$ by fixing the detector temperature at $280 \text{ }^{\circ}\text{C}$ and using temperature program as initial temperature at $60 \text{ }^{\circ}\text{C}$ (hold 1 min), final temperature at $240 \text{ }^{\circ}\text{C}$ (rate $20 \text{ }^{\circ}\text{C}/\text{min}$, hold 20

min). The highest peak areas of all analytes were obtained from 250 °C injector temperature because of highest volatilization of the sample, as shown in Figure 5.1.

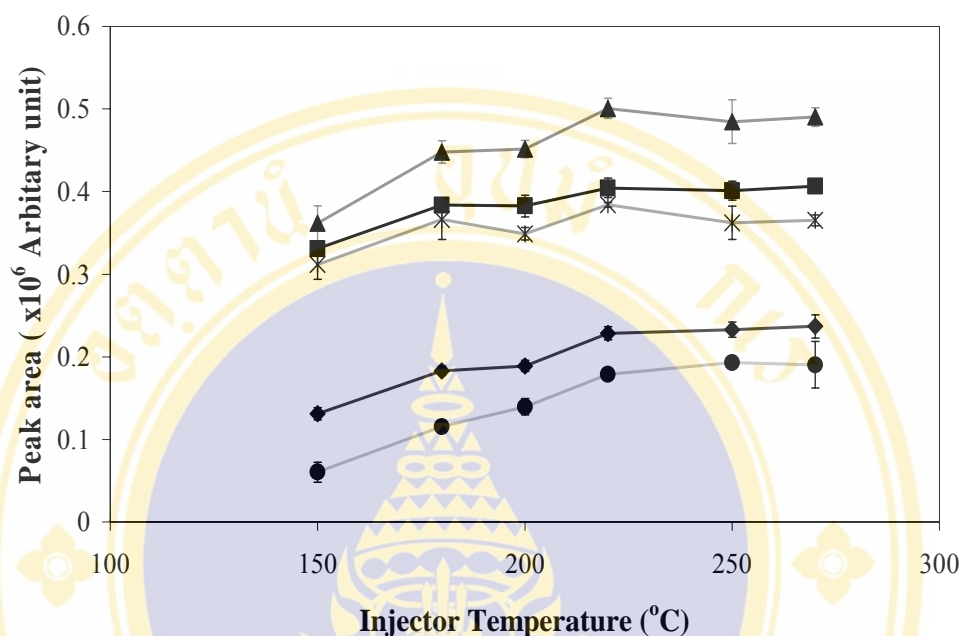


Figure 5.1 Effect of injector temperature on peak area: symbol; diazinon (■), fenitrothion (◆), malathion (▲), chlorpyrifos (*), triazophos (●).

5.1.2 Temperature program

The initial temperature was varied in the range of 50 - 100 °C by fixing the injector temperature at 250 °C and the detector temperature at 280 °C. Increasing of the initial temperature gave shorter retention time of all analytes. The higher temperature produced lower peak area due to partial decomposition of the OPPs. Therefore, the initial temperature of 80 °C was selected because of short retention time and high peak area, as shown in Figure 5.2.

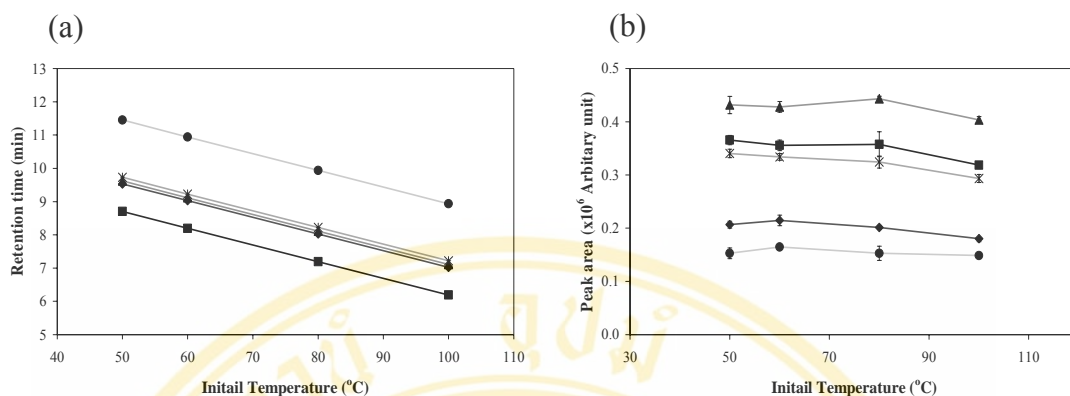


Figure 5.2 Effect of initial temperature on (a) retention time and (b) peak area: symbol; diazinon (■), fenitrothion (◆), malathion (▲), chlorpyrifos (*), triazophos (●).

Then, heating rate was varied in the range of 10 - 40 °C/min using the initial temperature at 80 °C. From Figure 5.3, the higher temperature program rate showed shorter retention time. The rate was selected at 20 °C. The higher temperature rate decreased peak resolutions of fenitrothion, malathion and chlorpyrifos.

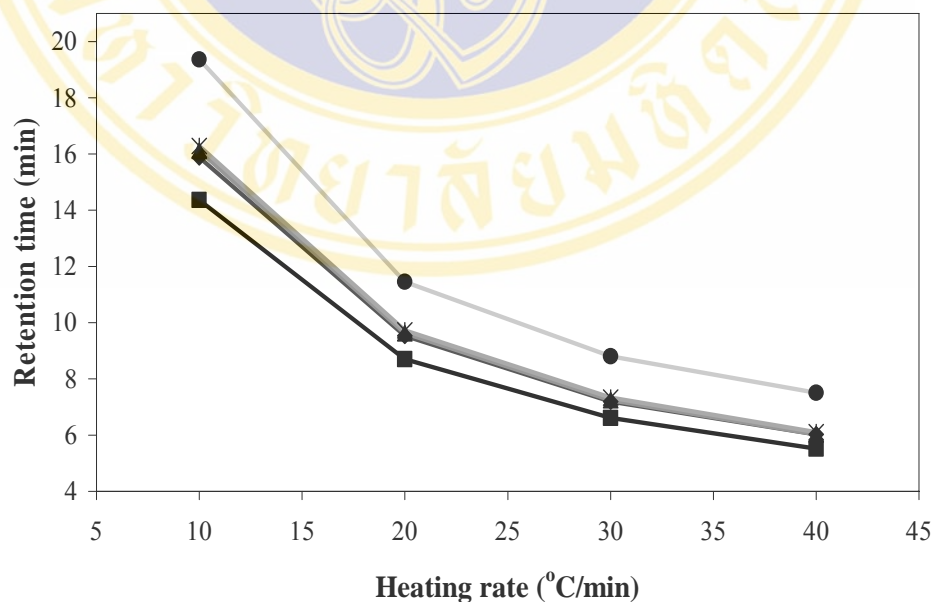


Figure 5.3 Effect of heating rate on retention time: symbol; diazinon (■), fenitrothion (◆), malathion (▲), chlorpyrifos (*), triazophos (●).

Therefore, multistage temperature programming was optimized to improve the separation. The usage of three-stage temperature rate gave shorter retention time than singlestage temperature programme. The chromatograms of both temperature programs are illustrated in Figure 5.4.

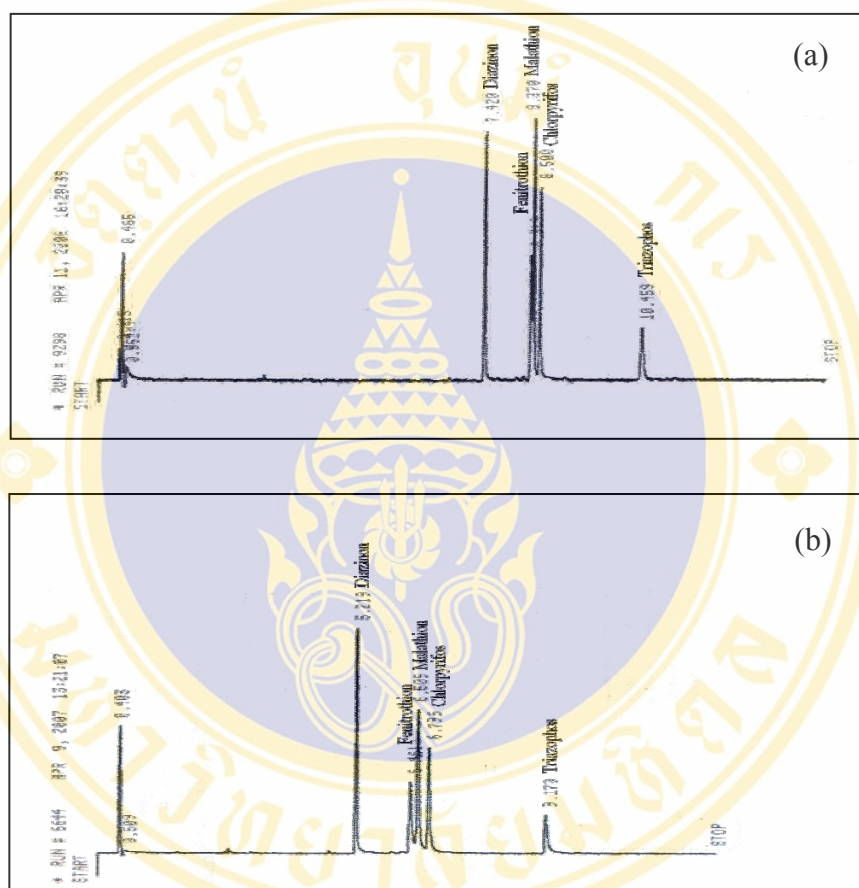


Figure 5.4 The GC chromatograms of OPPs obtained from (a) singlestage and (b) multistage temperature programme.

5.1.3 Carrier gas flow rate

The GC condition that obtained from setting the injector temperature at 250 °C and the detector temperature at 280 °C using temperature program as; initial temperature at 60 °C (hold 1 min), final temperature at 240 °C (rate 20 °C/min, hold 20 min) showed low peak resolutions of fenitrothion, malathion, and chlorpyrifos, as illustrated in Figure 5.5 (c). Then, the flow rate of He carrier gas was adjusted to

increase the peak resolutions of these analytes. Results found that the higher flow rate resulted in shifting retention time, while the peak resolutions of fenitrothion, malathion and chlorpyrifos showed no significant difference. The GC chromatograms derived from using various flow rates of carrier gas are shown in Figure 5.5.

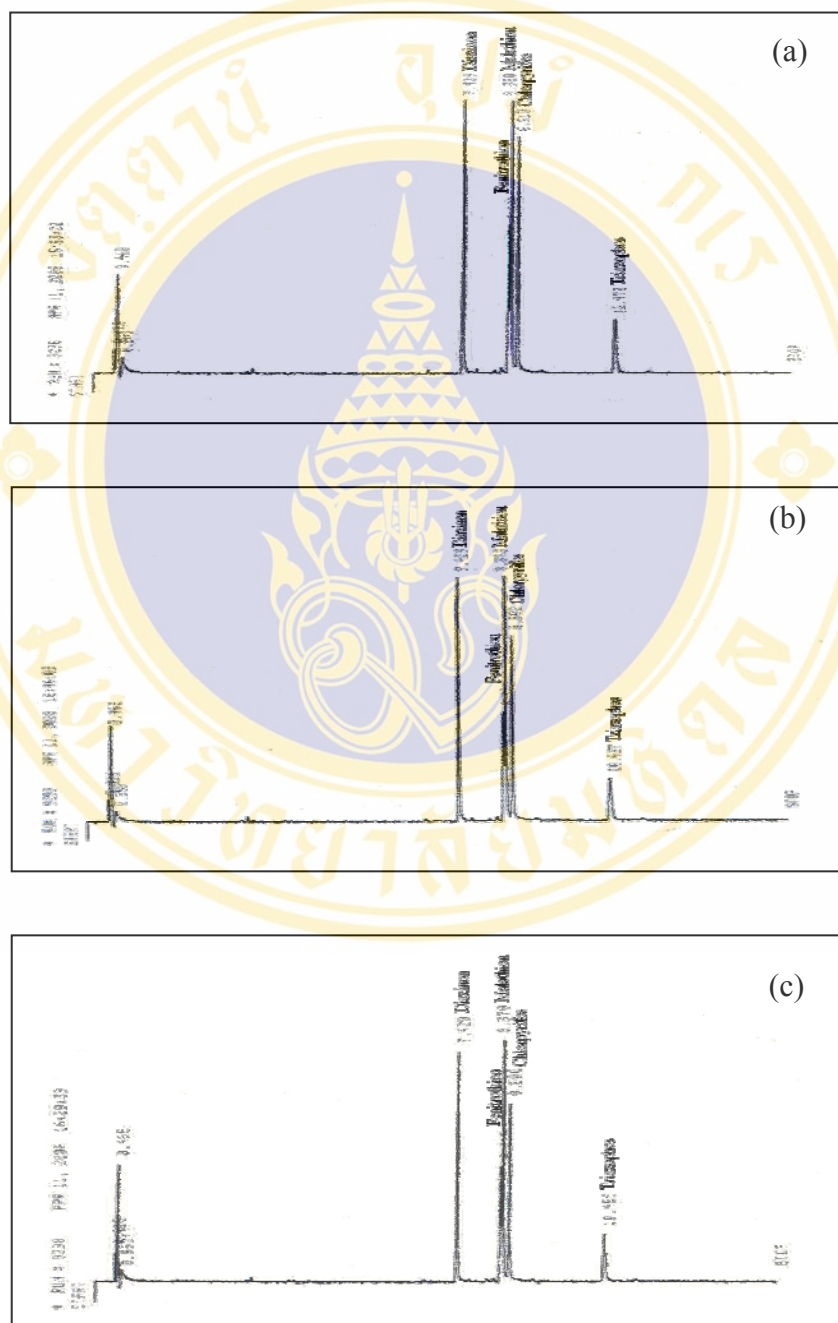


Figure 5.5 Effect of He carrier gas flow rate on separation; (a) 20.0 mLmin⁻¹, (b) 27.7 mLmin⁻¹ and (c) 36.7 mLmin⁻¹.

The optimum conditions of GC instrument for OPP compound analysis are described in Table 5.1.

Table 5.1 The GC conditions.

Parameter	Condition
Separation Column	HP-5 (5% crosslinked Methyl Phenyl Silicone) 25 m x 0.2 mm i.d. x 0.52 μm film thickness
Carrier gas	He 36.7 mLmin ⁻¹ at 80 °C
Fuel gas	H ₂ 150.5 mLmin ⁻¹
Oxidant gas	Air zero 75 mLmin ⁻¹
Make up gas	N ₂
Injection volume	1 μL
Injection mode	splitless mode
Injection temperature	250 °C
Detector temperature	280 °C
Temperature program	80 °C (1 min), rate 40 °C/min to 200 °C (2.5 min), rate 20 °C/min to 210 °C, rate 40 °C to 240 °C, maintained for 5 min

5.2 Analytical performance

Method validation confirms that the performing method is suitable and reliable for the analysis. All quantity parameters obtained from the analytical procedures were quantified by GC-FPD using the optimum conditions. The analytical performance consists of retention time (t_R), linear range, correlation coefficient, limit of detection (LOD) and percentage relative standard deviation (%RSD) of peak area which are concluded in Table 5.2.

5.2.1 Retention time

The retention times of diazinon, fenitrothion, malathion, chlorpyrifos and triazophos were 5.21 ± 0.01 , 6.50 ± 0.01 , 6.60 ± 0.01 , 6.84 ± 0.01 , 9.12 ± 0.01 min, respectively. The results showed that the standard deviations of the retention times ($n = 10$) were less than 0.02% for all analytes. The high repeatability of the retention times confirmed the identification of the analyte peaks on the chromatogram.

5.2.2 Linear range

The calibration curves of OPPs were plotted between OPP standard concentration and peak area of the analyte, as shown in Figure 5.6.

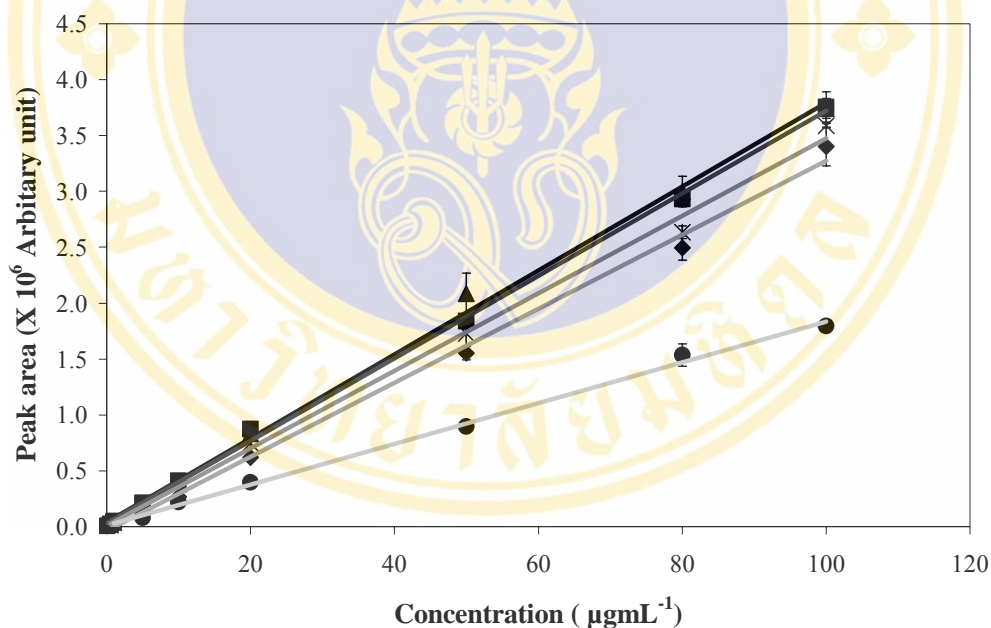


Figure 5.6 The calibration curves of diazinon, fenitrothion, malathion, chlorpyrifos and triazophos: filled symbols; diazinon (■), fenitrothion (◆), malathion (▲), chlorpyrifos (*), triazophos (●).

