SAMPLE PREPARATION BY MATRIX SOLID-PHASE DISPERSION FOR DETERMINATION OF ORGANOCHLORINE AND PYRETROID RESIDUES IN GARLICS

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การเตรียมตัวอย่างโดยการกระจายวัฏภาคของแข็งในเมทริกซ์สำหรับการตรวจวัดสารตกค้างชนิดออร์แกโนคลอรีนและไพรีทรอยด์ในกระเทียม

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การกระจายวัฏภาคของแข็งในเนื้อเมทริกซ์เป็นเทคนิคที่ง่าย รวดเร็วและใช้ปริมาณตัวทำละลายอินทรีย์น้อย โดยของแข็งดูดซับจะคลุกเคล้าเป็นเนื้อเดียวกับสารตัวอย่างเพื่อจับสิ่งรบกวนในเนื้อเมทริกซ์ตัวอย่างไว้ ในของแข็งดูดซับผสม จากนั้นชะสารที่ต้องการตรวจวัดออกด้วยตัวทำละลายอินทรีย์ งานวิจัยนี้ใช้เทคนิค MSPD ในการสกัดสารกำจัดศัตรูพืชกลุ่มออร์แกโนคลอรีนและไพรีทรอยด์ 26 ชนิดที่ตกค้างในกระเทียม และตรวจวัดด้วยเทคนิคแก๊สโครมาโทกราฟีที่มีเครื่องตรวจวัดชนิด μECD โดยศึกษาปัจจัยต่างๆที่มี ผลต่อประสิทธิภาพของวิธีการ MSPD ได้แก่ ชนิดของของแข็งดูดซับ ชนิดและปริมาณของตัวทำละลายที่ใช้ในการชะสารออกจากของแข็งดูดซับ สภาวะที่เหมาะสมคือใช้ของแข็งผสมฟลูริซิลและซิลิกาเจล 5.0 กรัม อัตราส่วน 1:1 (น้ำหนักต่อน้ำหนัก) และเฮกเซน 30 มิลลิลิตรเป็นตัวทำละลายที่ใช้ในการชะสารออกจากของ แข็งดูดซับ ทำการตรวจสอบความถูกต้องของวิธีการวิเคราะห์ได้ช่วงความเป็นเส้นตรงของการวิเคราะห์อยู่ในช่วง 0.005-0.5 ไมโครกรัมต่อลิตร ค่าขีดจำกัดต่ำสุดของการวิเคราะห์มีค่าอยู่ในช่วง 3.0 - 4.0 นาโนกรัมต่อกรัม และขีดจำกัดต่ำสุดในการหาปริมาณของวิธีการวิเคราะห์มีค่าอยู่ที่ 10.0 นาโนกรัมต่อกรัม ความเที่ยงของวิธีการวิเคราะห์และการเตรียมตัวอย่างมีประสิทธิภาพดี โดยเมื่อเติมสารมาตรฐานในกระเทียมที่ระดับความเข้มข้น 10, 50 และ 100 นาโนกรัมต่อกรัม ให้ค่าร้อยละของการคืนกลับอยู่ในช่วง 83.6 ถึง 107.0 สำหรับค่าสัมประสิทธิ์ความแปรผันของการวิเคราะห์อยู่ในเกณฑ์ดีน้อยกว่าร้อยละ 7.5 เมื่อเทียบกับค่าที่คำนวณได้จาก Horwitz equation และเมื่อนำสภาวะดังกล่าววิเคราะห์สารในตัวอย่างกระเทียมที่จำหน่ายในประเทศไทย 4 ตัวอย่าง พบว่าตัวอย่างทั้งหมดมีการปนเปื้อนของไซเพอร์เมทรินในระดับความเข้มข้นต่ำและไม่เกินข้อกำหนดของสหภาพยุโรป ดังนั้นวิธีการวิเคราะห์และการเตรียมตัวอย่างในงานวิจัยนี้สามารถนำมาเป็นวิธีมาตรฐานในการวิเคราะห์หาปริมาณสารกำจัดศัตรูพืชกลุ่มออร์แกโนคลอรีนและไพรีทรอยด์ที่ตกค้างในกระเทียม

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Matrix solid-phase dispersion (MSPD) is a simple and fast sample preparation technique that employs very small amount of organic solvent. Sample is dispersed uniformly in the solid support where the matrix and interferences are trapped within the sorbent and the target analytes can be eluted selectively with suitable organic solvent. This research developed a MSPD procedure for the extraction and cleanup of twenty six organochlorine and pyrethroid residues (OCPYs) in garlic for GC-μECD analysis. Optimization of key parameters included types of solid support, and types and volume of the elution solvent. The optimum MSPD condition used 5 g of sorbent 1:1 (w/w) florisil:siliga gel) and 30 mL of hexane to elute twenty six OCPYs. The method has been validated and achieved quantitative analysis down to their maximum residue limit. The linearity range from 0.005-0.50 mg/L, method detection limit is in range of 0.003-0.004 mg/kg and its method quantitation limit is 0.01 mg/kg. Intra –assayprecision, intermediate precision and relative standard deviation were less than 7.5 % when compared with the value from Horwitz equation. For all compounds at 0.01, 0.05 and 0.10 mg/kg, recoveries obtained were generally in the range of 83.6-107.0. The method was used to test 4 sample from 2 Thai markets. All of them were contaminated with Cypermethrin in low concentration and lower than MRLs of EU regulations. The approach can be used as a standard method for quantitative analysis of organochlorine and pyrethroid pesticides in Garlic.

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LIST OF SYMBOLS AND ABBREVIATIONS

α-BHC,HCH	alpha-1,2,3,4,5,6-hexachlorocyclohexane
b -Endosulfan	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine 3-oxide
%	Percentage
°C	degree Celsius
µL	microliter
2,4-D	2,4-dichlorophenoxy acetic acid
AED	atomic emission detector
Aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene
AOAC	association of Official Analytical Chemists
b-BHC,HCH	beta-1,2,3,4,5,6-hexachlorocyclohexane
Bifenthrin	2-methylbiphenyl-3-ylmethyl (1RS,3RS)-3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]
C18	octa decyl carbon
CDFA	The California Department of Food and Agriculture
CI	Chemical ionization
CO_2	carbon dioxide
Cyfluthrin	α-cyano-4-fluoro-3-phenoxybenzyl (1RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
Cypermthrin	α-cyano-3-phenoxybenzyl (1RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
d-BHC,-HCH	delta-1,2,3,4,5,6-hexachlorocyclohexane
Deltamethrin	α-cyano-3-phenoxybenzyl (1R)-cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane
Dicofol	2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol
Dieldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene
dSPE	dispersive solid phase extraction
ECD	electron capture detector
EI	Electron ionization
ELCE	electrolytic conductivity detector
EM	electron multiplier

Endosulfan sulfate	6,7,8,9,10,10-hexachloro-1,5,5A,6,9,9A-hexahydro-6,9-methano-2,3,4-benzodioxy
Endrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethano
EPC	electronic pneumatics control
eV	electron Volts
EU	The Europian Union
FAO	Food and Agriculture Organization of the Uniteds Nations
Fenvalerate	α-cyano-3-phenoxybenzyl (2RS)-2-(4-chlorophenyl)-3-methylbutyrate
FID	flame ionization detector
FPD	flame photometric detector
FSOT	fused-silica open tubular
g	gram
g –Chlordane	trans-1,2,4,5,6,7,8,8-Octachloro-3a,4,7,7a-tetrahydro-4,7-endo-methanoindan
g-BHC,HCH	gamma-1,2,3,4,5,6-hexachlorocyclohexane
GC	gas chromatography
GC-µECD	gas chromatography with micro electron capture detector
GC-MS	gas chromatography with mass spectrometer
GLC	Gas-Liquid chromatography
GPC	gel permeation chromatography
HED	high energy conversion dynode
Hept. Epoxide	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-. 4,7-methano-1H-indene
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
HPLC	high-performance liquid chromatography
I.D.	internal diameter
lamda –Cyhalothrin	α-cyano-3-phenoxybenzyl(1S,3S)-3-[(Z)-2-chloro-3,3,3 trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate and (S)-α-cyano-3-phenoxybenzyl(1R,3R)-3-[(Z)-2-chloro-3,3,3 trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate
LLE	liquid liquid extraction
LLP	liquid liquid partition
LODs	limit of detections
LOQs	limit of quantifications

m/z	mass per charge ratio
Methoxychlor	1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane
mg/kg	milligram per kilogram
mg/L	milligram per liter
min	minute
MIP	molecular imprinting
mL	milliliter
mL/min	milliliter per minute
mm	millimeter
MRL	maximum residue limits
MRMs	multi residue methods
MS	mass spectrometry
MSD	Mass Selective Detector
MSPD	matrix solid phase dispersion
NCI	Negative Chemical Ionization
ng/g	nanogram per gram
NIST-USA	National Institute of Standard
NPD	nitrogen phosphorus detector
o,p'-DDT	1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloroethane
OCPs	organochlorine pesticides
OCPYs	organochlorine and pyrethroid pesticides
ODS	Octadecylsilyl
OPPs	organophosphate pesticides
p,p'-DDD	1,1-dichloro-2,2-bis(4-chlorophenyl)ethane
p,p'-DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethane;2,2-dichloro-1,1-bis(4-chlorophenyl)ethane
p,p'-DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
PCI	Positive Chemical Ionization
Permethrin	3-phenoxybenzyl(1RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanec
PLOT	Porous Layer Open Tubular
PMT	photomultiplier tube
Psi	pound per square inch
PTV	Programmed temperature vaporizing injectors
R^2	correlation coefficient