Linear ranges of diazinon and triazophos were in the range of 0.1 - 100 μgmL^{-1} , whereas the linear ranges of fenitrothion, malathion and chlorpyrifos were in the range of 0.5 - 100 μgmL^{-1} . The sensitivity of all OPPs which was considered

from slope of the linear equation, showed no difference, except for triazophos showed lower value than the other pesticides. Correlation coefficient (r^2) of the studied compounds gave high linearity with these values more than 0.9900.

5.2.3 Limit of detection

The limit of detection (LOD) was calculated by using the signal to noise ratio at 3 S/N. LODs of diazinon, fenitrothion, malathion, chlorpyrifos and triazophos were 0.08, 0.10, 0.04, 0.07 and 0.50 $\mu\text{g mL}^{-1}$, respectively.

Table 5.2 Analytical performances of OPPs.

OPP	t_R (min)	Linear range ($\mu\text{g mL}^{-1}$)	Linear equation	R^2	LOD ($\mu\text{g mL}^{-1}$)	%RSD* peak area
Diazinon	5.2	0.1 - 100	$y = 36903x + 29103$	0.9989	0.08	3.97
Fenitrothion	6.5	0.5 - 100	$y = 33111x - 38079$	0.9967	0.10	4.31
Malathion	6.6	0.5 - 100	$y = 37525x + 41664$	0.9974	0.04	2.75
Chlorpyrifos	6.8	0.5 - 100	$y = 34654x + 8884$	0.9971	0.07	1.77
Triazophos	9.1	1.0 - 100	$y = 18248x + 11878$	0.9973	0.50	4.31

* %RSD = Relative standard deviation (n=3) at concentration 5 $\mu\text{g mL}^{-1}$

5.2.4 Compound confirmation

GC-MS analysis was used to validate the method which was obtained from GC-FPD analysis. Column type and the GC temperature program used in GC-MS were the same as used in GC-FPD. The GC-MS condition was used as shown in the section 4.4.3. The chromatogram of OPPs obtained from GC-MS had the same pattern as obtained from GC-FPD analysis, as shown in Figure 5.7.

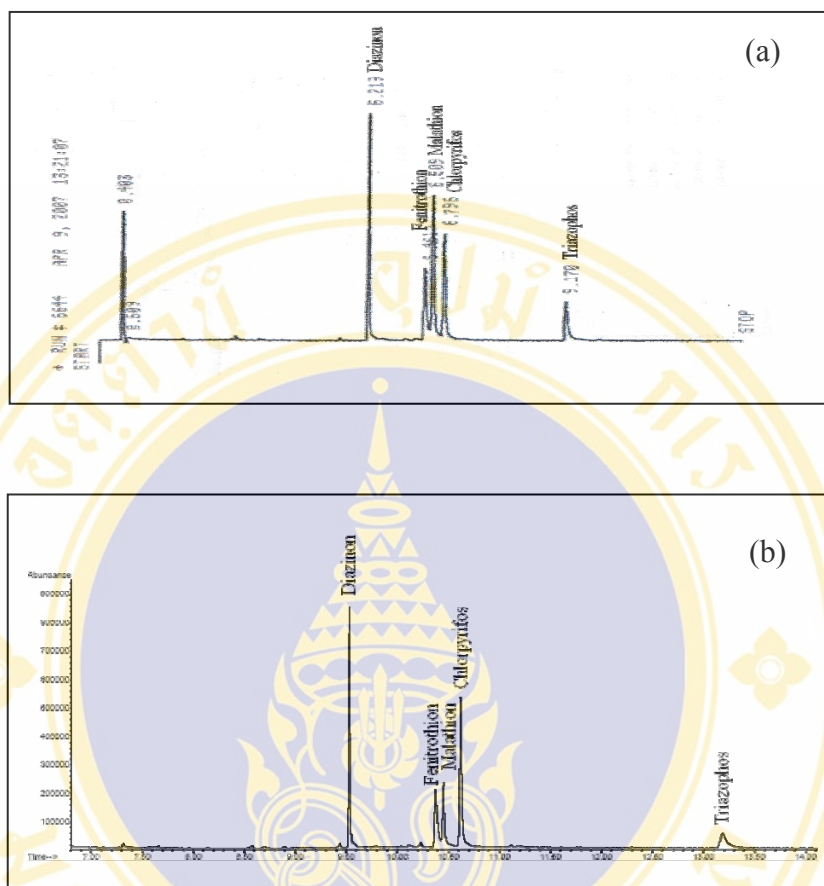


Figure 5.7 The GC chromatograms of OPPs when the OPP standard $10 \mu\text{g mL}^{-1}$ were injected to (a) GC-FPD and (b) GC-MS.

The main fragments obtained and their relative abundances are shown in Table 5.3 [Lacorte *et al.*, 1993]. Mass spectra are shown in Appendix A. The study of main fragments will help us to confirm the developed method of the analysis of OPP compounds.

Table 5.3 Fragment ions of OPPs obtained from GC-MS.

OPP	M.W. (gmol^{-1})	t_R (min)	m/z [main ion] ⁺
Diazinon	304	9.53	93 $[(\text{CH}_3\text{O})_2\text{P}]^+$ 137 $[\text{OH}(\text{C}_4\text{HN}_2)(\text{CH}(\text{CH}_3)_2)]^+$ 152 $[\text{OH}(\text{C}_4\text{N}_2)\text{CH}_3(\text{CH}(\text{CH}_3)_2)]^+$ 179 $[(\text{C}_2\text{H}_5\text{O})(\text{C}_4\text{N}_2)(\text{CH}_3)(\text{CH}(\text{CH}_3)_2)]^+$ 199 $[(\text{CH}(\text{CH}_3)_2)\text{O}(\text{PX}(\text{OC}_2\text{H}_5)(\text{OCH}_3))]^+$ 304 $[\text{M}]^+$
Fenitrothion	277	10.37	79 $[\text{CH}_3\text{OPOH}]^+$ 93 $[(\text{CH}_3\text{O})_2\text{P}]^+$ 109 $[(\text{CH}_3\text{O})_2\text{PO}]^+$ 125 $[(\text{CH}_3\text{O})_2\text{PS}]^+$ 260 $[\text{M-OH}]^+$ 277 $[\text{M}]^+$
Malathion	330	10.45	93 $[(\text{CH}_3\text{O})_2\text{P}]^+$ 125 $[(\text{CH}_3\text{O})_2\text{PS}]^+$ 127 $[173-\text{C}_2\text{H}_5\text{O}]^+$ 158 $[173-\text{CH}_3]^+$ 173 $[(\text{C}_2\text{H}_5\text{OOC})\text{CH}_2(\text{CHCOOC}_2\text{H}_5)]^+$
Chlorpyrifos	350	10.61	97 $[(\text{C}_2\text{H}_5\text{O})\text{PSH}]^+$ 125 $[(\text{CH}_3\text{O})_2\text{PS}]^+$ 197 $[\text{H}_2\text{OC}_5\text{Cl}_3\text{N}]^+$ 258 $[\text{M}-(\text{C}_2\text{H}_4)_2-\text{HCl}]^+$ 314 $[\text{M-HCl}]^+$
Triazophos	313	13.19	77 $[\text{C}_2\text{H}_5\text{OPH}]^+$ 97 $[(\text{HO})_2\text{PS}]^+$ 161 $[(\text{C}_6\text{H}_6)(\text{C}_2\text{N}_3)\text{OH}]^+$ 172 $[(\text{C}_2\text{H}_5\text{O})_2\text{POHSH}]^+$ 208 $[(\text{C}_2\text{N}_3\text{H})\text{OPS}(\text{C}_2\text{H}_5\text{O})(\text{OH})]^+$ 257 $[(\text{C}_6\text{H}_6)(\text{C}_2\text{N}_3)\text{O}]\text{PS}(\text{OH})_2]^+$ 285 $[(\text{C}_6\text{H}_6)(\text{C}_2\text{N}_3)\text{O}]\text{PS}(\text{OC}_2\text{H}_5)(\text{OH})]^+$ 313 $[\text{M}]^+$

5.3 The derivatization of OPPs

The derivatization step was performed to increase the sensitivity of the analysis. In this part, we use 2,3,4,5,6-pentafluorobenzyl bromide (PFB-Br) and 1-chloro-3-iodopropane which could react with OPPs to form ester derivatives, as illustrated in Figure 5.8. Derivative products may or may not be sensitive to the detector and enhance the sensitivity of the analysis. The derivative products may be employed to determine in other detectors such as flame ionization detection (FID) for comparing the method.

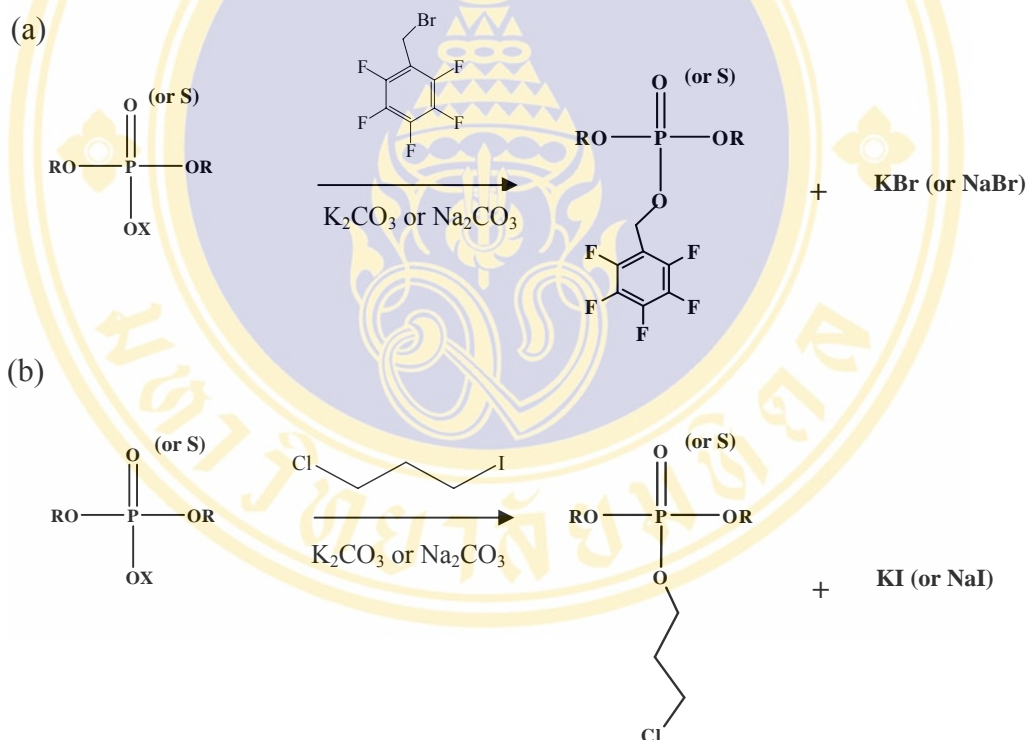


Figure 5.8 The derivatization reactions result in the formation of (a) chloropropyl ester and (b) pentafluorobenzyl ester of OPPs where X = living group, R = alkyl group.

The derivatization conditions were carried out by following the report of Hemakanthi De Alwis and Bardarov [Hemakanthi De Alwis *et. al.*, 2006; Bardarov and Mitewa, 1989]. Ester derivatives of both derivatizing agents reacted with the OPP

metabolites which consisted of -OH group as living group in their molecules i.e., dimethyl phosphate, dimethyl thiophosphate, dimethyl dithiophosphate, diethyl phosphate, diethyl thiophosphate and diethyl dithiophosphate. Pentafluorobenzyl and chloropropyl ester derivatives can be detected by several detectors such as flame photometric detector, flame ionization detector or mass spectrometry. These derivatizing agents were applied to derivatize the OPP analytes. The mixture of OPP standard $10 \mu\text{g mL}^{-1}$ was used for the derivatization. Na_2CO_3 or K_2CO_3 in acetonitrile were added at the reaction temperature of 65°C and 90°C for 2 - 3 hours. The derivatization conditions were optimized as shown in Table 4.2.

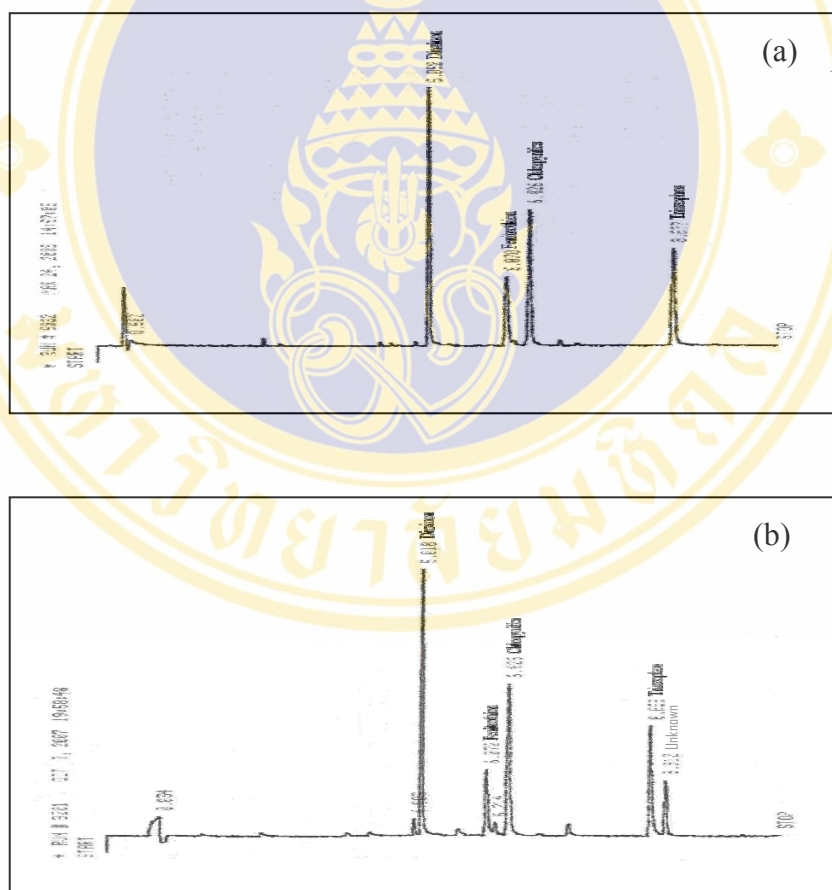


Figure 5.9 The GC chromatograms of OPPs obtained from (a) without and (b) with the addition of 1-chloro-3-iodopropane.

Figure 5.9a showed that the malathion peak ($t_R = 6.2$) was not observed on the chromatogram when added only K_2CO_3 without adding 1-chloro-3-iodopropane as derivatizing agent (condition 1). It can be deduced that salt of malathion may be occurred by reacting with K_2CO_3 and then precipitated, as illustrated in Figure 5.10(2). Consequently, an unknown peak was observed at 8.912 minute when the derivatizing agent was added, as shown in Figure 5.9b. This peak was a derivative of malathion. However, OPP derivatives of diazinon, fenitrothion, chlopyrifos and triazophos were not observed using 1-chloro-3-iodopropane as derivatizing agent. The derivatization reaction of malathion by adding 1-chloro-3-iodopropane as derivatizing agent was presented in Figure 5.10(3).

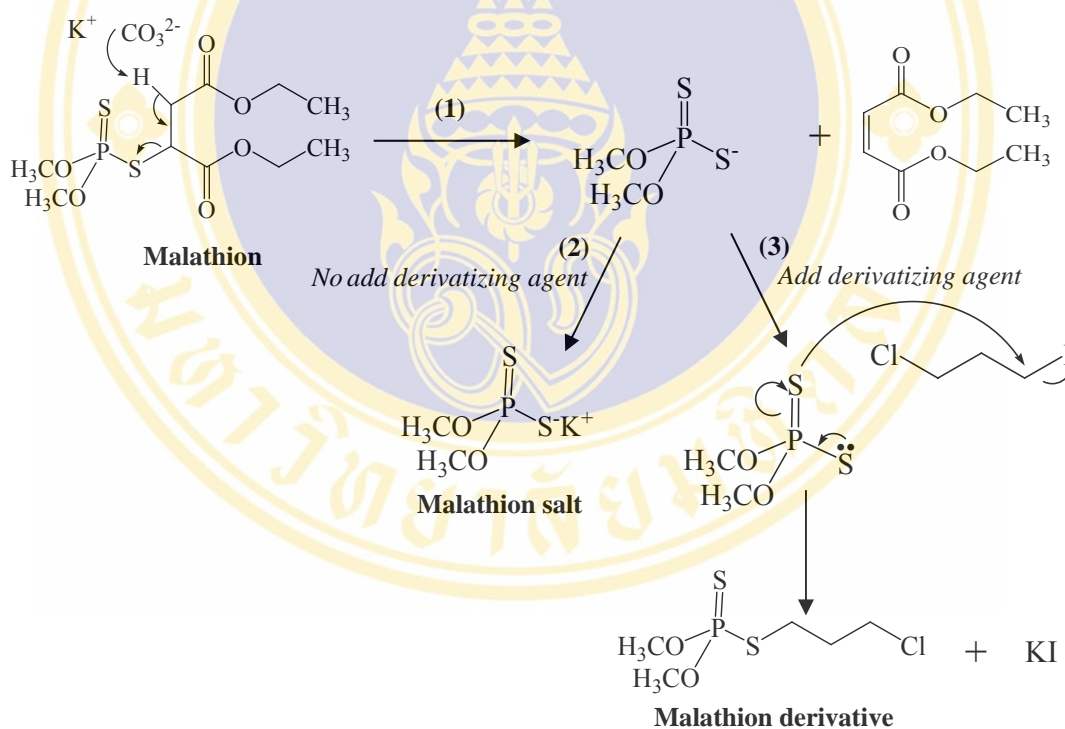


Figure 5.10 The derivatization reaction of malathion by using 1-chloro-3-iodopropane as derivatizing agent.