RF	radio frequency
RSD	relative standard deviations
S.D.	standard deviation
S/N	signal to noise ratio
SFE	Supercritical Fluid Extraction
SIM	selected ion monitoring
SPE	solid Phase Extraction
SPME	Solid phase microextraction
SRMs	single residue methods
TID	thermoinic detector
UHP	Ultra-High Purity
ULV	ultra low-volume
USEPA	U.S. environmental Protection Agency
UV	ultraviolet
w/w	weight by weight
WCOT	wall-coated open-tubular
α –Endosulfan	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxat
µg/kg	microgram per kilogram
µg/L	microgram per liter
µL	microliter
µm	micrometer
v/v	volume by volume

CHAPTER I

INTRODUCTION

1.1 Problem Definition

Currently, consumer behavior is showing an interest in natural products especially herbs. The herbs that can be consumed should have no poisonous residues nor should they cause any side effects to consumers health. In essence, they ought to have medicinal properties to cure medical problems in much the same way as the present day medicines. For all these advantages, the demand for herbs and extracted substances has increased tremendously.

Garlic *(Allium sativum L)* is used as a common food and also for the treatment of many diseases. Garlic has been used throughout history for culinary and medicinal purposes. It is well known for many health benefits such as preventing coronary heart disease, anticancer, antidiabetes, preventing hypercholesterolmia, and antihypertension. Allium is the thiosulfinates, largest and most important representative of garlic. The thiosulfinates, volatile sulfur compounds, which are found in garlic, are also responsible for their characteristic pungent aroma and taste. However, these compounds are very unstable compounds and give rise to further rearrangements leading to a wide variety of derived sulfur compounds. (1) Garlic is easy to grow in mild climates such as Thailand which produces more than 50,000 tons of garlic annually.

Pesticides such as pyrethroids are commonly applied in garlic cultivation to improve yield. Recently, there are concerns about the adverse health effects from pesticide residues that have led to strict regulations in many agricultural commodities. For example, the maximum residue limits (MRLs) of the European Union (EU) can be as low as 0.01 mg/kg depending on particular pesticide and matrix type (2) as shown in Table 1.1.

Sample extraction and cleanup is very important for a successful trace analysis of complex samples such as foods and agricultural products. A gas chromatography is commonly employed to perform a routine pesticide analysis of commodities. This is not a simple task for garlic because the enzyme allinase converts alliin to allicin when the clove is crushed during sample preparation.(1) Allicin is an unstable thiosulphinate that immediately degrades into several sulfur derivatives such as sulfides, sulfonic esters, and sulfonic ethers that contribute to the characteristic flavors and odors of garlic. These sulfur compounds overload the electron capture detector and mask the sensitivity of other compounds. Sample preparation techniques have been applied for the extraction of OCPYs (organochlorine and pyrethroid) in garlic prior to chromatographic analyses. Techniques such as supercritical fluid extraction, deactivation of enzyme by microwave, and liquid-liquid extraction were employed. (3-4) These extraction methods are lengthy, consume large quantity of organic solvent, and cannot effectively remove the interfering sulfur compounds which severely reduce the method recovery. A MSPD (matrix solid phase dispersion) procedure was optimized for the extraction of twenty six OCPYs in garlic prior to the analysis by GC-μECD in this work. Extraction parameters such as types of solid support; types and volumes of the eluent were studied. The data suggested that MSPD is more superior in the cleanup of garlic matrix than other commonly published methods. Only small volumes of sample and organic extractant are employed. The procedure is rapid and can effectively cleanup the sulfur interferences which made MSPD suitable for routine analysis purposes. The reduction of sulfur interferences also helps reduce the maintenance cost of the chromatographic system.

Table 1.1 Maximum Residue Limits (MRLs) of pesticide residues in garlic (2)

Pesticide	MRLs from EU regulations (ng/g)
α-BHC β-BHC δ-BHC	0.01
γ-BHC	0.01
Heptachlor Hept.Epoxide	0.01
Dicofol	0.02
Endrin	0.01
α-Endosulfan Endosulfan sulfate	0.05
α-Chlordane γ-Chlordane	0.01
Aldrin Dieldrin	0.01
p,p'-DDE p,p'-DDD o,p'-DDT p,p'-DDT	0.05
Bifenthrin	0.05
Methoxychlor	0.01
Lambda-Cyhalothrin	0.20
Permethrin	0.05
Cyfluthrin	0.02
Cypermethrin	0.1
Fenvalerate	0.02
Deltamethrin	0.1

1.2 Pesticides

Pesticides is a substance or group of substances made for the purpose of killing, preventing, destroying, repelling or otherwise deterring any pest or “pest” species. The word *pesticide* may refer to insecticides, herbicides, fungicides, or other pest control formulations. A pesticide may be a chemical substance, biological agent (such as a virus or bacterium), antimicrobial, disinfectant or device used against any pest (Pests include insects, plant pathogens, weeds, molluscs, birds, mammals, fish, nematodes (roundworms), and microbes) that destroy property, expand disease or are a vector for disease or cause an annoyance. Pesticides are toxic and often associated with adverse health effects in non-target organisms. Although there are benefits to the use of pesticides, there are also drawbacks, such as potential toxicity to humans and other animals.

FAO has defined the term of *pesticide* as: any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.(5)

Since 2000 BC, humans have utilized pesticides to protect their crops. The first known pesticide was elemental sulfur dusting used in ancient Sumer about 4,500 years ago in ancient Mesopotamia. By the 15th century, toxic chemicals such as arsenic, mercury and lead were applied to crops to kill pests. In the 17th century, nicotine sulfate was extracted from tobacco leaves for use as an insecticide.

The 19th century saw the introduction of two more natural pesticides, pyrethrum, which is derived from chrysanthemums, and rotenone, which is derived

from the roots of tropical vegetables.(6) Until the 1950s, arsenic-based pesticides were dominant.(7) Paul Müller discovered that DDT was a very effective insecticide. Organochlorines such as DDT were dominant, but they were replaced in the U.S. by organophosphates and carbamates by 1975. Since then, pyrethrin compounds have become the dominant insecticide. Herbicides became common in the 1960s, lead by "triazine and other nitrogen-based compounds, carboxylic acids such as 2,4-dichlorophenoxyacetic acid, and glyphosate.(7)

In 1940s, manufacturers began to produce large amounts of synthetic pesticides and their use became widespread.(8) Some sources consider the 1940s and 1950s to have been the start of the "pesticide era."(9) Pesticide use has increased 50-fold since 1950 and 2.3 million tonnes (2.5 million short tons) of industrial pesticides are now used each year.(6) Seventy-five percent of all pesticides in the world are used in developed countries, but use in developing countries is increasing. (10) In 2001 the EPA stopped reporting pesticide use statistics; the only comprehensive study of pesticide use trends was published in 2003 by the National Science Foundation's Center for Integrated Pest Management.(7)

In 1960s, it was discovered that DDT was preventing many fish-eating birds from reproducing, which was a serious threat to biodiversity. Rachel Carson wrote the best-selling book *Silent Spring* about biological magnification. The agricultural use of DDT is now banned under the Stockholm Convention on Persistent Organic Pollutants, but it is still used in some developing nations to prevent malaria and other tropical diseases by spraying on interior walls to kill or repel mosquitoes. (11)

Organochlorine pesticides (OCPY) are a large class of multipurpose chlorinated hydrocarbon chemicals. Organochlorine pesticides break down slowly in the environment and accumulate in the fatty tissues of animals. Thus, they stay in the environment and food web long after being applied. DDT, now banned in the United States because of its harm to the health of wildlife and people, is a notable example of an organochlorine pesticide. Many organochlorine pesticides are endocrine disrupting chemicals, meaning they have subtle toxic effects on the body’s hormonal systems. Endocrine disrupting chemicals often mimic the body’s natural hormones, disrupting normal functions and contributing to adverse health effects.

Organochlorine pesticides as a class are contain compounds that possess high affinity for some of the most persistent organic contaminants in thermal electrons and give strong electron-capture the environment. Although pesticides have low immediate toxicity, they easily accumulate in the body, where they can induce cancer and have a high endocrine disrupting potential. (12)

Pyrethroid is a synthetic chemical compound similar to the natural chemical pyrethrins produced by the flowers of pyrethrums (Chrysanthemum cinerariaefolium and C. coccineum). Pyrethroids constitute a major proportion of the synthetic insecticide market and are common in commercial products such as household insecticides. In the concentrations used in such products, they may also have insect repellent properties and are generally harmless to human beings in low doses but can harm sensitive individuals. They are usually broken apart by sunlight and the atmosphere in one or two days, and do not significantly affect groundwater quality.