The derivative product of malathion was not occurred by condition 8 and 9, as presented in Figure 5.11. It can be indicated that more ionic property of Na_2CO_3 caused less solubility compared with K_2CO_3 . In addition, PFB-Br could not form

derivative with the malathion in condition 9 because of its steric effect. Moreover, peak areas of their derivatives were lower than the peak area of standard malathion. Therefore, the chloropropyl derivatives of malathion could not increase the sensitivity of OPPs using flame photometric detection under the studied conditions.

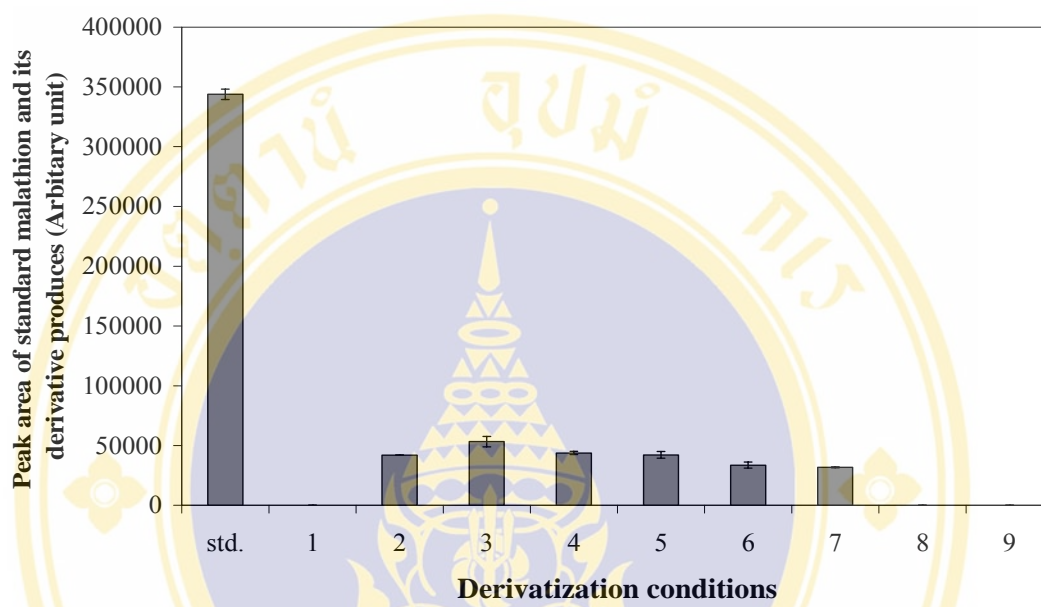


Figure 5.11 Peak areas of standard malathion and its derivative products obtained from the derivatization step following as condition 1-9.

The derivatization step was not necessary for these analytes when detected by FPD. Phosphorus and sulfur atoms in their molecules are sensitive to this detector which can determine the concentration of the analytes in the ppb level.

5.4 Sample preparation

Sample preparation for determining OPP residues in vegetable samples consists of sample extraction and clean-up steps. The extraction of pesticide residues depends on the polarity of pesticides as well as on the type of sample matrix.

The vegetable extracts usually compose of various interferences such as lipid, carbohydrate, pigment and a variety of polar and non-polar substances. The matrices

can interfere the analyte peak and accumulate in the injector and the front end of the capillary GC column that may increase the retention time, as well as resulting in the abnormally high recovery of certain analyte [Lal *et al.*, 2008; Hammarstrand, 1976]. Therefore, a clean-up step is necessary for removing the interferences in the extracted before GC analysis.

In this work, the sample preparation involved with solvent extraction and clean-up step using various organic solvents and SPE clean-up column. Then, the optimum condition of the sample preparation was compared with LLE method for determination of OPP residues in vegetable samples.

5.4.1 Sample extraction

Sample extraction was performed to extract target compounds from samples. In this section, the extraction parameters including type of solvent, ratio of the solvent mixture and extraction solvent volume were optimized to obtain the best efficiency of the analytes.

5.4.1.1 Type of solvent

The selection of the solvent for extracting OPPs was based on several criteria. The major consideration of solvent depends on the polarity of pesticides. The polarity of solvent must be sufficient to extract most of pesticides from the matrix samples [Patel *et al.*, 2004; Tekel and Hatrík, 1996; Cai *et al.*, 1995; Hernández Hernández *et al.*, 1990]. The solvent must extract all pesticides and can be rapidly evaporated in a large volume. The solvents used in this study were hexane, ethyl acetate, acetone and acetonitrile with the sample volume of 150 mL. The polarity indices of hexane, ethyl acetate, acetone and acetonitrile are 0, 4.3, 5.4 and 6.2, respectively. The water solubilities at 20 °C of diazinon, fenitrothion, malathion, chlorpyrifos and triazophos are 40, 30, 145, 0.73 and 40 mgL⁻¹ [PAN pesticide database, IPCSINTOX databank, World Health Organization]. The percentage recoveries of OPPs obtained from the extraction with four solvents are shown in

Figure 5.12 and in Appendix B (Table B1). The extraction with the mixture of ethyl acetate and hexane (1:1 v/v) gave the highest percentage recoveries for extracting all OPPs from cabbage sample (50 g) in the range of 71 - 78% with R.S.D < 5%.

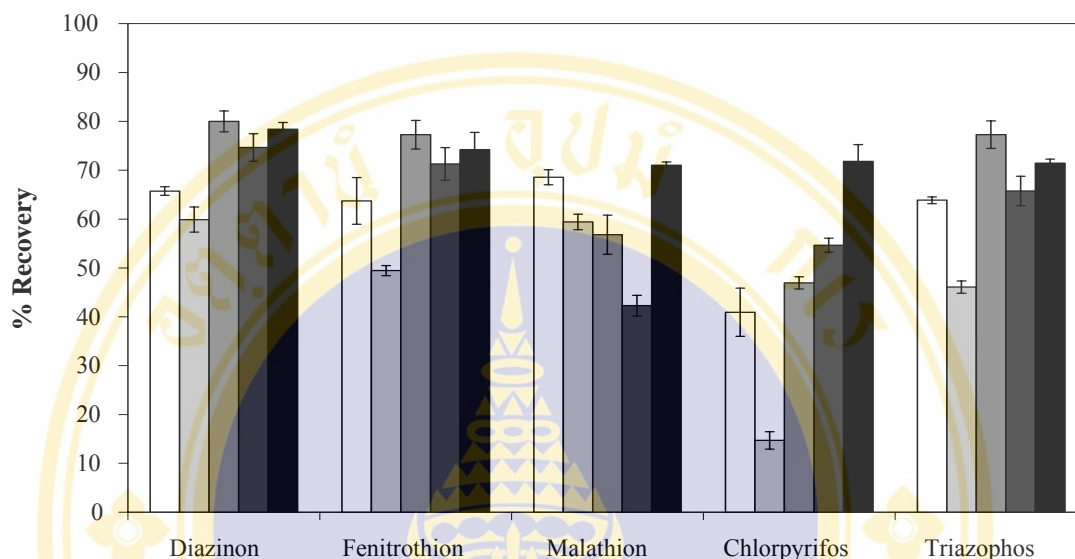


Figure 5.12 Percentage recoveries of solvent extraction obtained from varying organic solvent: symbol; acetonitrile (□), acetone (◻), ethyl acetate (◻), hexane (◻), ethyl acetate:hexane (1:1 v/v) (◼).

In addition, the extraction of OPPs with acetonitrile and acetone gave the lower percentage recoveries than ethyl acetate and hexane. Acetonitrile and acetone are able miscible with water in the extract and subsequently need to separate with immiscible solvent before the evaporation. This caused the loss of the analytes due to many preparation steps. Ethyl acetate extracted all OPPs as efficiency as hexane, but the extraction with both solvents gave low percentage recoveries of chlorpyrifos (47 - 55%). Then, the mixture of ethyl acetate and hexane (1:1 v/v) was used to adjust the suitable polarity of the solvent which increased the efficiency of the extraction with higher recovery, especially for chlorpyrifos (>70%).

5.4.1.2 Mixture of solvent

Consequently, the ratio of ethyl acetate and hexane was adjusted to gain the suitable polarity of the solvent extraction. The percentage recoveries are shown in Figure 5.13 and in Appendix B (Table B2). The ratio of ethyl acetate and hexane of 1:1 gave the highest percentage recoveries for the analytes in the range of 71 – 78% with R.S.D < 5%.

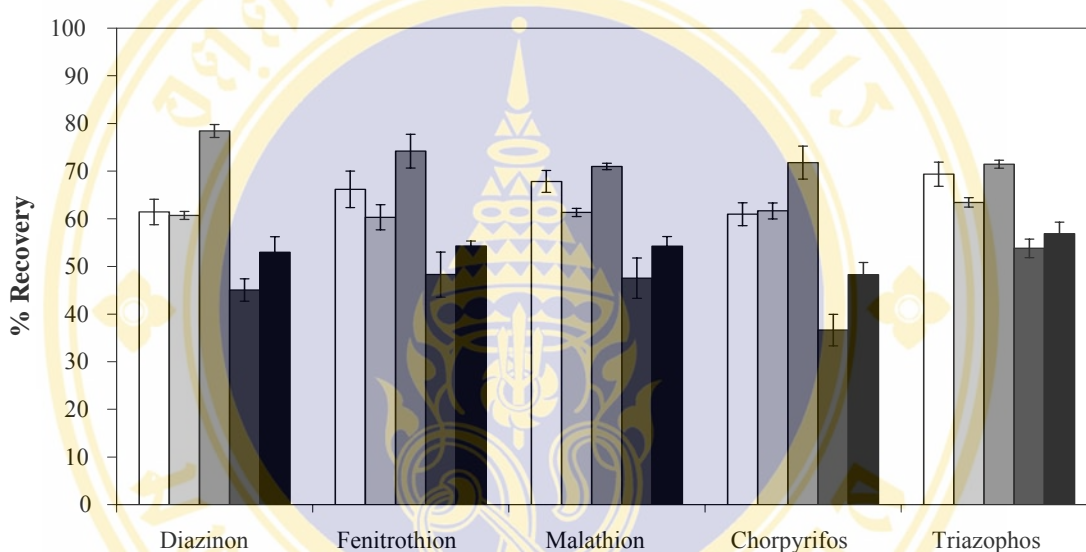


Figure 5.13 Percentage recoveries of adjusting the ratio of ethyl acetate and hexane mixture (v/v): symbol; 1:3 (□), 2:3 (▤), 3:3 (▥), 3:2 (▧), 3:1 (▨).

5.4.1.3 Extraction solvent volume

The solvent volume was studied to investigate the influence on the extraction efficiency. Large volume of extractant was used for extracting large amount of sample. Our preliminary study, 150 mL of the mixture of ethyl acetate and hexane (1:1 v/v) was used for extracting the sample of 50 g. Then, the solvent volume was varied in the range of 50 - 300 mL, as illustrated in Figure 5.13 and in Appendix B (Table B3). The percentage recoveries of all OPPs were not significantly different at the solvent volume of 100 mL (70 - 81%), while less extraction volume showed the lower recovery values for all analytes (61 - 65%).

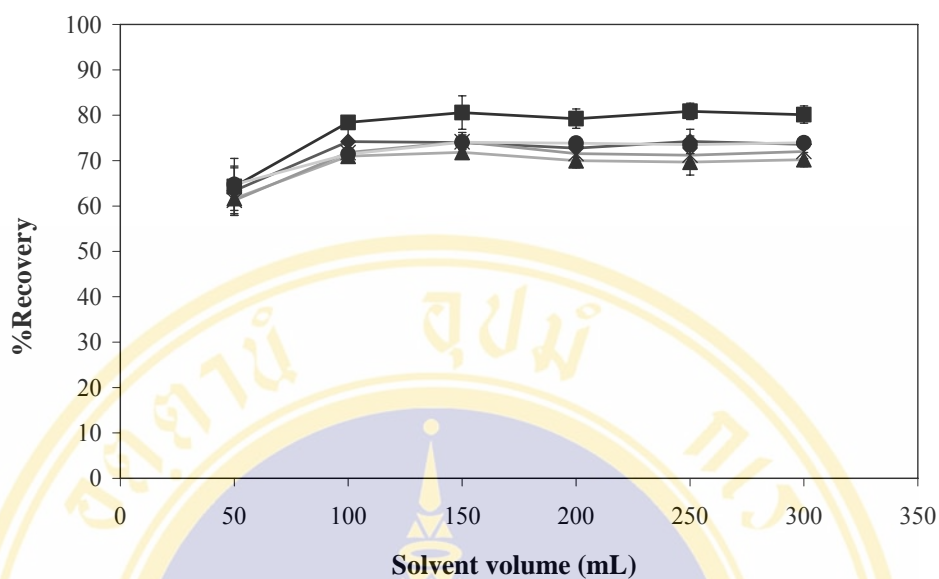


Figure 5.14 Effect of solvent volume on the extraction of cabbage sample: symbol; diazinon (■), fenitrothion (◆), malathion (▲), chlorpyrifos (*), triazophos (●).

In the extraction step, the mixture of ethyl acetate and hexane (1:1 v/v) of 100 mL was selected for the extraction of OPPs. This solvent gave the highest recoveries of the analytes and the usage of petroleum ether for separating the analytes from water in the vegetable extract before the evaporation was not necessary because the selected solvent was miscible with water.

5.4.2 SPE Clean-up of the extract

The clean-up step was used for removal of co-extract including lipid, carbohydrate, pigment and a variety of polar and non-polar substances in vegetable sample which can interfere with the analytes [Hammarstrand, 1976]. In this work, sample clean-up was accomplished by solid-phase extraction using octadecyl (C₁₈), florisil and silica gel column. The parameters of each sorbent including weight of sorbent, elution solvent and elution volume were optimized using the mixture of OPP standard 100 µg mL⁻¹. Percentage recovery was obtained by comparing between peak area of eluate fraction and OPP standard.

5.4.2.1 Weight of sorbent

Sorbent materials of the SPE clean-up column were varied in the range of 100 - 500 mg for loading the extracted sample of 0.5 mL. Then, the adsorbed OPPs were eluted by using 20 mL of acetone as an elution solvent.

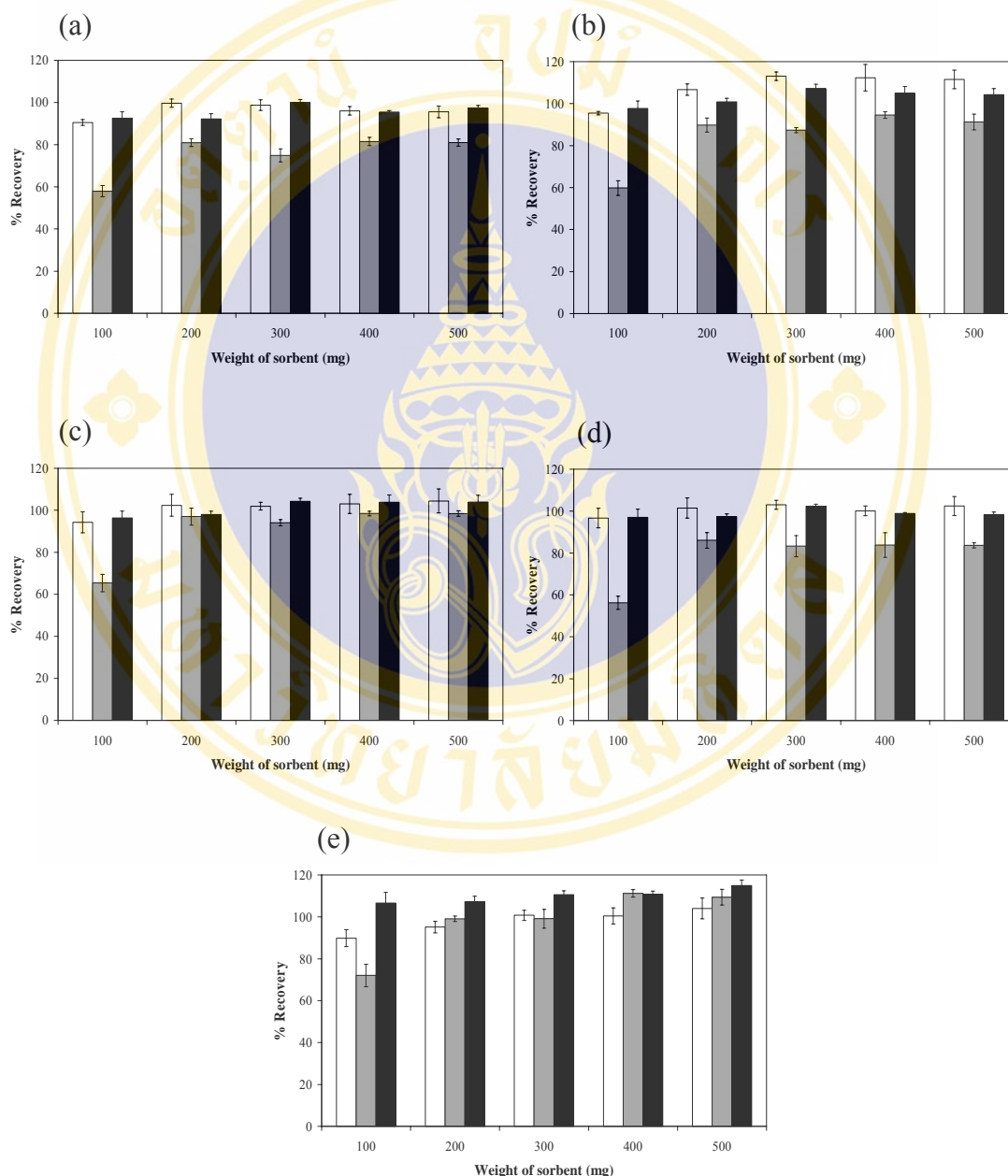


Figure 5.15 Percentage recoveries of (a) diazinon, (b) fenitrothion, (c) malathion, (d) chlorpyrifos and (e) triazophos obtained from various weights of sorbents: symbol; C₁₈-SPE(□), florisil-SPE (■), silica gel-SPE (■).