Pyrethroids are chemicals that kill insects, including mosquitoes. They can be an important tool in helping to prevent the spread of West Nile virus. Mosquito control professionals mix pyrethroids with water or oil and apply it as an ultra low-volume spray that kills flying adult mosquitoes. When used properly, pyrethroids have been found to pose very little risk to human health and the environment.

Pyrethroids are a group of man-made pesticides similar to the natural pesticide pyrethrum, which is produced by chrysanthemum flowers. Although more than 1,000 pyrethroids have been made, only a few are used in the United States.

Pyrethroids are found in many commercial products used to control insects, including household insecticides, pet sprays and shampoos. Some pyrethroids also are used as lice treatments applied directly to the head and as mosquito repellents that can be applied to clothes. Most pyrethroid mosquito control products can be applied only by public health officials and trained personnel of mosquito control districts. Mosquito control professionals apply pyrethroids as an ultra low-volume (ULV) spray. ULV sprayers release very tiny aerosol droplets that stay in the air and kill adult mosquitoes on contact. Pyrethroids are often mixed with water or oil and applied at rates less than 1/100th of a pound of active ingredient per acre. These pesticides are

approved by the U.S. Environmental Protection Agency (USEPA) for control of adult mosquitoes. After spraying, pyrethroids settle onto the ground and flat surfaces. Because pyrethroids are mixed with water or oil before being applied, the amount of residue left on surfaces is very small. Pyrethroids are broken down by sunlight and other chemicals in the atmosphere. Often, they last only one or two days in the environment. Pyrethroids are not easily taken up by the roots of plants because they bind to the soil. Because of this, pyrethroids usually do not get into groundwater and do not contaminate drinking water supplies. Pyrethroids are eventually broken down in the soil.

1.2.1 Classification of Pesticides

Pesticides can be classified by target organism, chemical structure, and physical state. Pesticides can also be classed as inorganic, synthetic, or biologicals (biopesticides), (13) although the distinction can sometimes blur. Biopesticides include microbial pesticides and biochemical pesticides.(14) Plant-derived pesticides, or "botanicals", have been developing quickly. These include the pyrethroids, rotenoids, nicotinoids, and a fourth group that includes strychnine and scilliroside. (15)

Many pesticides can be grouped into chemical families. Prominent insecticide families include organochlorines, organophosphates, and carbamates. Organochlorine hydrocarbons (e.g. DDT) can be separated into dichlorodiphenylethanes, cyclodiene compounds, and other related compounds. They operate by disrupting the sodium/potassium balance of the nerve fiber, forcing the nerve to transmit continuously. Their toxicities vary greatly, but they have been phased out because of their persistence and potential to bioaccumulate. (15) Organophosphate and carbamates largely replaced organochlorines. Both operate through inhibiting the enzyme acetylcholinesterase, allowing acetylcholine to transfer nerve impulses indefinitely and causing a variety of symptoms such as weakness or paralysis. Organophosphates are quite toxic to vertebrates, and have in some cases been replaced by less toxic carbamates. Thiocarbamate and dithiocarbamates are

subclasses of carbamates. Prominent families of herbicides include pheoxy and benzoic acid herbicides (e.g. 2,4-D), triazines (e.g. atrazine), ureas (e.g. diuron), and Chloroacetanilides (e.g. alachlor). Phenoxy compounds tend to selectively kill broadleaved weeds rather than grasses. The phenoxy and benzoic acid herbicides function similarly to plant growth hormones, and grow cells without normal cell division, crushing the plants nutrient transport system. Triazines interfere with photsynthesis. (15) Many commonly used pesticides are not included in these families, including glyphosate.

- Algicides or algaecides for the control of algae
- Avicides for the control of birds
- Bactericides for the control of bacteria
- Fungicides for the control of fungi and oomycetes
- Herbicides (e.g. glyphosate) for the control of weeds
- Insecticides (e.g. organochlorines, organophosphates, carbamates, and pyrethroids) for the control of insects - these can be ovicides (substances that kill eggs), larvicides (substances that kill larvae) or adulticides (substances that kill adults)
- Miticides or acaricides for the control of mites
- Molluscicides for the control of slugs and snails
- Nematicides for the control of nematodes
- Rodenticides for the control of rodents
- Virucides for the control of viruses

Pesticides can be classified based upon their biological mechanism function or application method. Most pesticides work by poisoning pests. (16) A systemic pesticide moves inside a plant following absorption by the plant. With insecticides and most fungicides, this movement is usually upward (through the xylem) and

outward. Increased efficiency may be a result. Systemic insecticides, which poison pollen and nectar in the flowers, may kill bees and other needed pollinators.

In 2009, the development of a new class of fungicides called paldoxins was announced. These work by taking advantage of natural defense chemicals released by plants called phytoalexins, which fungi then detoxify using enzymes. The paldoxins inhibit the fungi's detoxification enzymes. They are believed to be safer and greener. (17)

1.2.2 Toxicity of pesticides

1.2.2.1 Toxicity of Organochlorine pesticides

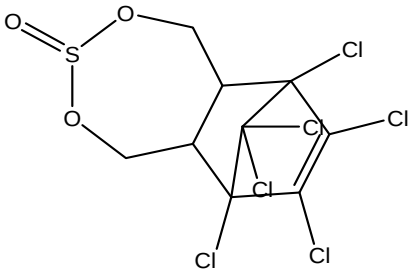
The chlorinated hydrocarbons are all contact poisons, although they penetrate insect cuticle at differing rates. Because they are insoluble in water, they are not translocated within plants. They show a high affinity for fats, and are concentrated in fatty tissues of animals. In varying degrees, chlorinated hydrocarbons are absorbed from the gut and also by the lung and skin. The efficiency of dermal absorption is variable. Endosulfan is efficiently absorbed across the skin, while docofol is not. Lindane is absorbed even more efficiently across abraded skin, which becomes important when taking into account its use on children with severe dermatitis associated with scabies. The chief acute toxic action of the chlorinated hydrocarbons is on the nervous system. Acute symptoms of neurologic toxicity include tremor and involuntary muscular movement, which is due to the prolonged recovery phase of the affected neuron. There has been interest in the interaction of chlorinated hydrocarbons with endocrine receptors, particularly estrogen and androgen receptors. Some experimentation has shown that the function of the endocrine system may be altered by these interactions. (18) This in turn may alter the reproductive development and success of animals and humans. In addition, some chlorinated hydrocarbons may inhibit lactation and may also be developmental toxicants. (19) Besides environmental persistence, evidence of carcinogenic potential of some chlorinated hydrocarbons added more evidence for the Environmental Protection Agency to ban or restrict their uses.

1.2.2.2 Toxicity of Pyrethroid pesticides

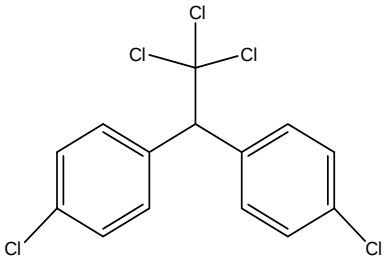
Pyrethroids are synthetic (human-made) forms of pyrethrins. There are two types that differ in chemical structure and symptoms of exposure.

Pyrethroids are one of the least acutely toxic insecticides to mammals because they are quickly deactivated by metabolic processes. However, rats fed high doses (1,000 mg/kg of body weight) showed liver damage. (19) Toxicity by inhalation and dermal absorption is low. Sensitization sometimes occurs in some individuals after a single exposure which causes either an asthmatic condition or a skin rash or inflammation. After the initial exposure to the sensitizing agent, the sensitized individual responds to a dose smaller than the initial dose. Symptoms are more common with exposure to the pyrethroids whose structures include cyano-groups. Sensations are described as stinging, burning, itching, and tingling, progressing to numbness, with the face most commonly affected. Persons treated with permethrin for lice or flea infestations sometimes experience itching and burning at the site of application, but this is more of a reaction to the effects of the parasites themselves. Due to the inclusion of certain solvents, some formulations of fluvalinate are corrosive to the eyes. Scientists have no data from work-related, accidental poisonings, or epidemiological studies that indicate whether or not pyrethrins are likely to cause cancer in humans. There were no birth defects in pups of rabbits exposed to pyrethrins. (20) However, rat pups born to rats fed very high doses of pyrethrins for three weeks prior to mating were of low body weights. Pyrethrins are highly toxic to fish and tadpoles. They affect their skin touch receptors and balance organs. (21)

1.2.3 Structure and chemistry



α-BHC



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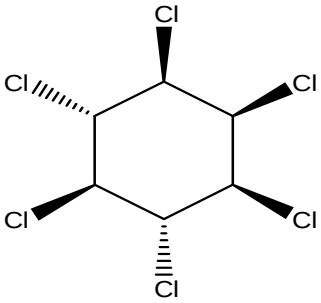

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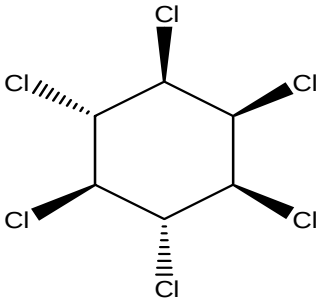