Loading capacity is an important parameter for the SPE clean-up column. The increasing of the sample loading is related to the increasing of the sorbent mass. According to Figure 5.15, the suitable weights of C₁₈, florisil and silica gel were 300, 400 and 300 mg, respectively. These weights provided the highest percentage recoveries of all analytes. Less amount of sorbent gave lower recovery values because of the analyte desorption occurring. The sample loading capacity of low amount of sorbent was not sufficient to retain all analytes in the SPE cartridge. The recovery data of OPPs obtained from varying the sorbent weight are presented in Appendix B (Table B4 - B6).

5.4.2.2 Elution solvents

Solvents used in the study were hexane, ethyl acetate, acetone and acetonitrile to elute the adsorbed OPPs by using the elution volume of 20 mL. Amount of C₁₈, florisil and silica gel were 300, 400 and 300 mg, respectively.

Figure 5.16 showed that acetone and ethyl acetate gave the highest percentage recoveries in the range of 85 - 100% with R.S.D < 10% using C₁₈-SPE. However, acetone was selected as an elution solvent because it has a lower boiling point and higher vapor pressure compared to ethyl acetate. In addition, acetone was also used for florisil-SPE while acetonitrile was suitable for silica gel-SPE. It gave the best percentage recoveries of the analytes. Percentage recoveries of the analytes using acetone for florisil-SPE and acetonitrile for silica gel-SPE were in the range of 86 - 105% and 90 - 110%, respectively with R.S.D < 10%. The recovery data of OPPs obtained from the study of elution solvent are presented in Appendix B (Table B7 - B9)

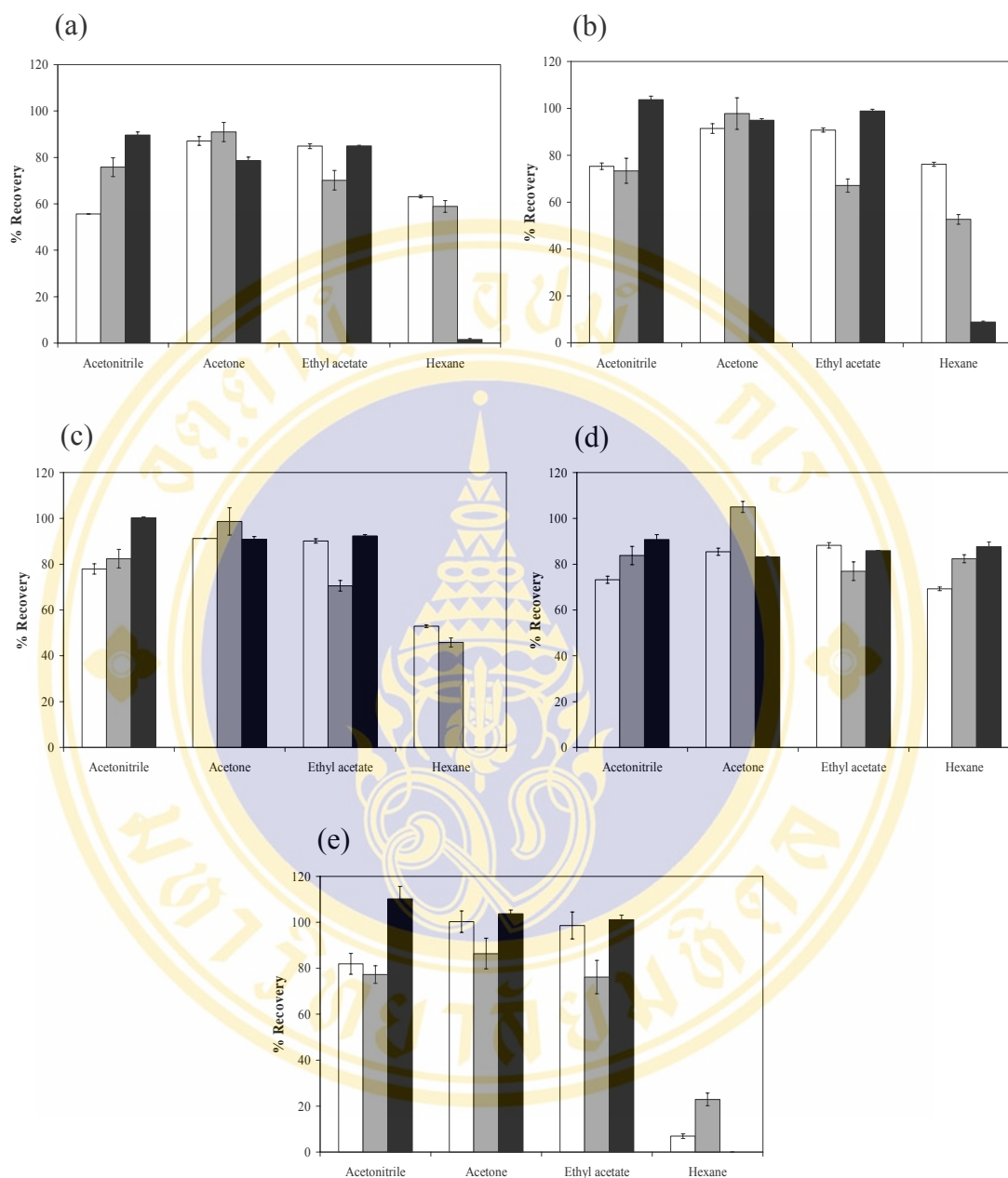


Figure 5.16 Percentage recoveries of (a) diazinon, (b) fenitrothion, (c) malathion, (d) chlorpyrifos and (e) triazophos obtained from various elution solvents: symbol; C₁₈-SPE (□), florisisil-SPE (■), silica gel-SPE (■).

5.4.2.3 Elution volume

The elution volume was performed to obtain the optimum volume for eluting the adsorbed analytes. Amount of C₁₈, florisisil and silica gel used in

this study were 300, 400 and 300 mg, respectively. Acetone was used as elution solvent for C₁₈-SPE and florisil-SPE, while acetonitrile was used for silica gel-SPE.

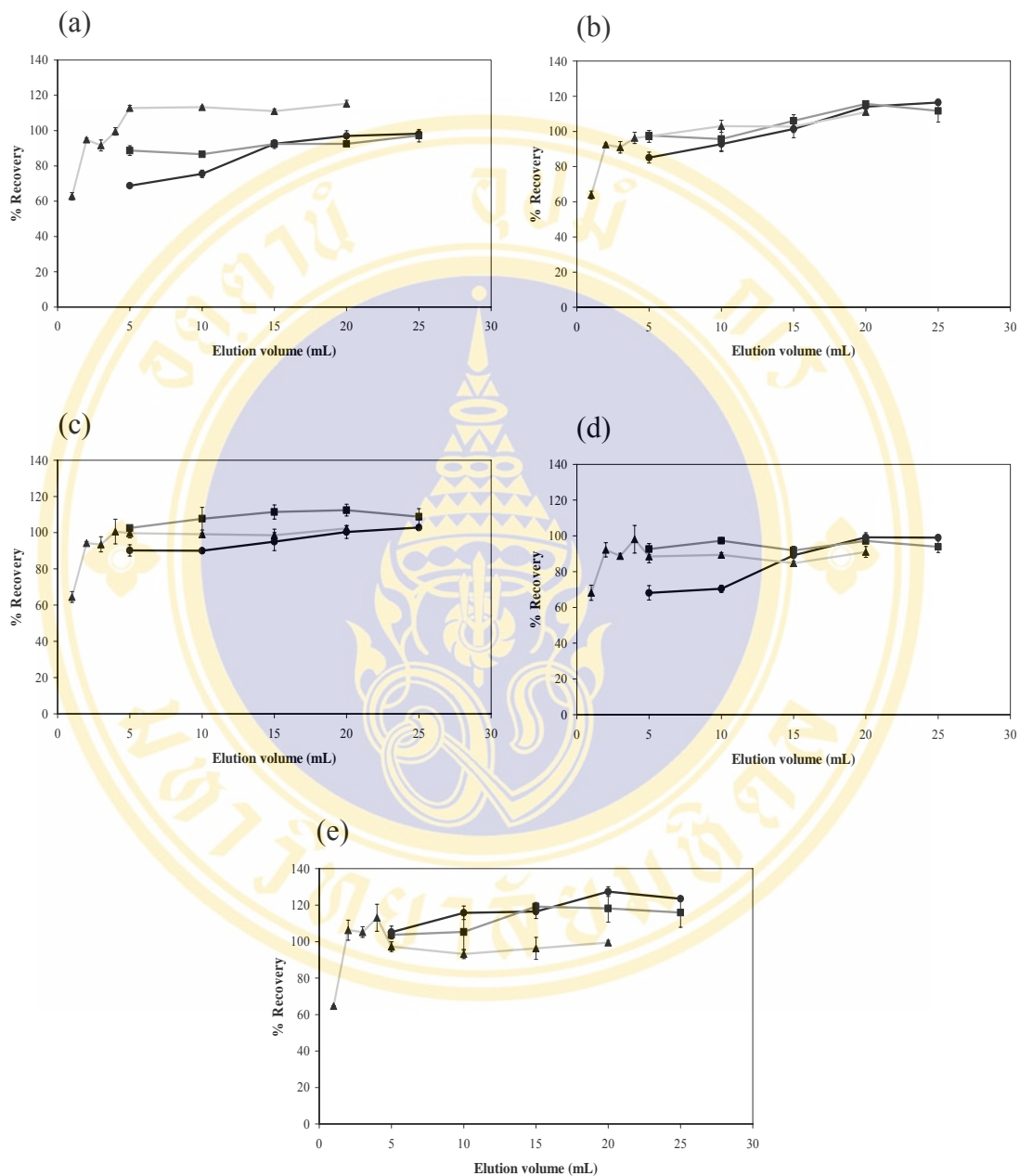


Figure 5.17 Percentage recoveries of (a) diazinon, (b) fenitrothion, (c) malathion, (d) chlorpyrifos and (e) triazophos obtained from various elution volumes: symbol; C₁₈-SPE (□), florisil-SPE (■), silica gel-SPE (■).

The elution volumes for C₁₈-SPE and florisil-SPE were studied in the range of 5 - 20 mL, while the elution volumes for silica gel-SPE were

studied in the range of 1 - 20 mL. From Figure 5.17 found that the percentage recoveries of OPPs increased with the increasing of the elution volume. The suitable elution volumes of C₁₈, florisil and silica gel were 20, 20 and 4 mL, respectively. Higher elution volume showed no significant difference between the recovery values (> 92%). The recovery data of OPPs obtained from the study of elution volume are shown in Appendix B (Table B10 - B12).

The optimum parameters consisted of sample volume, weight of sorbent, type of elution solvent, elution volume and percentage recoveries of all analytes are summarized in Table 5.4.

Table 5.4 Summary of the optimized parameters of SPE clean-up column.

Parameter	Type of sorbent		
	C ₁₈	Florisil	Silica gel
Sample volume (mL)	0.5	0.5	0.5
Weight of sorbent (mg)	300	400	300
Elution solvent	Acetone	Acetone	Acetonitrile
Elution volume (mL)	20	20	4
% Recovery of OPPs	97-127%	92-118%	96-109%

The GC chromatograms of cabbage sample obtained from using the optimum condition of C₁₈, florisil and silica gel-SPE are shown in Figure 5.18.

then partition with petroleum ether. Polar and medium polar interferences are in a mixture of acetonitrile and water layer, while the pesticides are partition to petroleum ether layer [Hammarstrand, 1976]. Percentage recoveries of fortified OPPs from cabbage sample from the LLE method are shown in Table 5.5.

Table 5.5 Percentage recoveries of fortified OPPs from cabbage sample obtained from the LLE method.

Concentration (mgkg ⁻¹)	Recovery* (%)				
	Diazinon	Fenitrothion	Malathion	Chlorpyrifos	Triazophos
0.1	63.69 ± 7.85	73.88 ± 3.37	56.67 ± 5.54	50.67 ± 5.03	69.28 ± 3.01
0.2	64.40 ± 9.13	78.41 ± 6.81	59.29 ± 4.12	47.47 ± 4.31	63.17 ± 1.71
0.5	62.75 ± 2.57	73.19 ± 3.71	59.58 ± 2.19	69.80 ± 2.93	65.43 ± 3.94
1.0	66.90 ± 3.59	73.86 ± 1.59	52.16 ± 1.32	51.46 ± 1.35	63.30 ± 3.82

* mean ± SD (n = 3)

Percentage recoveries of all fortified OPPs were in the range of 51 - 78% with R.S.D. < 10%. This clean-up method can remove the interferences as SPE procedure, as shown in Figure 5.19, but it gave lower recoveries for all OPPs. The lower percentage recoveries may occur from the loss of the analytes in many sample preparation steps. Therefore, the silica gel-SPE was chosen for sample clean-up because of its simplicity, saving cost and reducing time consuming.

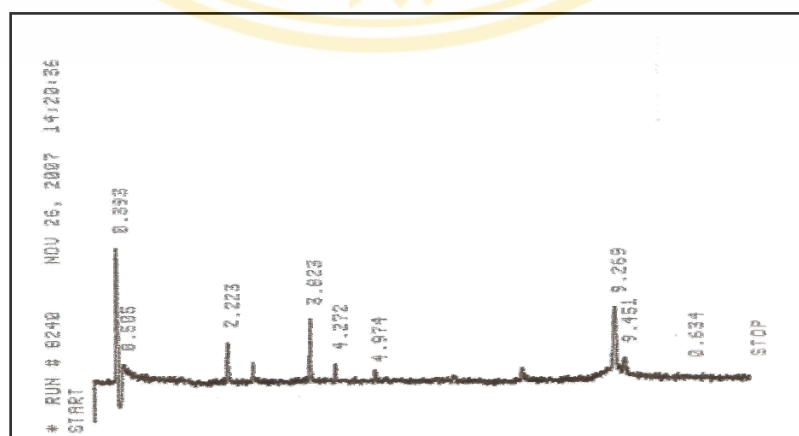


Figure 5.19 The GC chromatogram of cabbage sample obtained from LLE method.

In addition, silica gel-SPE and LLE clean-up methods were compared using statistical t-test with 95% confidence interval, as shown in Table 5.6. It showed significantly difference between both methods ($t_{\text{stat}} > t_{\text{critical}}$), except fenitrothion.

Table 5.6 Statistical test (t-test) for SPE and LLE recovery values.

OPP	t Stat	Correlation	P (T≤t) two-tail	t Critical two-tail
Diazinon	33.1921	0.8142	0.0001	3.1824
Fenitrothion	0.7964	-0.4517	0.4840	3.1824
Malathion	5.0616	-0.9722	0.0149	3.1824
Chlorpyrifos	4.1285	-0.0919	0.0258	3.1824
Triazophos	3.8186	-0.3628	0.0316	3.1824

A significant difference for OPPs between two clean up methods using t-test (2-tailed, paired) at *P* value of 0.05.

Consequently, the optimum conditions of the extraction and silica gel-SPE clean-up step were applied to other vegetable samples, i.e., tomato and cucumber. These vegetables are a fruiting vegetable-edible peel groups which have low chlorophyll and oil content, classified by Codex Alimentarius and Ambrus [Tekel and Hatrík, 1996]. The extraction with the mixture of ethyl acetate and hexane (1:1 v/v) and clean-up by silica gel-SPE were also suitable for these vegetables. Silica gel-SPE can remove interferences from the cucumber and tomato extracts. This sample preparation procedure provided high percentage recoveries of all analytes for both samples (> 70%). The GC chromatograms of cucumber and tomato samples with and without silica gel-SPE treatment are shown in Figure 5.20. The percentage recoveries of OPPs using the optimum condition of silica gel-SPE are illustrated in Table 5.7.