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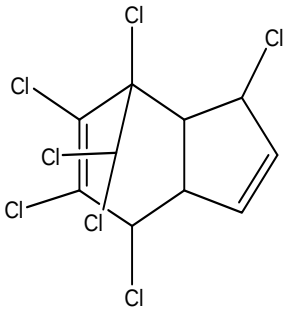
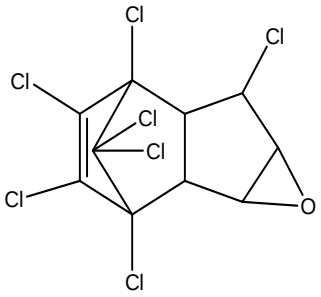
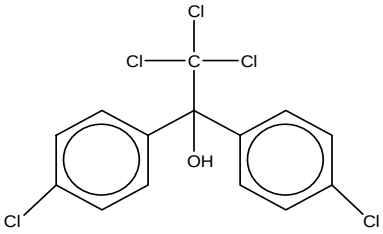


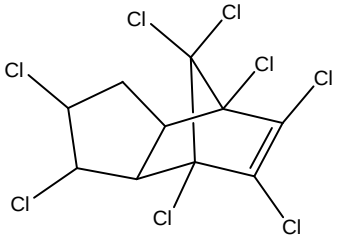
Heptachlor



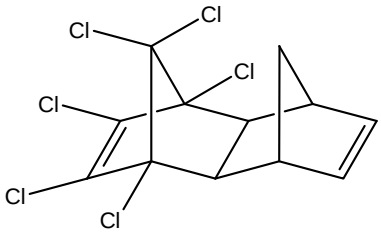
Heptachlor Epoxide



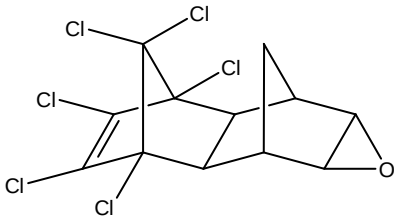
Dicofol



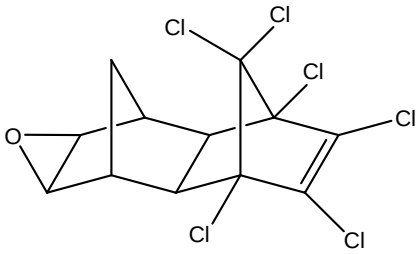
γ , α-Chlordane



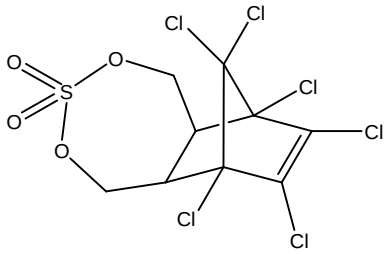
Aldrin



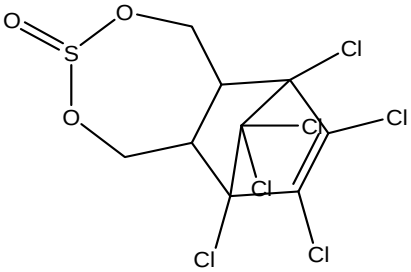
Dieldrin



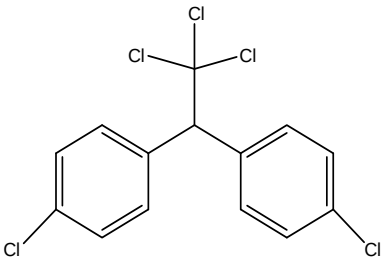
Endrin



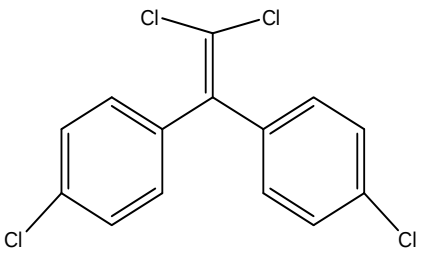
Endosulfan sulfate

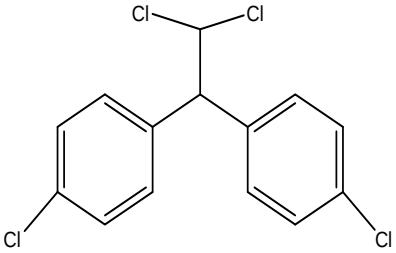


α, β -Endosulfan

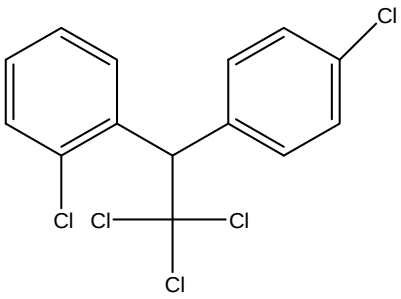


p,p'-DDT

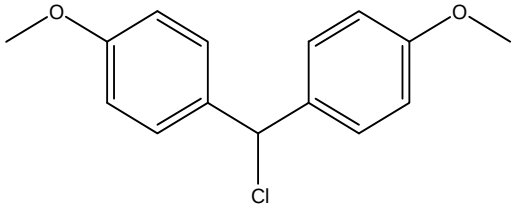




p,p'-DDE



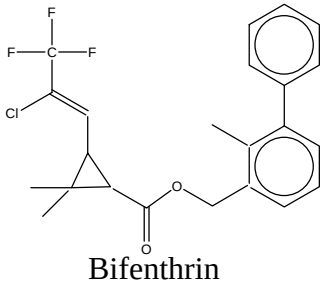
p,p'-DDD



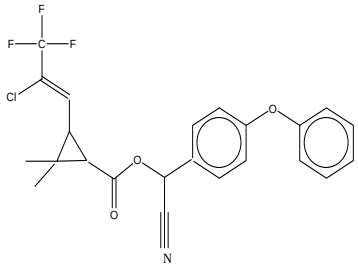
o,p'-DDT

Methoxychlor

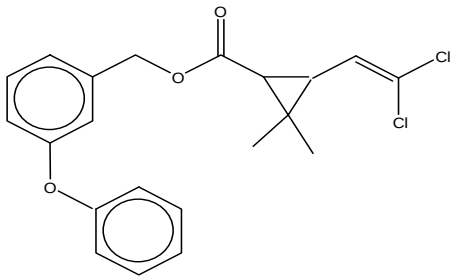
Figure 1.1 Organochlorine pesticides



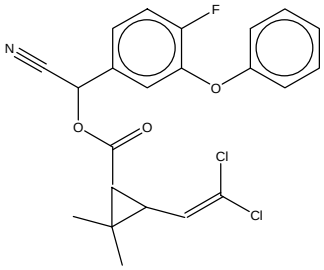
Bifenthrin



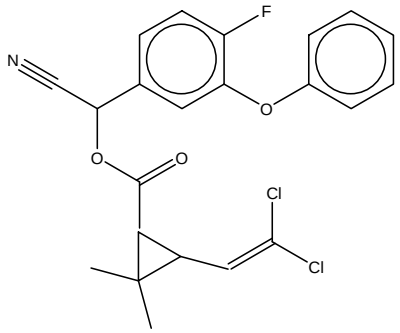
lambda Cyhalothrin



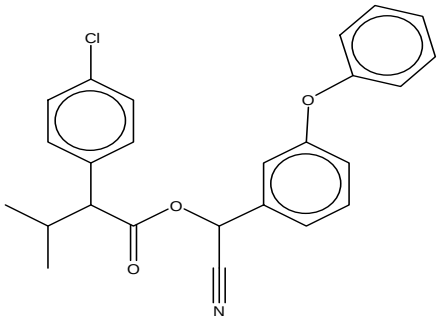
Permethrin



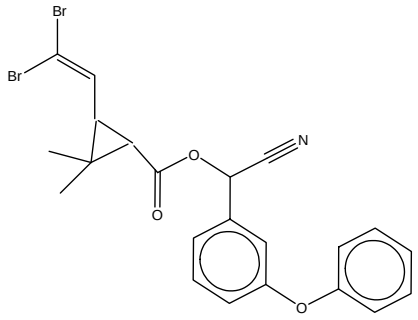
Cyfluthrin



Cypermethrin



Fenvalerate



Deltamethrin

Figure 1.2 Pyrethroid pesticides

1.3 Garlic (*Allium sativum* Linn.)

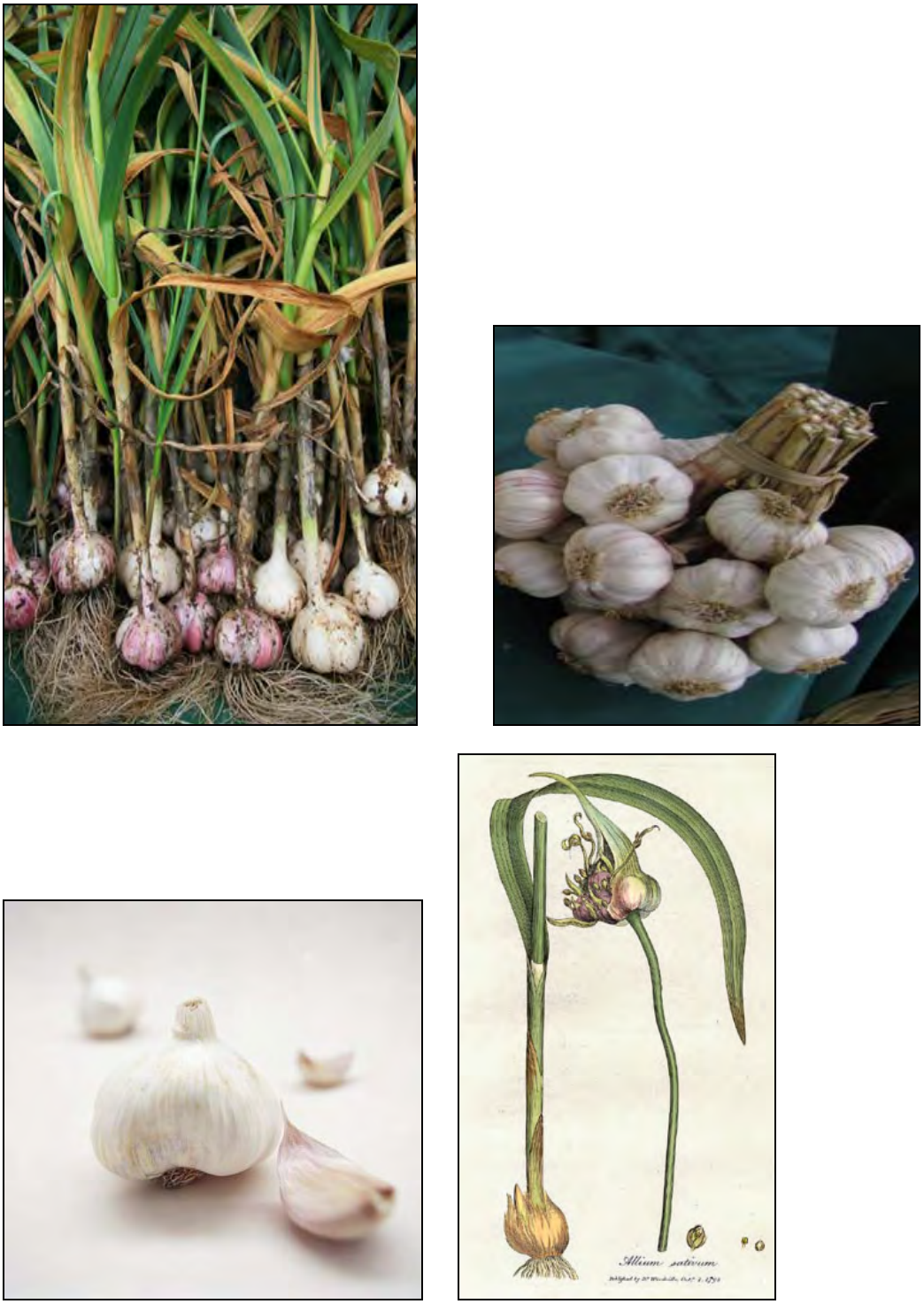


Figure 1.3 Garlic ***(****Allium sativum Linn****.)***

Allium sativum Linn, which belongs to the Alliaceae family, Allium Genus and A. sativum species. It has many common names, such as garlic. Garlic has been used throughout history for both culinary and medicinal purposes. The garlic plant's bulb is the most commonly used part of the plant. With the exception of the single clove types, the bulb is divided into numerous fleshy sections called cloves. The cloves are used for cloning, consumption (raw or cooked), or for medicinal purposes, and have a characteristic pungent, spicy flavor that mellows and sweetens considerably with cooking. (22) The leaves, and flowers (bulbils) on the head (spathe) are also edible, and being milder in flavor than the bulbs, they are most often consumed while immature and still tender. Additionally, the immature flower stalks (scapes) of the hardneck and elephant types are sometimes marketed for uses similar to asparagus in stir-fries. (23)

The first citation of these plants is found in the Codex Ebers (1550), an Egyptian medical papyrus reporting several therapeutic formulas based on garlic as a useful remedy for a variety of diseases such as heart problems, headache, bites, worms and tumours. (24) Cloves of garlic have been found in the tomb of Tutankhamen and in the sacred underground temple of the bulls of Saqqara. Egyptians thought garlic and onions aided endurance and assumed large quantities of them. Raw plants were routinely given to asthmatics and to those people suffering bronchial-pulmonary complains. Later on, these food plants were known by Greeks and Romans, who used them as important healing agents just as they still are used from most of the people of the Mediterranean area. (25) Allium species are a rich source of phytnutrients, useful for the treatment or prevention of a number of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, diabetes type 2, hypertension, cataract and disturbances of the gastrointestinal tract (e.g. colic pain, flatulent colic and dyspepsia). (26-30)

1.3.1 Phytochemical of Garlic

The main phytochemicals in garlic are alliin, methiin and S-allyl cysteine. When garlic is damaged or crushed the sulphur components are transformed in different organosulfur compounds. Enzymes in garlic will convert alliin into allicin, which has antimicrobial action. Allicin is not very stable, so it will have no biological effect when ingested by humans. Even when large quantities are consumed no allicin is detectable either in the serum or urine. S-allylcysteine on the other hand is bioavailable and has the ability to lower cholesterol, act as an antioxidant, inhibit the cancer process and protect the liver from toxins. S-allylcysteine is present in aged garlic extract and has been well researched. The safety of aged garlic extract has been confirmed by many studies.

Many of these biological effects are related to the thiosulfinates, volatile sulfur compounds, typical of the Allium plants, which are also responsible for their characteristic pungent aroma and taste. However, these compounds are unstable and give rise to transformation. For this reason, recent attention has been focused on polar compounds that are more stable for cooking and for the storage. Among these compounds, sapogenins, saponins, and flavonoids are the main classes found. This growing interest follows a general trend that is oriented to the analysis of secondary metabolites from foods. These compounds, recently named with the terms of “nutraceutical” or “phytochemicals” are classified as non-essential micronutrients and are able to contribute to human homeostasis, playing a role in the maintenance of health. (31) Such interest was due to the results of epidemiological studies that have correlated a semi-vegetarian diet and a decreased incidence of chronic- and acute-inflammatory diseases such as arteriosclerosis and cancer. The identification of organic compounds responsible for these activities has increased scientific studies of food plants. (32) Thus, nutritional sciences usually interested in quality and safety of foods has moved to the issue of promotion of well-being, with increased attention to the analysis of secondary metabolites from edible plants. In this intense scientific debate garlic and onion also received a renewed attention.

Allium is the largest and most important representative genus of the Alliaceae family. These molecules originate from *S*-alk(en)yl-l-cysteine-*S*-oxide (1a–1d, Fig. 1),

located in the cytoplasm, through an enzymatic reaction catalyzed by alliinase, a C-S lyase present in the vacuoles, giving initially sulfenic acids (2a–2d, Fig. 1). These are highly reactive intermediates that immediately produce thiosulfinates by condensation reaction (3a–3k, Fig. 1.4)

Allicin is garlic's defence mechanism against attacks by pests. When the garlic plant is attacked or injured it produces allicin by an enzymatic reaction. The enzyme alliinase, converts the chemical alliin to allicin, which is toxic to insects and microorganisms. The antimicrobial acivity of allicin was discovered in 1944 by Cavallito. Purified allicin is not sold commercially because it is not stable and has an offensive odour. Allicin extracted from garlic loses its beneficial properties within hours and turns into other sulphur containing compounds. Diallyl trisulfade, which is similar to allicin but is chemically produced, is stable and is used for treatment of bacterial, fungal and parasitic infections

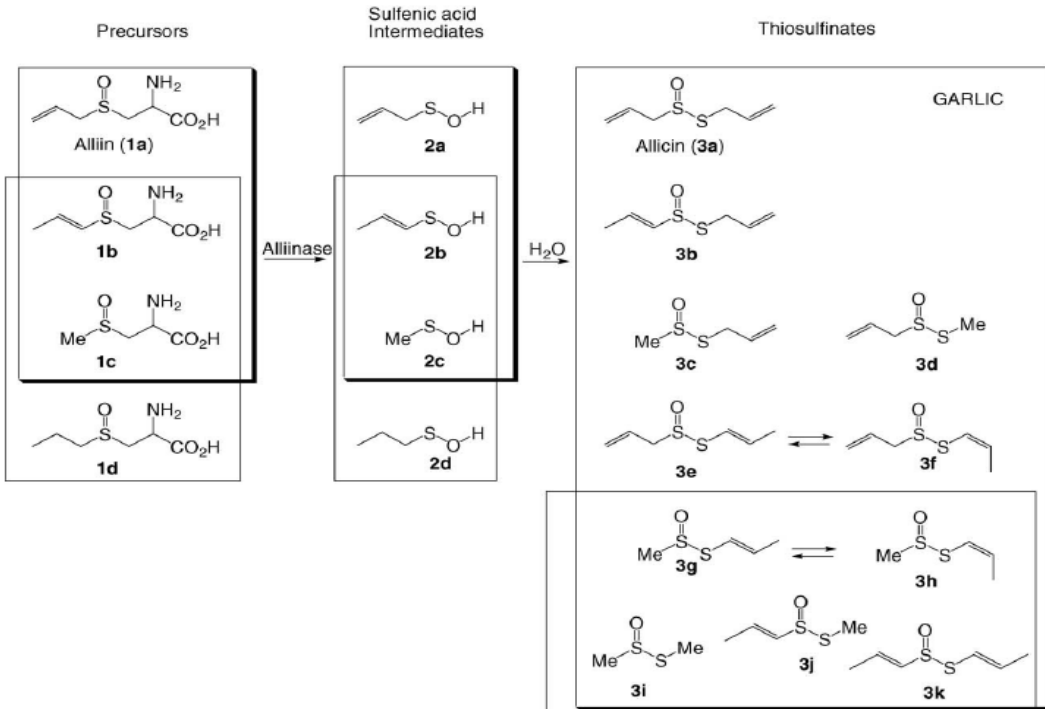


Figure 1.4 Biosynthetic pathway of Thiosulfinate (1)