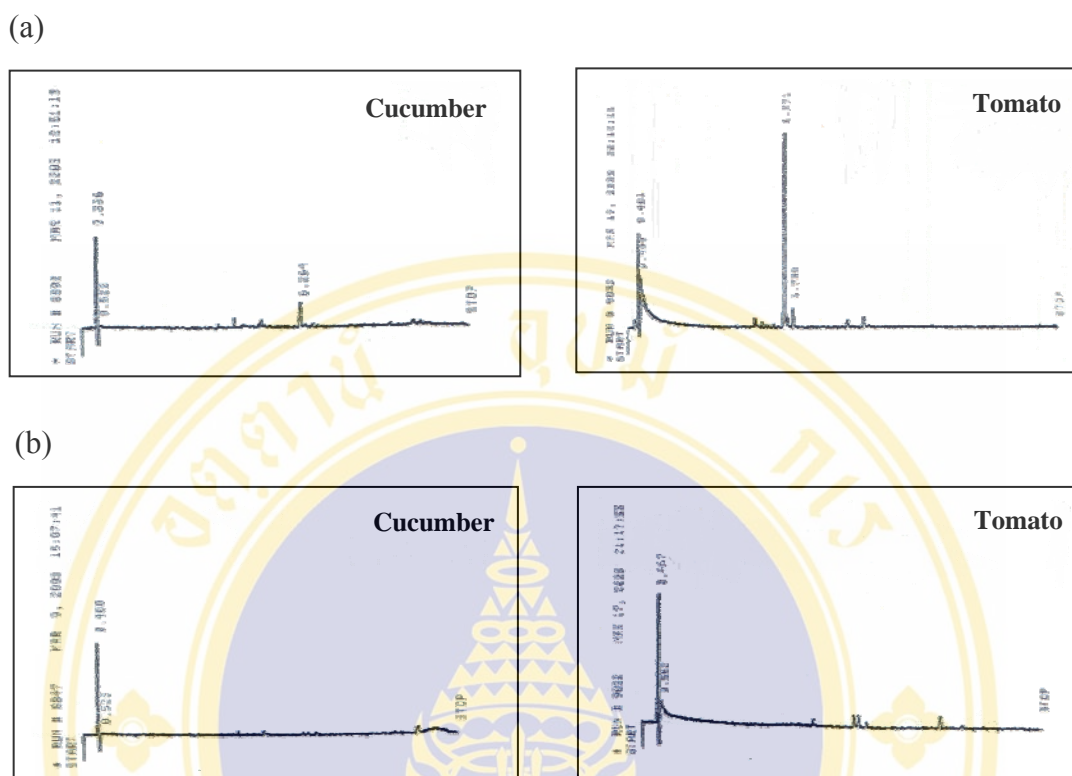


Figure 5.20 The GC chromatograms of cucumber and tomato samples (a) no clean-up and (b) clean-up with silica gel-SPE.

5.4.4 Recovery study

The recoveries of extraction were performed by spiking OPP standard into vegetable sample with five consecutive concentrations, as shown in Table 5.7. The recoveries of all OPPs were in the range of 72 - 95%, 72 - 98%, and 68 - 107% for cabbage, cucumber and tomato, respectively with R.S.D. < 10%. Therefore, the extraction procedure gave good precision and high reliability for determination of OPPs in cabbage, cucumber and tomato samples.

Table 5.7 Recoveries of fortified OPPs from cabbage, cucumber, and tomato by SPE method.

OPP	Added (mgkg ⁻¹)	Cabbage		Cucumber		Tomato	
		Recovery* (%)	RSD (%)	Recovery* (%)	RSD (%)	Recovery* (%)	RSD (%)
Diazinon	0.02	89.92 ± 2.77	1.02	97.54 ± 4.50	4.61	94.42 ± 3.98	4.21
	0.10	80.95 ± 2.31	7.39	91.61 ± 1.93	2.10	70.00 ± 1.08	1.54
	0.20	80.58 ± 3.68	4.57	86.74 ± 1.47	1.70	70.38 ± 2.00	2.84
	0.50	81.47 ± 6.02	2.86	92.68 ± 1.65	1.78	71.16 ± 3.32	4.66
	1.00	76.13 ± 0.78	5.42	84.43 ± 4.96	5.56	72.00 ± 0.83	1.15
Fenithothion	0.02	70.39 ± 2.30	0.33	87.96 ± 2.88	3.28	106.94 ± 8.72	8.16
	0.10	79.25 ± 0.90	1.52	92.39 ± 2.10	2.28	73.76 ± 1.84	2.50
	0.20	73.30 ± 1.11	3.00	94.71 ± 1.01	1.07	92.95 ± 5.71	9.11
	0.50	73.87 ± 1.14	1.13	94.93 ± 1.27	1.34	77.04 ± 4.24	5.51
	1.00	80.08 ± 0.26	3.27	86.13 ± 2.81	3.26	77.31 ± 0.26	0.34
Malathion	0.02	73.70 ± 2.17	2.95	85.49 ± 0.32	0.37	74.18 ± 1.60	2.16
	0.10	74.40 ± 1.94	0.77	87.85 ± 0.98	1.11	71.07 ± 0.55	0.77
	0.20	71.80 ± 0.71	0.99	82.22 ± 0.50	0.61	73.82 ± 2.21	2.99
	0.50	73.20 ± 0.56	2.61	89.32 ± 1.78	1.99	70.22 ± 3.49	4.97
	1.00	80.29 ± 0.46	2.95	87.09 ± 2.11	2.43	72.47 ± 0.34	0.47
Chlorpyrifos	0.02	71.04 ± 4.33	0.30	73.80 ± 3.36	4.56	79.47 ± 1.23	1.55
	0.10	77.35 ± 2.11	0.98	82.03 ± 2.00	2.44	75.00 ± 1.03	1.37
	0.20	74.01 ± 1.37	1.70	82.08 ± 0.90	1.09	72.74 ± 2.08	2.86
	0.50	76.21 ± 0.75	2.72	81.95 ± 2.21	2.70	68.05 ± 2.57	3.78
	1.00	82.06 ± 2.05	6.09	81.21 ± 1.75	2.15	69.72 ± 0.28	0.40
Triazophos	0.02	95.24 ± 3.61	1.55	71.65 ± 1.09	1.51	81.70 ± 2.98	3.65
	0.10	74.23 ± 1.67	1.06	94.87 ± 1.04	1.10	88.47 ± 1.14	1.29
	0.20	76.88 ± 1.57	2.24	83.37 ± 1.67	2.01	76.88 ± 1.99	2.59
	0.50	83.98 ± 0.89	2.25	89.73 ± 2.04	2.28	74.83 ± 2.34	3.12
	1.00	83.84 ± 1.30	3.79	87.75 ± 2.07	2.36	75.10 ± 0.92	1.22

*mean ± SD (n=3)

5.5 Determination of OPPs in vegetable samples

External standard and standard addition methods are widely used for quantity pesticide residues in various vegetable samples to ensure that their agricultural products have the amount of the pesticide residues not over the maximum residue limit (MRLs) values for consumer's health safety [Ostroukhova and Zenkevich, 2006].

The study was carried out to assess the developed method of determining OPP residues in cabbage, cucumber and tomato. The comparison of the amounts of OPPs which were obtained from external standard and standard addition methods was evaluated to employ the suitable method.

5.5.1 External standard method

Calibration curves of OPPs were constructed by using standard OPPs. The observed peak area of OPP in the sample was used to compare with the peak area of OPP standard. The observed concentrations of OPPs in vegetable samples using external standard method are shown in Table 5.8.

Table 5.8 The observed concentrations of OPPs in vegetable samples using external standard method.

OPP	Concentration (mgkg ⁻¹)					
	Cabbage		Cucumber		Tomato	
	Observed*	MRL	Observed*	MRL	Observed*	MRL
Diazinon	0.51 ± 0.045	0.5	0.09 ± 0.004	0.1	0.09 ± 0.006	0.5
Fenitrothion	0.40 ± 0.018	-	0.09 ± 0.005	-	0.09 ± 0.005	-
Malathion	0.32 ± 0.029	-	0.06 ± 0.005	0.2	0.08 ± 0.005	0.5
Chlorpyrifos	0.39 ± 0.019	1	0.08 ± 0.004	-	0.13 ± 0.005	0.5
Triazophos	0.51 ± 0.028	0.1	0.12 ± 0.007	-	0.11 ± 0.006	-

* = Mean ± SD (n = 5), MRL established by Codex Alimentarius

5.5.2 Standard addition method

Standard addition was performed by adding the concentrations of OPP standard in the range of 5 - 50 $\mu\text{g mL}^{-1}$ in cabbage sample and 2 - 10 $\mu\text{g mL}^{-1}$ in cucumber and tomato samples. The observed concentrations of OPPs in vegetable samples using standard addition method are shown in Table 5.9 and in Appendix C.

Table 5.9 The observed concentrations of OPPs in vegetable samples using standard addition method.

OPP	Concentration (mg kg^{-1})					
	Cabbage		Cucumber		Tomato	
	Observed*	MRL	Observed*	MRL	Observed*	MRL
Diazinon	0.64 ± 0.062	0.5	0.11 ± 0.003	0.1	0.11 ± 0.005	0.5
Fenitrothion	0.47 ± 0.041	-	0.11 ± 0.002	-	0.12 ± 0.011	-
Malathion	0.37 ± 0.038	-	0.08 ± 0.007	0.2	0.11 ± 0.001	0.5
Chlorpyrifos	0.54 ± 0.022	1	0.09 ± 0.007	-	0.15 ± 0.007	0.5
Triazophos	0.69 ± 0.062	0.1	0.11 ± 0.008	-	0.10 ± 0.009	-

* = Mean \pm SD (n=3), MRL established by Codex Alimentarius

5.5.3 Comparison of the method

The results of concentration values showed that the concentration of OPPs obtained from the standard addition method was slightly higher than the external standard method. Standard addition method can compensate matrix effect and reduce the systematic error of the determination [Ostroukhova and Zenkevich, 2006].

Statistical tests for comparing between external standard and standard addition methods are shown in Table 5.10. It showed no significant difference between both methods ($t_{\text{stat}} < t_{\text{critical}}$). Therefore, both methods were suitable for determining OPPs in cabbage, cucumber and tomato samples.

Table 5.10 Statistical test (t-test) for comparing between external standard and standard addition methods.

Sample	T Stat	Correlation	P ($T \leq t$) two-tail	t Critical two-tail
Cabbage	2.0630	0.7923	0.1081	2.7764
Cucumber	2.1464	0.7654	0.0984	2.7764
Tomato	2.6716	0.7156	0.0557	2.7764

A significant difference for OPPs between two determination methods using t-test (2-tailed, paired) at P value of 0.05.

However, the external standard method should be used for the routine analysis due to less amount of sample, saving cost and reducing time and solvent usage in the sample preparation step.

5.6 Degradation of OPPs in water

OPPs are normally unstable in aqueous media. However, accidental leaching may occur from treated area into river and lake. This study can be applied for determination of OPP contaminants in water. OPPs can be degraded by many pathways such as hydrolysis, photolytic degradation, chemical oxidation, or microbial transformation. All these natural occurring degradation processes are exploited in research and development of treatments for purification of polluted water containing OPPs.

The OPPs in aqueous system can not be directly determined using gas chromatography because it may damage the capillary GC column. Therefore, the SPE method was applied for extracting OPPs from water sample before injection to GC system.

5.6.1 SPE condition

The breakthrough volume causes by overloading of the sample volume. The same amount of analyte is spiked in each different sample volume and loaded into each SPE cartridge. If breakthrough of SPE does not occur, the amount of the analyte preconcentrated is constant, but when breakthrough of SPE occurs, it is decreased by increasing the sample volume [Dopico-García *et.al.*, 2005]. In this study, the mixture of OPP standard $100 \mu\text{g mL}^{-1}$ of $100 \mu\text{L}$ was spiked in the various sample volume in the range of 1 - 1000 mL. The breakthrough of C_{18} , florisil and silica gel-SPE are shown in Figure 5.21.

The percentage recoveries of OPP analytes using C_{18} , florisil and silica gel-SPE continuously decreased when increased the sample volume because of breakthrough of the SPEs. In case of C_{18} -SPE, the breakthrough volume of diazinon, chlorpyrifos and triazophos was more than 250 mL, while the breakthrough volume of malathion and fenitrothion was more than 300 mL. The percentage recoveries of the analytes obtained from loading water sample in the range of 50 - 250 mL were in the range of 95 - 120% with R.S.D < 10%. At sample volume of 1000 mL gave the lowest percentage recoveries of the analytes in the range of 17 - 36% with R.S.D < 10%. Therefore, the sample volume should be less than 250 mL using C_{18} -SPE cartridge. For florisil and silica gel-SPE, the breakthrough volume of all analytes was only 5 mL. The elution of both SPEs is accomplished with more polar solvents, so the high polarity of water sample can elute the analytes from the sorbents in the sample loading step. From this study, we can conclude that the C_{18} -SPE method was suitable for OPP determination in water sample. The recovery data of breakthrough volume study are presented in Appendix B (Table B10 – B12).

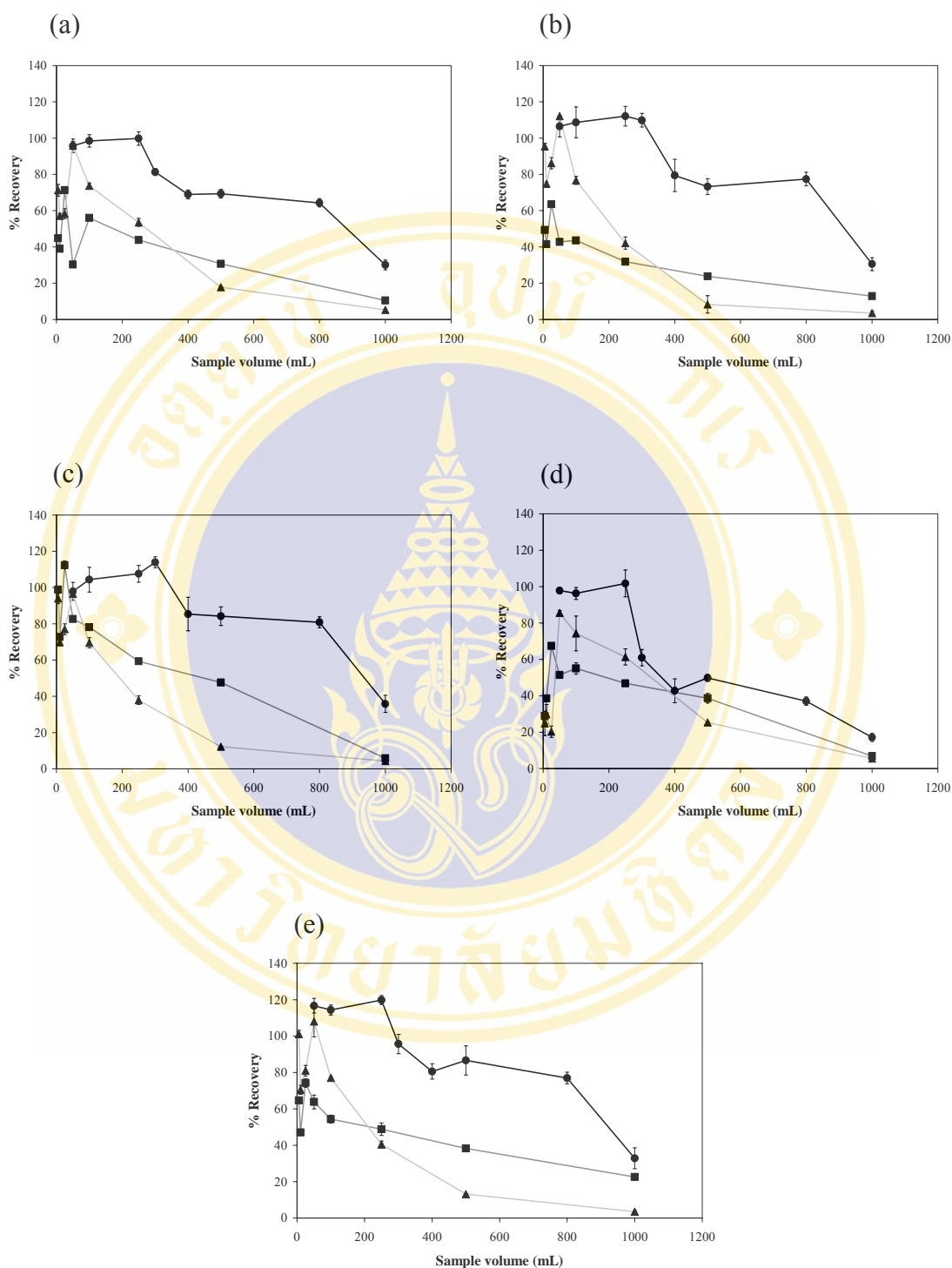


Figure 5.21 Breakthrough volume of (a) diazinon, (b) fenitrothion, (c) malathion, (d) chlorpyrifos and (e) triazophos in different SPEs: symbol; C₁₈-SPE (●), florisil-SPE (■), silica gel-SPE (▲).

For the study of OPP degradation in water sample, the sample volume of 5 mL was loaded in C₁₈-SPE cartridge. The percentage recoveries of all OPPs were studied by spiking OPP standard into water sample with three consecutive concentrations, as demonstrated in Table 5.11. The percentage recoveries of all analytes were in the range of 74 - 132% with R.S.D < 10%.

Table 5.11 Percentage recoveries of OPPs at three spiked level of concentrations obtained from C₁₈-SPE cartridge.

Spiked Concentration (µgmL ⁻¹)	Recovery* (%)				
	Diazinon	Fenitrothion	Malathion	Chlorpyrifos	Triazophos
0.5	82.95 ± 0.90	87.48 ± 2.96	92.75 ± 4.46	75.97 ± 0.54	132.44 ± 0.29
1.0	74.01 ± 1.99	92.05 ± 4.71	92.41 ± 1.49	76.61 ± 0.50	93.32 ± 0.90
2.0	84.78 ± 0.63	107.57 ± 4.40	105.58 ± 5.47	83.01 ± 3.18	104.76 ± 9.36

* = Mean ± SD (n=3)

5.6.2 Degradation study

The degradation study of OPPs in water is a useful information for drinking water and aquatic organism. Most OPP metabolites were occurred by many pathways such as hydrolysis, photolytic degradation, chemical oxidation or biodegradation, as described in Section 3.4 [Shemer and Linden, 2006; Bavcon *et al.*, 2003].

The study focused on the hydrolysis and photolytic degradation of OPPs in water. The samples were prepared in aqueous system pH 5.5 and divided into 2 groups: (1) exposed to sunlight and (2) kept in darkness during the 31 day experiment, as shown in Figure 5.22.

The results showed that the concentrations of OPPs in water sample obtained from keeping the sample in darkness continuously decreased from their initial concentrations. It can be deduced that the degradation of OPPs was occurred by

hydrolysis process. In addition, the effect of photolytic degradation of OPPs was also studied by exposing the sample to sunlight. The results found that the OPP degradation in both conditions showed no significantly difference, even if the OPP degradation in sunlight should more rapidly degraded than the OPP degradation in darkness due to the effect of hydrolysis process and photolytic degradation by UV absorption of OPP molecule, as shown in Appendix D (Figure D1). It can be deduced that the rate of photodegradation may be extremely slower than the rate of hydrolytic degradation. Therefore, the degradation of OPPs in water is caused by hydrolysis process mainly which corresponded to the literature data [Bavcon *et al.*, 2003].

Moreover, the concentration of diazinon rapidly decreased from its initial concentrations compared to the other pesticides. This phenomenon demonstrated that the hydrolytic degradation of diazinon rapidly occurred in acidic condition (pH 5.5) which corresponded to the literature data [Shemer and Linden, 2006; Bavcon *et al.*, 2003], indicating that pH of media is an important factor for degradation of diazinon via hydrolysis process. The half-life of OPP degradation in water sample at different concentrations is shown in Appendix D (Table D1-D3).

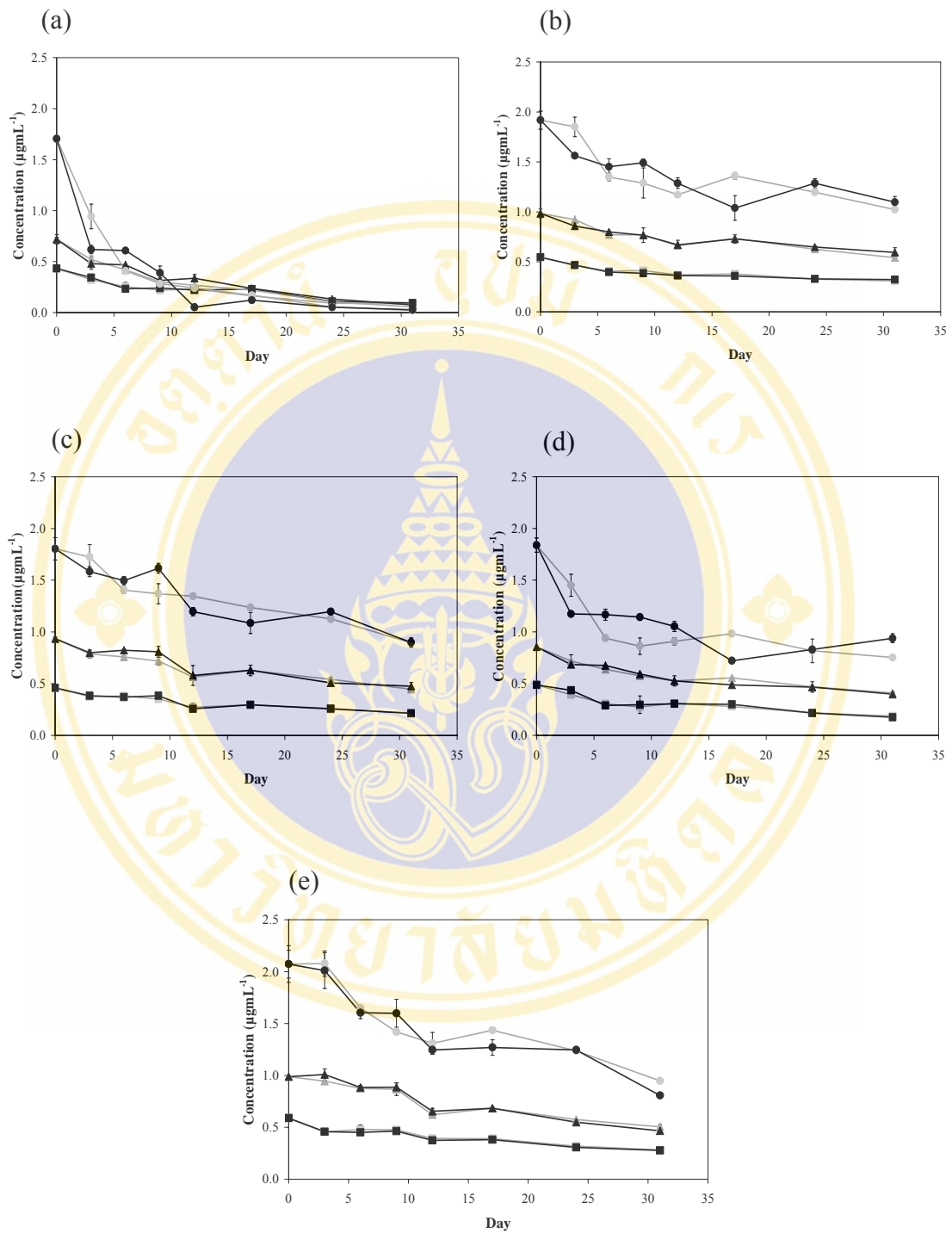


Figure 5.22 Degradation study of (a) diazinon, (b) fenitrothion, (c) malathion, (d) chlorpyrifos and (e) triazophos in aqueous system exposed to sunlight condition: light symbols; 0.5 $\mu\text{g mL}^{-1}$ (\square), 1.0 $\mu\text{g mL}^{-1}$ (\triangle), 2.0 $\mu\text{g mL}^{-1}$ (\circ) and kept in the darkness: dark symbol; 0.5 $\mu\text{g mL}^{-1}$ (\blacksquare), 1.0 $\mu\text{g mL}^{-1}$ (\blacktriangle), 2.0 $\mu\text{g mL}^{-1}$ (\bullet).

CHAPTER VI

CONCLUSION

Nowadays, organophosphorus compounds are one class of pesticides which are extensively used as insecticides to increase the agricultural production. The merit of using these pesticides is a low environmental persistence due to their easily decomposition. However, these pesticides are highly toxic to nervous system because they inhibit the activity of acetylcholine esterase enzyme by the interruption of the nerve working. To protect consumer's health, many countries have established legal directives to control their used levels in the agricultural products and their contaminants in the water, through the maximum residue limits for pesticides. This research focused on mainly organophosphorus compounds including diazinon, fenitrothion, malathion, chlorpyrifos and triazophos. These pesticides were successfully separated within 10 minutes on HP-5 column using splitless injection and flow rate of 36.7 mLmin^{-1} . The temperature program of $80 \text{ }^{\circ}\text{C}$ (hold 1 min), rate $40 \text{ }^{\circ}\text{C/min}$ to $200 \text{ }^{\circ}\text{C}$ (hold 2.5 min), rate $20 \text{ }^{\circ}\text{C/min}$ to $210 \text{ }^{\circ}\text{C}$, rate $40 \text{ }^{\circ}\text{C}$ to $240 \text{ }^{\circ}\text{C}$ (hold 5 min), injector temperature at $250 \text{ }^{\circ}\text{C}$ and detector temperature at $280 \text{ }^{\circ}\text{C}$ were used in the study. Linear ranges of diazinon and triazophos were in the range of $0.1 - 100 \mu\text{g mL}^{-1}$, whereas the linear ranges of fenitrothion, malathion and chlorpyrifos were in the range of $0.5 - 100 \mu\text{g mL}^{-1}$. LODs of the analytes were in the range of $0.04 - 0.50 \mu\text{g mL}^{-1}$.

Derivatization of OPPs was studied to increase the sensitivity of the analysis using GC-FPD. Pentafluorobenzyl bromide and 1-chloro-3-iodopropane were used as derivatizing agents. The results showed that only derivative of malathion was observed using 1-chloro-3-iodopropane as derivatizing agent, but it was not sensitive to the FPD detector. However, the derivatization step was not necessary for these compounds when detected by flame photometric detector. The molecules of the analytes containing phosphorus and sulfur atoms were sensitive to this detector.

The sample preparation step for OPP determination consists of sample extraction and clean-up steps. The study was optimized only cabbage sample. Various solvents including acetonitrile, acetone, ethyl acetate and hexane were studied to select the suitable solvent for extracting OPPs from vegetable sample. The mixture of ethyl acetate and hexane (1:1 v/v) of 150 mL was an appropriate extraction solvent. This condition gave the highest percentage recoveries in the range of 70 - 81% with R.S.D. < 5%. The sample extracted consisting of co-extract such as lipid, pigment and a variety of polar and non-polar substances was subjected to the SPE column for clean-up the interferences before GC analysis. Packing materials of SPEs including C₁₈, florisil and silica gel were studied. The clean-up step was carried out by silica gel-SPE because it showed more efficiency to reduce the interferences in the sample extracted comparing to other sorbents. The condition of SPE column was packing with silica gel of 300 mg and eluting with acetonitrile of 4 mL. Percentage recoveries of all OPPs using silica gel-SPE were in the range of 96 - 109% with R.S.D. < 10%. LLE procedure was also used to compare with the SPE procedure. It can remove the interference as SPE procedure but it gave the lower recoveries (51 - 78 %). Consequently, the optimized sample preparation was applied for other vegetable samples, i.e., tomato and cucumber. This extraction and clean-up procedures were suitable for these vegetables because it gave high percentage recoveries of all OPPs as well as cabbage sample. Recoveries of this method were in the range of 70 - 95%, 72 - 98% and 68 - 107% with R.S.D < 10% for cabbage, cucumber and tomato, respectively. In this study, external standard and standard addition methods were applied for determining OPPs in cabbage, cucumber and tomato. The concentrations of OPP residues in cabbage, cucumber and tomato showed no significantly difference between both methods. However, the external standard method was suitable for the routine analysis due to saving cost, less time consuming and reducing solvent used.

OPP residues in water were studied by the SPE method. First, breakthrough volume of water loading was carried out. The column of C₁₈-SPE was selected to extract OPP analytes from the water sample and preconcentration because the breakthrough volume of the analytes was higher than silica gel and florisil-SPE column. The breakthrough volume of the analytes for C₁₈-SPE was 250 mL, while the

breakthrough volume of the analytes for florisil and silica gel-SPE were only 5 mL. So, the sample volume should be used less than 250 mL when applied the C₁₈-SPE column. The condition of SPE was packing with C₁₈ of 300 mg and eluting with acetone of 20 mL. Recoveries of all OPPs were in the range of 74 - 132% with R.S.D. < 10%. Then, the investigation of OPP degradation in water sample was carried out. This study indicated that the hydrolysis was the major process of OPP degradation and there was no effect of sunlight on the hydrolytic degradation of OPPs in water. For the determination of OPPs in vegetable samples with high water content, the homogenized sample should be extracted immediately before the loss of analytes from hydrolysis process.

Suggestion for future work:

This developed method should be applied for other OPPs and pesticides (organochlorine, carbamate and pyrethroid) in various kinds of vegetable samples. This method can be applied for determining pesticide residues in the agricultural products to protect the consumer's health and to set the legislation value for the products. For the degradation of OPPs in water, the degradation products of OPPs should be studied to evaluate the toxicity and the degradation pathway which is important for the development of purification and removal of OPPs from water. However, the degradation products of OPPs were not observed in this study. This is caused by the higher polarity of most OPP degradation products which may be eluted with water in the loading step. Therefore, the LLE method may be used to extract OPP degradation products from the eluent fraction of the loading step. The degradation products of OPPs may be determined by other separation technique such as HPLC because of high polarity of these compounds.

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APPENDIX A

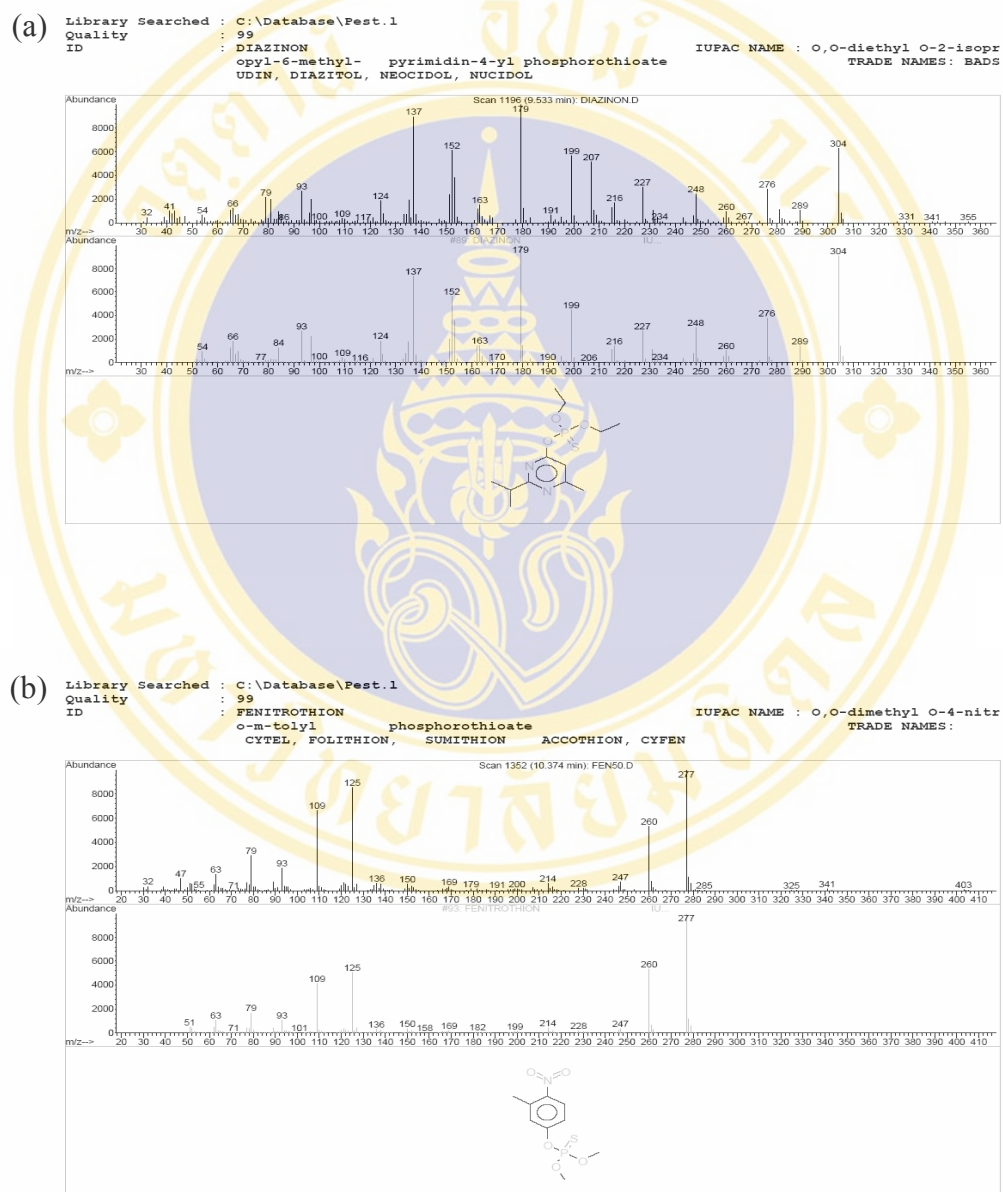


Figure A1 MS spectra of (a) diazinon, (b) fenitrothion, (c) malathion, (d) chlorpyrifos and (e) triazophos obtained from pesticide database (upper) and GC-MS chromatogram (below).