1.3.2 Activities of Garlic (33)

Allicin, one of the sulfur compounds of garlic, possesses antioxidant activity and is shown to cause a variety of actions potentially useful for human health. Allicin exhibits hypolipidemic, antiplatelet, and procirculatory effects. It demonstrates antibacterial, anticancer and chemopreventive activities. In addition, aged garlic extract possesses hepatoprotective and neuroprotective. But a factor that will limit the biological activity of allicin is its instability. Fresh crushed garlic cloves generate antibacterial activity and chemically detectable allicin, but this activity declines on a daily basis in aqueous and ethanol solutions. Allicin is also not bioavailable and will not get absorbed in the blood, even after ingesting large amounts of allicin.

1.3.2.1 Antimicrobial Activities

The antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes. The phytochemical inhibits bacteria and viruses, but also yeasts such as Candida. By its antimicrobial activity, allicin may be an effective therapeutic candidate to promote ulcer healing. In vitro-studies have demonstrated the antimicrobial activity against various pathogens, such as Helicobacter pylori, Staphylococcus aureaus, Escherichia coli and Lancefield group B streptococci.

1.3.2.2 Anticancer Activities

In vitro studies show that allicin inhibits the invasion and metastasis of human colon carcinoma cells. The phytochemical also exhibits antigenotoxic action. But the anticancer effect of allicin in humans remains uncertain, because of its low stability and poor bioavailability.

1.3.2.3 Heart health Activities

Garlic has been suggested to improve heart health by lowering blood pressure, but scientific studies have provided conflicting results. One study showed that the protective role of allicin against atherosclerosis, is not only the direct result of its antioxidant activity but also of other mechanisms, such as lipoprotein modification, inhibition of LDL uptake and degradation by macrophages.

1.4 Literature review

Organochlorine and pyrethroid pesticide residues may be recovered from agricultural or other biological samples by exhaustive extraction with a variety of solvents. Analytical methods are needed to screen, quantify, and confirm pesticide residues in fruit and vegetables. Multi residue methods (MRMs) and single residue methods (SRMs) generally consist of the same basic steps. However, MRMs are more preferred than SRMs for the analysis of pesticides, because MRMs provide the capability of determining different pesticide residues in a single analysis. (34) The MRMs concept is raised to a higher dimension when a single extract is examined with more than one chromatographic deternative step, each providing the coverage of residues in a different class, e.g. chlorinated hydrocarbon, organophosphates and carbamate. (35)

The MRMs is potentially capable of determining any residue extracted by its extraction step. Each determinative step in MRMs provides coverage for a particular group of residues in the extract, and each clean up-step is designed to purify the extract sufficiently to permit accurate determination. Over 30 years ago, among the more widely used MRMs were those of Mills (36);Mills, Onley and Gaither (37); Storherr (38) Luke (39); and Krause. (40)

A sample preparation by multi-residues method for pesticides in fruits, vegetables, soils and water, began in 1963-Mills, Gaither-MOG procedure. The extraction was done with acetone or acetonitrile, partitioning with petroleum ether, cleaning up with florisil, and detection with GC-ECD and paper and thin layer chromatography. The scope of the method did not include the more polar organonitrogen and organophosphorous pesticides. (41)

The method of the Association of Official Analytical Chemists (AOAC), typifies the international recognized MRMs. The method has made it possible to determine many extractions and laborious clean-up. The methods, generally consist of an extraction step with a water miscible solvent, follow by a clean-up step with an organic solvent of limited water capacity to achieve the removal of interferences present in the sample extract, and/or solid phase clean-up with silica or florisil.

Finally, analytic determination is performed by gas chromatography (GC) or high-performance liquid chromatography (HPLC) with selective detectors. (42) These methods detect approximately 325 pesticides and pesticide-related compounds and most of them have undergone rigorous multi-laboratory calibration studies, such as those needed to obtain the official acceptance by the AOAC. (43) However, its continued use still presents some disadvantages, such as (i) the amount of chemicals and toxic solvents used (ii) their inefficiency as a screening method. The method is too complex, and it did not allow the generation of relevant data in time to prevent contaminated foods from entering the marketplace, because the procedures were time-consuming and laboriously intensive. In addition, the newly developed groups of pesticides are getting more and more polar and/or thermo degradable, and they should be incorporated into the existing MRMs.

To avoid the general drawbacks of the classical methods, methylene chloride partition is applied after two petroleum ether partitions. More polar pesticides such as mevinphos can be determined with a newly developed thermoinic detector (TID) for phosphate and nitrogen residues. In 1972, modification was made to analyze even more polar compounds; particularly methamidophos. The petroleum ether partitioning was eliminated. Water was not added to acetone to obtain a better recovery of the water-soluble compounds. TID is used to analyze P and N containing residues. ECD is used after florisil clean-up. In 1975, the acetone in partitioning step was replaced by petroleum ether to eliminate the precipitate from occurring during the analysis of some fruits. Acetone extraction was usually preferred since it was suitable for both non- polar and polar pesticides, (44-45) as demonstrated in different comparative studies performed by GC and HPLC. Acetone has low toxicity. It is easy to purify, evaporate and filter. And, above all, it is cheap. Fruit and vegetable extracts in acetone are usually cleaner than those obtained with other solvents of similar polarity. The National Food Administration of Sweden (46) also used acetone extraction which was Followed by partitioning with hexane-dichloromethane, and twice with dichloromethane. After the clean-up method on an SX-3 permeation chromatography column, residues were determined by GC using ECD, NPD, FPD and FID. In Germany, pesticides analysis in fruits and vegetables is mainly performed with

MRMs. This method was developed to obtain extracts suitable for GC determination with selective detectors, mainly ECD, NPD and FPD. Fruits and vegetables are extracts with acetone-dichloromethane, and pesticide residues are detected after the clean-up by gel permeation chromatography (GPC) and mini-silica gel column fractionation in up to six fractions. The data about elution and recoveries of more than 400 pesticides and their metabolites as well as a few common pollutants are well documented. (47-48)

In 1981, the flame photometric detector (FPD) that could be used as P or S mode with The Hall electrolytic conductivity detector (ELCE) which could be used in a halogen or nitrogen detection mode was developed. So that the florisil clean-up step for determination of the chlorinated pesticides could be eliminated for the first time after the use of ELCD in halogen mode.

In 1982, the process of simultaneous extraction and clean-up in chromatographic column mixing or blending aqueous samples with silica gel or alumina was introduced to remove lipids prior to liquid-liquid partitioning and florisil clean-up.

A rapid and efficient multi-residue extraction procedure using ethyl acetate and sodium sulfate, followed by GPC on a SX-3 column, was first reported by Roos et al. (49) Recoveries better than 90% were obtained for OCPs and OPPs. The ethyl acetate and sodium sulfate extraction without further clean-up was applied as screening method for the analysis using GC-FPD and GC-NPD. Interfering chromatographic peaks were decreased and the analysis time and use of solvent reduced, resulting in cheaper analysis.

In 1985, the application of GPC for a clean-up of more than 300 pesticides were reported that they could be used to simultaneously clean chemical residues. In the same year there appeared the development of solid phase extraction (SPE) C-18 bonded silica gel cartridges, XAD resins, Tenax-GC cartridges that were applied for extraction and preconcentration of pesticides from air and water samples. To replace classical LLP, and to reduce analysis costs and pollution, the SPE method has been developed. In this process, the compound is isolated from a liquid sample by

differences in the elative solubilities between a liquid mobile phase and a stationary phase. The modified Mill's method, consisting of acetonitrile and clean-up on C-18 (50) was developed by The California Department of Food and Agriculture (CDFA).

The pH of the filtrate is adjusted to neutral with phosphate, and the acetonitrile layer is separated from the the aqueous layer by a salting out process. This method was Evaluated by analysis for seven OCPs at 0.1 – 0.2 ug/g in six representative fruits and Vegetables using GC and HPLC. SPE cartridges, containing. normal or reversed-phase supports, have become available commercially and offered the potential of simplifying the purification of the initial extract as well as educing the amount of Solvent consumed. C-18 commercial cartridges were examined for the clean-up of crop extracts on the determination of fungicide and OCPs.