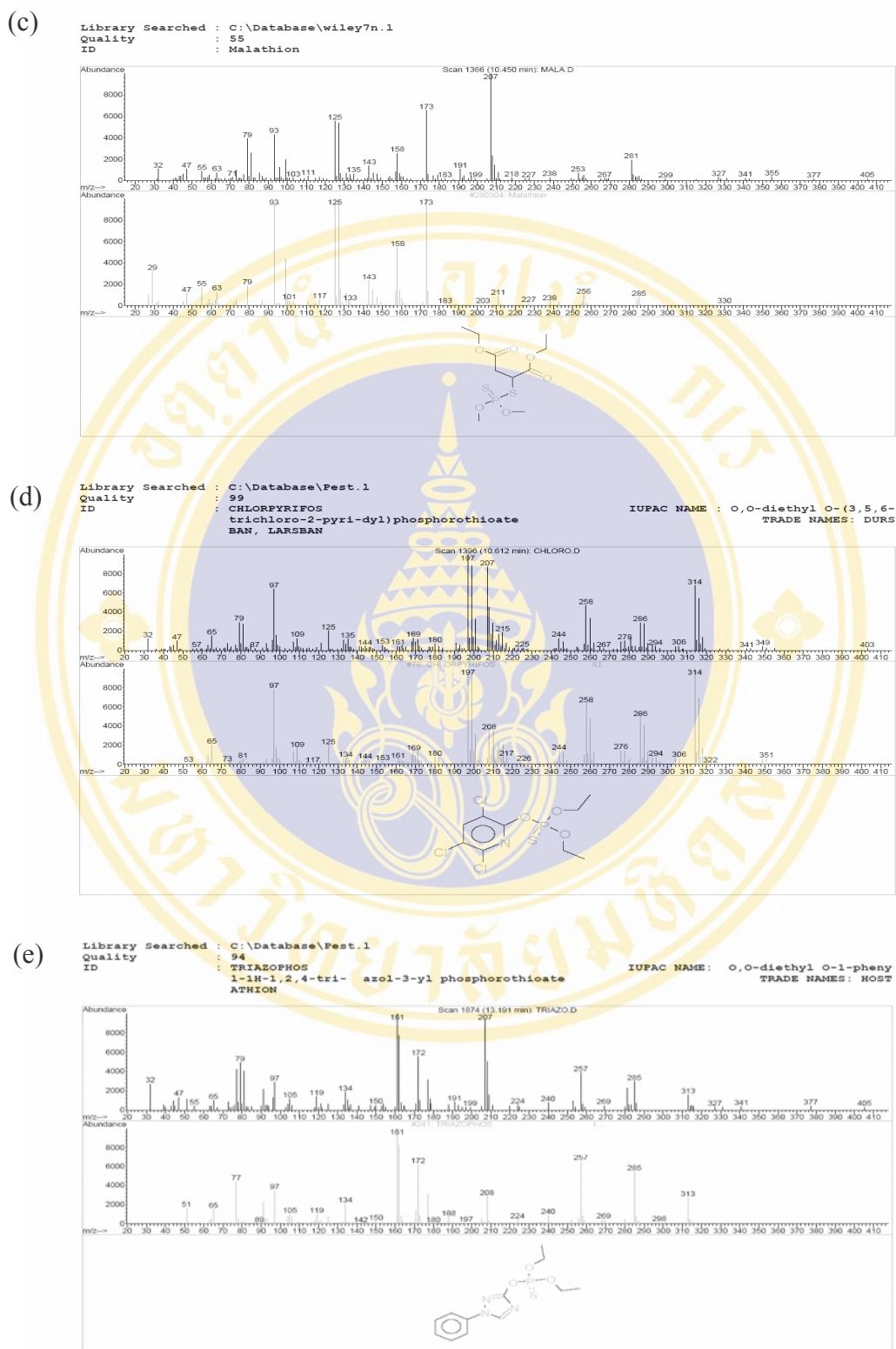


Figure A1 (continue) MS spectra of (a) diazinon, (b) fenitrothion, (c) malathion, (d) chlorpyrifos and (e) triazophos obtained from pesticide database (upper) and GC-MS chromatogram (below).

APPENDIX B

Table B1 The percentage recoveries of OPPs obtained from varying extraction solvent for cabbage sample.

OPP	Acetonitrile		Acetone		Ethyl acetate		Hexane		Ethyl acetate: Hexane (1:1)	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	65.72±0.89	1.35	59.88±2.58	4.31	80.01±2.13	2.66	74.65±2.80	3.75	78.42±1.37	1.74
Fenitrothion	66.73±2.61	3.91	49.46±1.04	2.10	77.26±2.94	3.80	71.27±3.34	4.69	74.19±3.53	4.76
Malathion	68.56±1.55	2.26	59.41±1.60	2.69	56.80±4.00	7.05	42.28±2.10	4.96	70.99±0.68	0.95
Chlorpyrifos	40.92±4.94	12.07	14.68±1.80	12.26	46.94±1.23	2.63	54.63±1.44	2.63	71.79±3.47	4.83
Triazophos	63.86±0.68	1.07	46.10±1.26	2.73	80.59±3.07	3.81	65.75±3.02	4.59	71.45±0.84	1.18

Table B2 The percentage recoveries of OPPs obtained from varying the mixture ratio of extraction for cabbage sample.

OPP	1:3		2:3		1:1		3:2		3:1	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	61.42±2.67	4.34	60.69±0.82	1.36	78.42±1.37	1.74	45.06±2.35	5.23	52.95±3.29	6.21
Fenitrothion	66.18±2.66	4.40	60.31±2.66	4.40	74.19±3.53	4.76	48.28±4.73	9.80	54.29±1.04	1.92
Malathion	67.83±2.29	3.38	61.33±0.85	1.38	70.99±0.68	0.95	47.54±4.24	8.92	54.25±2.03	3.74
Chlorpyrifos	60.95±2.41	3.95	61.65±1.68	2.73	71.79±3.47	4.83	36.63±3.32	9.06	48.25±2.59	5.36
Triazophos	69.35±2.55	3.68	63.44±1.00	1.58	71.45±0.84	1.18	53.80±1.94	3.61	56.87±2.43	4.28

Table B3 The percentage recoveries of OPPs obtained from varying the extraction solvent volume for cabbage sample.

OPP	50 mL		100 mL		150 mL	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	64.29±4.13	6.42	78.42±13.37	1.74	80.58±13.68	4.57
Fenitrothion	63.37±5.43	8.57	74.19±3.53	4.76	73.96±2.22	3.00
Malathion	61.65±3.33	5.41	70.99±0.68	0.95	71.82±0.71	0.99
Chlorpyrifos	61.25±3.84	6.27	71.79±3.47	4.83	71.15±1.26	1.70
Triazophos	64.75±5.72	8.83	71.45±0.84	1.18	74.04±1.66	2.24
OPP	200 mL		250 mL		300 mL	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	79.25±2.15	2.71	80.86±1.81	2.24	80.15±1.90	2.37
Fenitrothion	72.74±2.06	2.83	74.24±2.67	3.60	73.60±0.75	1.01
Malathion	69.98±1.55	2.86	69.67±2.86	4.10	70.20±1.58	2.25
Chlorpyrifos	71.57±1.28	1.79	71.22±2.62	3.68	72.22±4.82	6.70
Triazophos	73.86±1.18	1.59	73.49±1.93	2.63	73.93±1.31	1.78

Table B4 The percentage recoveries of OPPs obtained from varying sorbent weight for C₁₈-SPE.

OPP	100 g		200 g		300 g		400 g		500 g	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	90.51±1.35	1.49	99.71±1.95	1.96	98.75±2.54	2.57	96.02±2.02	2.10	95.53±2.80	2.93
Fenitrothion	95.47±0.81	0.85	106.64±2.70	2.54	113.04±2.01	1.77	112.30±6.30	5.61	111.49±4.43	3.97
Malathion	94.30±4.97	5.27	102.38±5.19	5.07	101.96±1.87	1.83	112.30±6.30	4.40	104.44±5.71	5.47
Chlorpyrifos	96.71±4.65	4.81	101.46±4.80	4.73	103.02±2.15	2.08	103.05±4.54	2.32	102.40±4.54	4.43
Triazophos	89.81±4.06	4.52	95.12±2.72	2.86	100.76±2.47	2.45	100.48±3.88	3.86	104.03±4.97	4.97

Table B5 The percentage recoveries of OPPs obtained from varying sorbent weight for florisisil-SPE.

OPP	100 g		200 g		300 g		400 g		500 g	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	57.93±2.68	4.62	80.95±1.87	2.31	74.92±3.12	4.16	81.51±1.99	2.45	81.02±1.73	2.13
Fenitrothion	59.81±3.49	5.84	89.72±3.35	3.73	87.35±1.21	1.39	94.56±1.56	1.65	91.28±3.72	4.08
Malathion	65.38±4.16	6.37	96.99±4.11	4.24	94.07±1.48	1.57	98.48±1.07	1.09	98.44±1.29	1.31
Chlorpyrifos	56.21±3.20	5.69	86.02±3.75	4.36	83.28±4.99	5.99	83.81±5.88	7.01	83.65±1.22	1.46
Triazophos	72.05±5.37	7.45	99.07±1.30	8.39	99.15±4.52	4.56	111.25±1.80	4.00	109.41±3.73	3.41

Table B6 The percentage recoveries of OPPs obtained from varying sorbent weight for silica gel-SPE.

OPP	100 g		200 g		300 g		400 g		500 g	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	92.61±2.94	3.18	92.23±2.48	2.69	99.96±1.39	1.39	95.51±0.65	0.68	97.41±1.17	1.20
Fenitrothion	97.69±3.53	3.61	100.78±1.76	1.75	107.20±2.04	1.90	105.00±3.17	3.01	104.19±2.98	2.86
Malathion	96.31±3.32	3.45	97.97±1.53	1.56	104.20±1.56	1.49	103.80±3.48	3.35	103.90±3.21	3.09
Chlorpyrifos	97.01±3.98	4.11	97.33±1.37	1.41	102.35±0.93	0.90	98.88±0.44	0.45	98.23±1.37	1.39
Triazophos	106.61±5.18	4.86	107.27±2.63	2.46	110.60±1.85	1.67	110.83±1.45	1.31	114.94±2.69	2.34

Table B7 The percentage recoveries of OPPs obtained from varying elution solvent for C₁₈-SPE.

OPP	Acetonitrile		Acetone		Ethyl acetate		Hexane	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	55.64±0.12	0.21	87.10±1.94	4.34	84.86±1.06	1.25	63.10±0.57	0.90
Fenitrothion	75.32±1.39	1.85	91.41±2.05	2.24	90.76±0.87	0.96	76.15±0.87	1.14
Malathion	77.89±2.23	2.86	91.22±0.18	0.20	90.10±0.93	1.03	52.88±0.58	1.10
Chlorpyrifos	73.18±1.53	2.10	85.42±1.58	1.84	88.23±1.19	1.35	69.22±0.76	1.10
Triazophos	81.93±4.52	5.52	100.25±4.69	4.68	98.59±5.86	5.94	6.92±0.99	14.30

Table B8 The percentage recoveries of OPPs obtained from varying elution solvent for florisil-SPE.

OPP	Acetonitrile		Acetone		Ethyl acetate		Hexane	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	75.80±4.03	5.31	90.98±4.13	4.54	70.16±4.17	5.59	58.89±2.55	4.33
Fenitrothion	73.36±5.34	7.28	97.80±6.72	6.87	67.06±2.86	4.26	52.64±2.09	3.97
Malathion	82.40±4.07	4.94	98.65±5.95	6.03	70.55±2.29	3.25	45.82±1.94	4.23
Chlorpyrifos	83.78±4.03	4.81	104.99±2.42	2.31	76.96±4.06	5.27	82.38±1.75	2.12
Triazophos	77.23±3.84	4.97	86.40±6.67	7.72	76.16±7.27	9.54	22.90±2.77	12.11

Table B9 The percentage recoveries of OPPs obtained from varying elution solvent for silica gel-SPE.

OPP	Acetonitrile		Acetone		Ethyl acetate		Hexane	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	89.63±1.32	1.47	78.63±1.46	1.86	84.97±0.24	0.29	1.56±0.38	24.03
Fenitrothion	103.66±1.58	1.52	94.92±0.75	0.79	98.86±0.81	0.82	8.81±0.43	4.93
Malathion	100.17±0.46	0.46	90.89±1.20	1.32	92.28±0.66	0.72	0.00±0.00	0.00
Chlorpyrifos	90.74±2.15	2.37	83.15±0.28	0.33	85.93±0.06	0.07	87.64±2.05	2.34
Triazophos	110.15±5.52	4.92	103.72±1.60	1.54	101.13±2.05	2.03	0.00±0.00	0.00

Table B10 The percentage recoveries of OPPs obtained from varying elution volume for C₁₈-SPE.

OPP	5 mL		10 mL		15 mL		20 mL		25 mL	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	68.81±0.87	1.26	75.47±2.11	2.80	92.52±1.70	1.84	97.05±2.83	2.92	98.18±1.28	1.31
Fenitrothion	85.14±3.09	3.62	92.80±3.77	4.06	101.35±4.91	4.84	114.05±0.09	4.11	116.51±1.04	1.37
Malathion	90.16±3.11	3.45	89.99±0.44	0.49	95.05±4.93	5.19	100.39±3.57	3.55	102.79±1.15	1.12
Chlorpyrifos	68.13±4.02	5.91	70.39±1.97	2.80	89.06±5.17	5.81	99.20±2.65	2.67	99.04±1.19	1.20
Triazophos	105.15±3.30	3.14	115.83±3.72	3.21	116.50±3.78	3.24	127.42±2.66	2.09	123.55±0.71	1.20

Table B11 The percentage recoveries of OPPs obtained from varying elution volume for florisisl-SPE.

OPP	5 mL		10 mL		15 mL		20 mL		25 mL	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	88.67±2.74	3.09	86.58±1.20	1.38	92.42±1.70	1.84	92.48±0.49	0.53	97.18±3.54	3.64
Fenitrothion	97.68±0.83	0.85	95.67±6.33	7.40	106.15±3.39	3.19	115.73±0.45	4.58	111.75±6.41	5.73
Malathion	102.59±1.23	1.20	107.71±5.88	5.88	111.40±3.91	3.51	112.42±3.26	2.90	108.83±4.41	4.05
Chlorpyrifos	92.63±3.12	3.37	97.34±1.64	1.69	91.90±1.12	5.81	99.20±2.65	1.21	93.93±3.24	3.45
Triazophos	103.70±2.26	2.18	105.32±10.17	9.65	119.24±2.30	1.93	118.19±7.62	6.45	115.86±3.24	6.85

Table B12 The percentage recoveries of OPPs obtained from varying elution volume for silica gel-SPE.

OPP	1 mL		2 mL		3 mL		4 mL	
	%ofrecovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%ofrecovery (mean±SD)	% RSD	%ofrecovery (mean±SD)	% RSD
Diazinon	62.77±2.13	3.39	94.86±0.83	0.87	91.59±3.14	3.42	99.52±2.16	2.17
Fenitrothion	63.87±2.18	3.41	92.55±0.83	0.90	90.98±3.26	3.58	96.33±3.25	3.37
Malathion	64.46±3.00	4.66	94.20±0.91	0.97	93.53±4.18	4.47	100.59±6.77	6.73
Chlorpyrifos	68.22±4.21	6.17	92.18±4.00	4.34	88.72±1.45	1.63	98.07±7.72	7.87
Triazophos	64.72±0.02	0.02	106.27±5.50	5.11	105.23±2.94	2.71	113.11±7.38	6.57
OPP	5 mL		10 mL		15 mL		20 mL	
	%ofrecovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%ofrecovery (mean±SD)	% RSD	%ofrecovery (mean±SD)	% RSD
Diazinon	97.23±1.30	1.34	97.70±0.62	0.63	95.68±1.15	1.20	99.31±1.72	1.73
Fenitrothion	93.61±3.27	3.50	99.17±3.35	3.38	98.98±3.27	3.31	106.90±0.70	0.65
Malathion	101.70±3.27	2.65	101.03±0.74	0.73	100.59±3.35	3.33	104.32±1.39	1.34
Chlorpyrifos	98.69±3.92	3.98	99.66±1.27	1.27	94.42±1.20	1.28	101.42±3.35	3.30
Triazophos	109.15±3.00	2.73	104.57±2.86	2.66	108.06±6.75	6.23	111.66±1.35	1.20

Table B13 The percentage recoveries of OPPs obtained from breakthrough volume study for C₁₈-SPE.

OPP	50 mL		100 mL		250 mL		300 mL	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	95.74±3.69	3.86	98.50±3.42	3.47	99.77±3.65	3.66	81.31±1.87	2.29
Fenitrothion	106.44±5.80	5.45	108.67±8.46	7.78	112.11±5.43	4.85	109.86±3.79	3.45
Malathion	97.93±4.96	5.07	104.35±6.79	6.50	107.60±4.66	4.33	113.91±3.00	2.64
Chlorpyrifos	97.76±1.58	1.62	96.25±3.26	3.39	101.74±7.36	7.24	60.94±4.47	7.33
Triazophos	116.70±4.06	5.46	114.35±2.72	7.41	119.78±2.47	15.53	95.70±5.29	5.52
OPP	400 mL		500 mL		800 mL		1000 mL	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	68.96±2.51	3.64	69.32±2.35	3.39	64.33±2.33	3.62	30.11±2.69	8.92
Fenitrothion	79.44±8.83	11.12	73.23±4.37	5.97	77.41±3.72	4.80	30.56±3.57	11.69
Malathion	85.35±9.29	10.89	84.16±5.16	6.13	80.84±3.01	3.73	35.85±4.70	13.10
Chlorpyrifos	42.78±6.58	15.38	49.79±1.93	3.87	37.05±2.27	6.13	17.16±2.18	12.68
Triazophos	80.58±4.25	15.27	86.66±8.01	9.25	76.99±3.18	4.13	32.86±5.70	17.36

Table B14 Percentage recoveries of OPPs obtained from breakthrough volume study for florisil-SPE.

OPP	50 mL		100 mL		250 mL		300 mL	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	44.90±0.30	0.66	38.97±1.45	3.72	71.38±1.76	2.47	30.27±0.83	2.73
Fenitrothion	49.29±1.44	2.93	41.49±0.32	0.78	63.53±1.34	2.10	42.75±1.24	2.89
Malathion	98.90±1.51	1.53	72.77±0.32	0.44	112.25±2.40	2.10	82.71±1.72	2.08
Chlorpyrifos	28.90±0.73	2.53	38.55±0.75	1.94	67.45±1.38	2.05	51.38±1.11	2.17
Triazophos	64.68±1.54	2.37	47.06±1.74	3.69	74.25±2.37	3.19	63.80±3.79	5.94
OPP	400 mL		500 mL		800 mL		1000 mL	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	56.00±0.45	0.81	43.77±0.56	1.27	30.70±0.51	1.67	10.52±0.52	4.94
Fenitrothion	43.63±1.05	2.40	31.77±0.61	1.91	23.85±1.37	5.73	12.83±1.51	11.77
Malathion	78.10±1.54	1.97	59.34±0.35	0.59	47.61±1.65	3.47	5.83±1.45	24.87
Chlorpyrifos	55.02±3.12	5.67	46.90±0.79	1.68	38.63±2.60	6.72	6.89±1.39	20.17
Triazophos	54.40±2.21	4.06	48.83±3.45	7.06	38.30±1.21	3.17	22.53±1.34	5.95

Table B15 Percentage recoveries of OPPs obtained from breakthrough volume study for silica gel-SPE.

OPP	5 mL		10 mL		25 mL		50 mL	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	71.25±3.22	4.52	57.12±1.33	2.33	58.32±2.73	4.68	95.93±2.20	2.29
Fenitrothion	95.31±1.94	2.04	74.74±1.01	1.36	86.14±3.11	3.61	112.05±0.32	0.29
Malathion	93.97±2.79	2.97	69.77±1.94	2.79	77.01±3.00	3.90	96.49±1.51	1.56
Chlorpyrifos	24.84±6.65	6.79	29.92±5.32	17.78	20.25±3.08	15.22	85.59±1.24	1.45
Triazophos	101.18±2.09	2.06	70.44±2.48	3.52	81.02±2.93	3.62	108.02±8.41	7.79
OPP	100 mL		250 mL		500 mL		1000 mL	
	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD	%recovery (mean±SD)	% RSD
Diazinon	73.75±1.49	2.02	53.50±2.19	4.10	17.85±0.13	0.75	5.27±0.33	6.22
Fenitrothion	76.70±2.23	2.91	42.13±3.39	8.04	8.31±4.80	1.05	3.53±1.10	31.18
Malathion	69.53±2.82	4.06	37.88±2.38	6.30	12.16±0.25	2.08	4.27±0.18	4.23
Chlorpyrifos	74.26±9.58	12.90	61.38±4.41	7.18	25.30±0.55	2.16	5.51±0.22	3.97
Triazophos	77.05±0.63	0.82	40.45±1.89	4.67	13.12±0.07	0.53	3.52±0.50	14.11

APPENDIX C

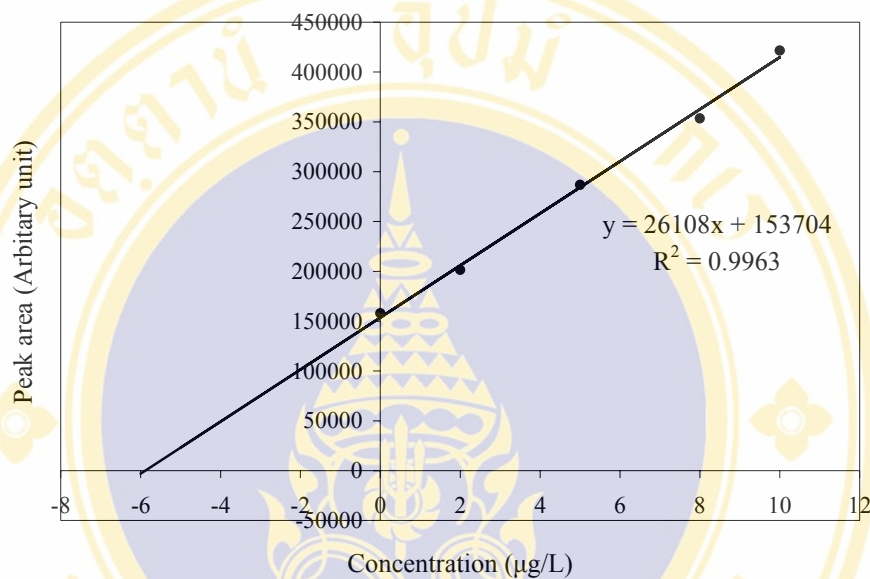


Figure C1 Standard addition assay for determining OPP residues in vegetable samples. Analyses were performed for original sample and series of five samples spiked with increasing concentrations of target compounds. Linear regression analysis of relative responses as a function of spiked concentration; negative intersection with x axis corresponds to detected concentration in sample.

Table C1 Linear regressions of standard addition for cucumber sample.

OPP	Linear equation	R ²	Intersection x axis (= detected concentration in µg mL ⁻¹)
Diazinon	$y = 26860x + 852721$	0.9987	31.7468
Fenitrothion	$y = 22877x + 618942$	0.9980	27.0552
Malathion	$y = 30912x + 568498$	0.9957	18.3901
Chlorpyrifos	$y = 20716x + 556350$	0.9972	26.8561
Triazophos	$y = 14852x + 509697$	0.9990	34.3184

Table C2 Linear regressions of standard addition for cucumber sample.

OPP	Linear equation	R ²	Intersection x axis (= detected concentration in µg mL ⁻¹)
Diazinon	$y = 27098x + 160843$	0.9996	5.9356
Fenitrothion	$y = 26318x + 149530$	0.9988	5.6817
Malathion	$y = 25388x + 105927$	0.9992	4.1723
Chlorpyrifos	$y = 22001x + 98822$	0.9994	4.4917
Triazophos	$y = 19807x + 110584$	0.9975	5.5830

Table C3 Linear regressions of standard addition for tomato sample.

OPP	Linear equation	R ²	Intersection x axis (= detected concentration in µg mL ⁻¹)
Diazinon	$y = 28509x + 152555$	0.9949	5.3511
Fenitrothion	$y = 24028x + 139654$	0.9991	5.8121
Malathion	$y = 26692x + 140015$	0.9971	5.2456
Chlorpyrifos	$y = 22847x + 174506$	0.9982	7.6380
Triazophos	$y = 19752x + 102353$	0.9940	5.1819

APPENDIX D

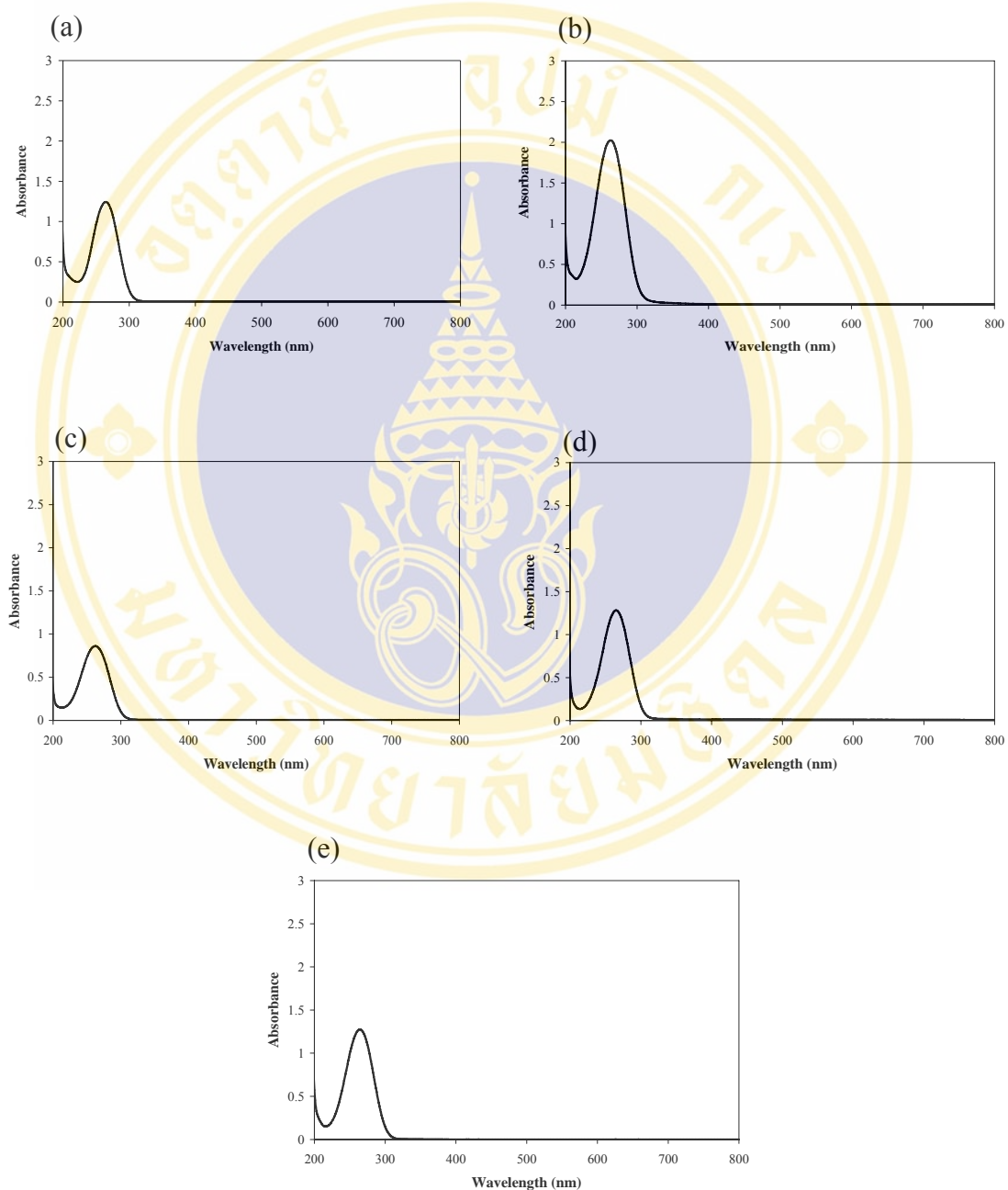


Figure D1 Absorption spectra in the range of 200 - 800 nm of (a) diazinon, (b) fenitrothion, (c) malathion, (d) chlorpyrifos and (e) triazophos in water media.

Table D1 Half-life of OPPs degradation in water at concentration of 0.5 µg/mL⁻¹.

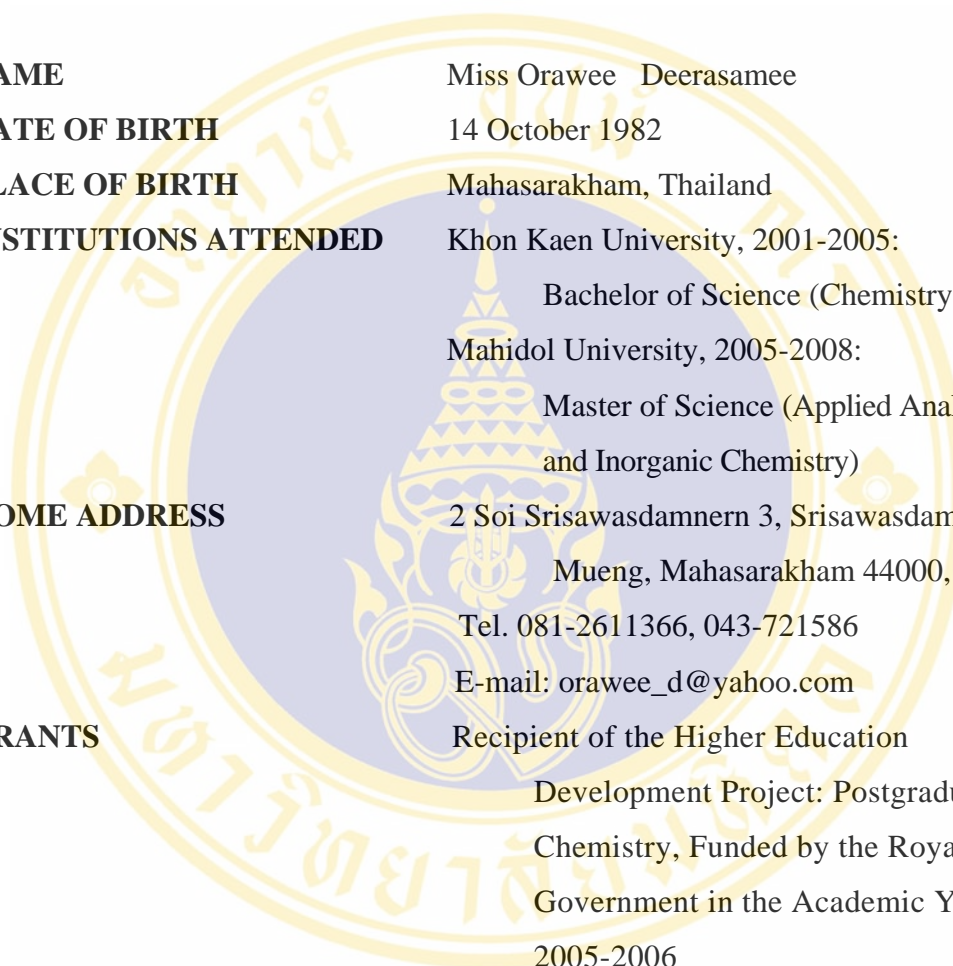
OPP	Sunlight			Darkness		
	Exponential equation	k	Half life (Day)	Exponential equation	k	Half life (Day)
Diazinon	$y = 0.385e^{-0.0522x}$ $R^2 = 0.9598$	0.0522	15.03	$y = 0.3858e^{-0.0461x}$ $R^2 = 0.9117$	0.0461	13.28
Fenitrothion	$y = 0.4868e^{-0.0161x}$ $R^2 = 0.8702$	0.0161	46.82	$y = 0.4723e^{-0.0148x}$ $R^2 = 0.7814$	0.0148	43.04
Malathion	$y = 0.4201e^{-0.0222x}$ $R^2 = 0.9029$	0.0222	30.66	$y = 0.4244e^{-0.0226x}$ $R^2 = 0.8421$	0.0226	31.22
Chlorpyrifos	$y = 0.4135e^{-0.0268x}$ $R^2 = 0.8746$	0.0268	23.81	$y = 0.4318e^{-0.0291x}$ $R^2 = 0.8676$	0.0291	25.86
Triazophos	$y = 0.5428e^{-0.0216x}$ $R^2 = 0.9291$	0.0216	31.50	$y = 0.5317e^{-0.022x}$ $R^2 = 0.9216$	0.0220	32.08

Table D2 Half-life of OPP degradation in water at concentration of 1.0 µgmL⁻¹

OPP	Sunlight			Darkness		
	Exponential equation	k	Half-life (Day)	Exponential equation	k	Half-life (Day)
Diazinon	$y = 0.6855e^{-0.0784x}$ $R^2 = 0.9876$	0.0784	8.88	$y = 0.6759e^{-0.0685x}$ $R^2 = 0.9789$	0.0685	10.12
Fenitrothion	$y = 0.9205e^{-0.0173x}$ $R^2 = 0.8723$	0.0173	40.06	$y = 0.8947e^{-0.0141x}$ $R^2 = 0.8518$	0.0141	49.15
Malathion	$y = 0.8598e^{-0.0212x}$ $R^2 = 0.8989$	0.0212	32.69	$y = 0.8964e^{-0.0221x}$ $R^2 = 0.8882$	0.0221	31.36
Chlorpyrifos	$y = 0.754e^{-0.0208x}$ $R^2 = 0.8922$	0.0208	33.32	$y = 0.7567e^{-0.0222x}$ $R^2 = 0.9144$	0.0222	31.22
Triazophos	$y = 0.9808e^{-0.0224x}$ $R^2 = 0.9006$	0.0224	30.94	$y = 1.0281e^{-0.0259x}$ $R^2 = 0.9410$	0.0259	26.76

Table D3 Half-life of OPP degradation in water at concentration of 2.0 µgmL⁻¹

OPP	Sunlight			Darkness		
	Exponential equation	k	Half-life (Day)	Exponential equation	k	Half-life (Day)
Diazinon	$y = 1.2413e^{-0.1303x}$ $R^2 = 0.9730$	0.1303	5.32	$y = 0.9955e^{-0.1262x}$ $R^2 = 0.8362$	0.0461	5.49
Fenitrothion	$y = 1.6988e^{-0.0171x}$ $R^2 = 0.697$	0.0171	40.53	$y = 1.6623e^{-0.0153x}$ $R^2 = 0.6772$	0.0153	45.29
Malathion	$y = 1.7315e^{-0.0204x}$ $R^2 = 0.9445$	0.0204	33.97	$y = 1.7213e^{-0.0204x}$ $R^2 = 0.8455$	0.0204	33.97
Chlorpyrifos	$y = 1.3608e^{-0.0225x}$ $R^2 = 0.6074$	0.0225	30.80	$y = 1.3705e^{-0.0195x}$ $R^2 = 0.551$	0.0195	35.54
Triazophos	$y = 1.9784e^{-0.0231x}$ $R^2 = 0.8684$	0.0231	30.00	$y = 2.016e^{-0.0272x}$ $R^2 = 0.8935$	0.0272	25.48

BIOGRAPHY

NAME Miss Orawee Deerasamee

DATE OF BIRTH 14 October 1982

PLACE OF BIRTH Mahasarakham, Thailand

INSTITUTIONS ATTENDED Khon Kaen University, 2001-2005:
Bachelor of Science (Chemistry)
Mahidol University, 2005-2008:
Master of Science (Applied Analytical
and Inorganic Chemistry)

HOME ADDRESS 2 Soi Srisawasdamnern 3, Srisawasdamnern Rd.,
Mueng, Mahasarakham 44000, Thailand
Tel. 081-2611366, 043-721586
E-mail: orawee_d@yahoo.com

GRANTS Recipient of the Higher Education
Development Project: Postgraduate in
Chemistry, Funded by the Royal Thai
Government in the Academic Year
2005-2006
Recipient of a Teaching Assistance Scholarship
from the Department of Chemistry,
Faculty of Science, Mahidol University in
the Academic Year of 2006-2007