Matrix solid-phase dispersing (MSPD) is a new extraction and clean-up technique, that has been developed to avoid the general drawbacks of the LLE, such as the use of large amounts of solvent, the occurrence of troublesome emulsion with certain fruit or vegetable matrices and their slowness. (51) This method constitutes a significant advance in simplicity and efficiency that make it possible to screen more samples. Kadenski et al. demonstrated the applicability of MSPD to a large number of fruit and vegetable matrices for pesticide residues. In most cases, samples were added with distilled water, if necessary, for proper blending. Plant material was mixed with florisil and, after that, extracted with methylene chloride-acetone or ethyl acetate. The analytical performance of the method and the MRLs were established by the EuropenaUnion. Recently, Supercritical Fluid Extraction (SFE) has been recognized in the field of Pesticide residue analysis. Lehotay et al. (52) demonstrated in an initial study, that the Extraction of various pentachloronitrobenzene pesticides from vegetables by SPE was clean enough for direct injection to GC-MS in EI mode. The selection of the appopiate SPE conditions such as co_2 density, temperature modifier, type of solid phase used for happing the analytes, and elution solvent was manipulated to overcome most chromatographic methods. In their later work, same authors used a SPE multi-residue method for the determination of 46 pesticides of different polarities and physico-chemical properties from fruits and vegetables, followed by GC-MS. Recoveries obtained were over 80% except for methamidephos, which was not recovered at all.

For the development of instrumental analysis, in recent years, capillary columns have almost completely replaced the packed column owing to their high resolving power, which allows the separation of a large number of pesticides with similar physico- chemical characteristic. (53) The most frequently used detectors include ECD,NPD, FPD and MSD. The last one has becomes the standard confirmatory technique MIP-AED (molecular imprinting with atomic emission detector, which allows the specific detection of many elements, has recently been applied to the determination of pesticides. In the past 30 years, the ECD has been the detector mostly used in pesticide residue analysis. It presents a very high sensitivity to other halogenated pesticides but its selectivity is rather poor. A new detector introduced in 1989 is the AED, used for its selective detection of the elements fluorine, chlorine, bromine, iodine, phosphorous, sulfur and nitrogen. Mass Spectrometer Detector can be employed to achieve selective detection, by full scanning or selective ion monitoring, of target pesticides in the presence of the complex matrix. Quantification is usually achieved by the technique of selected ion monitoring (SIM). With this technique selectivity is also improved. In addition, a multi-residue method for screening OPP residues in fruit and vegetable samples, with an ion trap mass spectrometer in the chemical ionization mode, has been developed. Solid phase micro extraction (SPME) is a sample preparation technique that, since its development by Pawliszyn and co-workers in the early 1990s (54-55) has received increasing attention. Advantages of this new solvent – free extraction technique, as simple or capable of injecting the whole extracted sample, have been discussed in several papers.(56-59) Development of an adequate SPME procedure for pesticide determination should allow us to achieve a reduction in sample manipulation, even eliminating the need for clean - up steps. Several applications of SPME to pesticide determination in biological samples can be found for organochlorine (60) and organophosphorous pesticides. (61-62)

In 1998, levels of 5 organochlorine pesticide in garlic were using supercritical fluid extraction (SFE) and quantified by GC-μECD. Recoveries were measured from 85.0% to 110% with the relative standard deviations (RSD) below 7.2%. (3)

In 2000, levels of 14 organochlorine pesticides in spices powder that were not Curcumin were extracted with n- hexane-dichloromethane (4:1) and the extracts were

cleaned in single step on a cartridge packed with silica and florisil, quantified by GC-MS in SIM mode. Recoveries were measured from 60%for dieldrin and endrin to 97% for other pesticides. (4)

In 2008, levels of 16 herbicides in onion were extracted with acetonitrile and preventing formation of sulfur-containing compounds in onion by microwave inactivation of the enzyme alliinase. The extracts were cleaned by solid-phase extraction quantified by GC-MS in SIM mode. Recoveries were measured from 69.2% to 105% with the relative standard deviations (RSD) below 10.7%. (63)

1.5 Purpose of the study

The sample preparation and quantitative analysis of organochlorine and pyrethroid pesticide residues in each country have different methods which is one of the reasons for this trade-barrier. In Thailand, there has been no standard method for garlic sample preparation to analyze for contaminated organochlorine and pyrethroid pesticide residues. There are several problems involved in sample preparation and quantitative analysis of organochlorine and pyrethroid pesticide residues. Interferences in garlic include chemical substances such as organosulfur compounds alliin, allicin, methiin S-allyl cysteine and volatile oils. This matrix is very complex and requires a more challenging clean- up process for trace analysis. Garlic contains a large amount of volatile oils and organosulfur compound from those of low to more polarities. Obviously, this leads to the fact that the sample preparation of pesticides from garlic is more likely to be difficult too.

From literature review, organochlorine and pyrethroid pesticide residues were analyzed with various sample preparation and detection techniques. In garlic sample, organochlorine and pyrethroid pesticide residues were analyzed with supercritical fluid extraction (SFE) and liquid-liquid extraction (LLE). GC-μECD and GC-MS are a common detection technique because of their selectivity and high sensitivity. However, these extraction methods are lengthy, consume large quantity of organic solvent, and could not effectively remove the interfering sulfur compounds which severely reduce the method recovery.

Matrix solid phase dispersion (MSPD) has found particular application as an analytical process for the preparation, extraction and fractionation of solid, semi-solid and/or highly viscous biological samples. Its simplicity and flexibility have been cited as contributing to it being chosen over more classical methods for these purposes. Indeed, MSPD is based on several simple principles of chemistry and physics, involving forces applied to the sample by mechanical blending to produce complete sample disruption and the interactions of the sample matrix with a solid support bonded-phase (SPE) or the surface chemistry of other solid support materials.

In this work, MSPD based on matrix solid phase dispersion was chosen for the determination of organochlorine and pyrethroid pesticide residues in garlic and compared with those of QuEChERS Dispersive SPE used as a purification procedure and liquid liquid extraction (LLE) and pretreated interference of sulfur-containing compounds with microwave heating, water bath steaming. The organochlorine and pyrethroid pesticide ; alpha-Hexachlorocyclohexane (α-HCH), beta-Hexachlorocyclohexane (β-HCH), gamma-Hexachlorocyclohexane (γ-HCH), delta-Hexachlorocyclohexane (δ-HCH), Heptachlor, Aldrin, Dicofol, Heptachlor epoxide, gamma-Chlordane, alpha-Endosulfan (Endosulfan I), p,p-DDE, Dieldrin, Endrin, beta-Endosulfan (Endosulfan II), p,p-DDD, o,p-DDT, Endosulfan sulfate, p,p-DDT, Methoxychlor, Bifenthrin, lamda Cyhalothrin, Permethrin, Cyfluthrin, Cypermethrin, Fenvalerate and Deltamethrin; are commonly used as pesticide residues in fruit and vegetables easily transferred to the environment. Their residues usually exist in low amounts and induce the difficulty to extract from complex matrices. The structure and property of these pesticides are shown in Table 1.2.

In MSPD, the sample was dispersed homogeneous in a mixture of solid support and the interference in the sample was trapped in the sorbent. Then, the sorbent was eluted by organic solvent. After extraction, the extracting solutions was placed in freezer approximate 6 hours, these solutions were separating into two layers. Finally, the upper layer solution was directly injected to GC-μECD. MSPD can simultaneous enrich and clean-up analytes from sample matrix. The related parameters were optimized such as type of solid support, type and volume of elution solvent. The

optimized MSPD method was applied with various extraction methods for extracted organochlorine and pyrethroid pesticide in garlic obtained from a local market.

Table 1.2 The studied Organochlorin and Pyrethroid pesticides properties

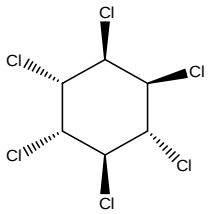
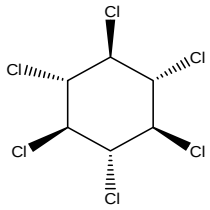
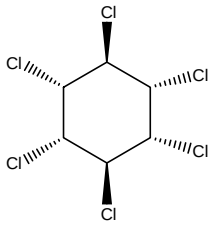
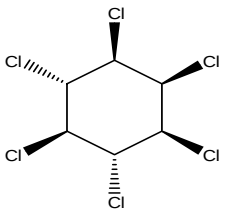
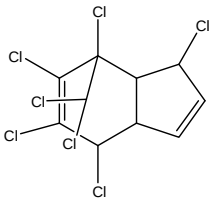
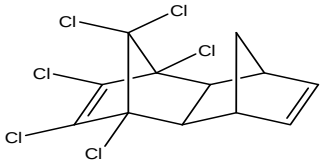
Analyte	Chemical structure	Chemical formula	Molecular mass (g/mol)
α-HCH		$C_6H_6Cl_6$	288
β-HCH		$C_6H_6Cl_6$	288
γ-HCH		$C_6H_6Cl_6$	288
δ-HCH		$C_6H_6Cl_6$	288
Heptachlor		$C_{10}H_5Cl_70$	386
Aldrin		C12H8Cl6	362

Table 1.2 continued

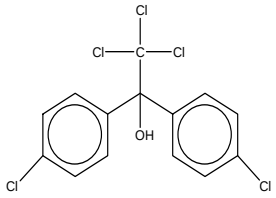
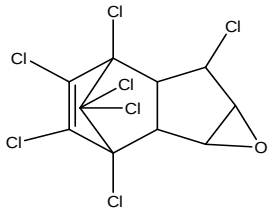
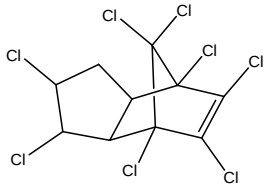
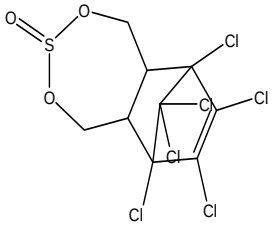
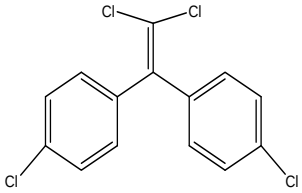
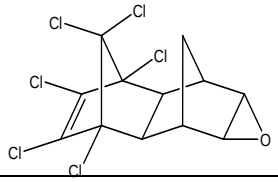
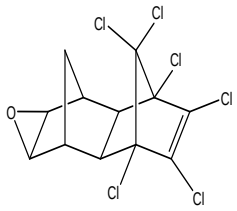
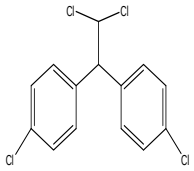
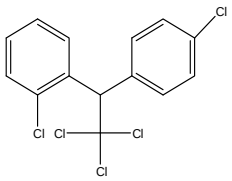
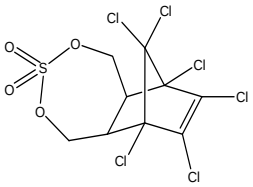
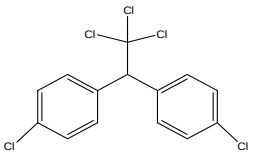
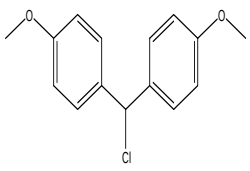
Analyte	Chemical structure	Chemical formula	Molecular mass (g/mol)
Dicofol		C13H8Cl20	250
Hept. epoxide		C10H5Cl70	386
α,γ-Chlordane		C10H6Cl8	406
α-Endosulfan		C9H6Cl6O3S	404
p,p-DDE		C14H8Cl4	316
Dieldrin		C12H8Cl6O	378

Table 1.2 continued

Analyte	Chemical structure	Chemical formula	Molecular mass (g/mol)
Endrin		C12H8Cl6O	378
p,p-DDD		C14H10C14	318
o,p-DDT		C14H9Cl5	352
Endosulfan sulfate		C9H6Cl6O4S	420
p,p-DDT		C14H9Cl5	352
Methoxychlor		C16H15Cl302	344

able 1.2 continued

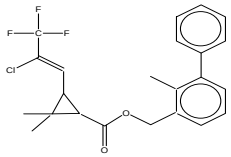
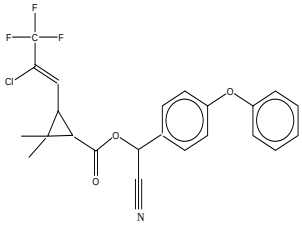
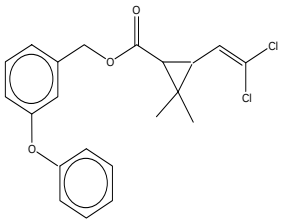
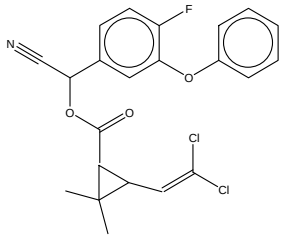
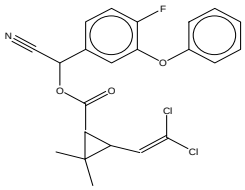
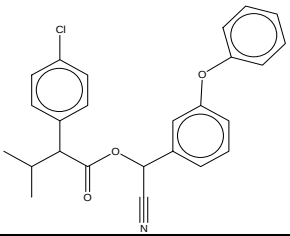
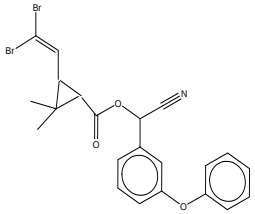
Analyte	Chemical structure	Chemical formula	Molecular mass (g/mol)
Bifenthrin		C23H22ClF3O2	422
lamda - Cyhalothrin		C23H19ClF3NO3	449
Permethrin		C21H20Cl2O3	390
Cyfluthrin		C22H18Cl2FNO3	433
Cypermthrin		C22H19C12NO3	415
Fenvalerate		C25H22ClNO3	419

Table 1.2 continued

Analyte	Chemical structure	Chemical formula	Molecular mass (g/mol)
Deltamethrin		C22H19Br2NO3	503

CHAPTER II

THEORY

Sample preparation is the crucial first step in the analysis. The determination of trace contaminants in complex matrices, such as food, often requires extensive sample extraction and preparation regimes prior to instrumental analysis. The amount of sample preparation needed depends on the sample matrix and the properties and level of analyte to be determined. The typical steps within sample preparation include sampling/homogenization, extraction, clean-up and concentration followed by the final analysis. Another step that can be included at several points is derivatisation. For the determination of trace organics, the final analysis is invariably achieved using a powerful separation technique, typically chromatographic, combined with an appropriate detector. The selective extraction of analytes is based on differences in their chemical and physical properties. These typically include molecular weight, charge, solubility (hydrophobicity), polarity, or differences in volatility. Some extraction methods, such as immunoaffinity and imprinted polymers, utilise selectivity for specific structural groupings or mimic a biological selectivity.

2.1 Solvent extraction (Liquid-liquid extraction)

Liquid-liquid extraction (LLE), also known as solvent extraction and partitioning, is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid phase into another liquid phase. Liquid-liquid extraction is a basic technique in chemical laboratories, where it is performed using a separator funnel. This type of process is commonly performed after a chemical reaction as part of the work-up. Analytes in solution or liquid samples can be extracted by direct partitioning with an immiscible solvent. Liquid–liquid extraction is based on the relative solubility of an analyte in two immiscible phases and is governed by the equilibrium distribution/ partition coefficient. Extraction of an analyte is achieved by the differences in solubilising

power (polarity) of the two immiscible liquid phases. Liquid–liquid extraction is traditionally one of the most common methods of extraction, particularly for organic compounds from aqueous matrices. Typically a separating funnel is used and the two immiscible phases are mixed by shaking and then being allowed to separate. To avoid emulsions, in some cases, salt may be added and centrifugation can be used if necessary.

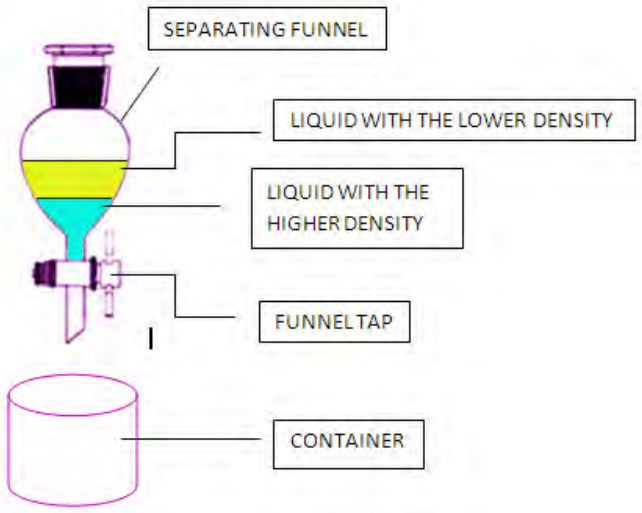


Figure 2.1 Two layers separating during a liquid-liquid extraction

2.9 Solid phase extraction

Solid-phase extraction (SPE) is a separation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties. Analytical laboratories use solid phase extraction to concentrate and purify samples for analysis. Solid phase extraction can be used to isolate analytes of interest from a wide variety of matrices. SPE uses the affinity of solutes dissolved or suspended in a liquid or a solid through which the sample is passed to separate a mixture into desired and undesired components. The result is that either the desired analytes of interest or undesired impurities in the sample are retained on the stationary phase. The portion that passes through the stationary phase is collected or discarded, depending on

whether it contains the desired analytes or undesired impurities. If the portion retained on the stationary phase includes the desired analytes, they can then be removed from the stationary phase for collection in an additional step in which the stationary phase is rinsed with an appropriate eluent.

Solid phase extraction cartridges and disks are available in a variety of stationary phases, each of which can separate analytes according to different chemical properties. Most stationary phases are based on silica that has been bonded to a specific functional group. Some of these functional groups include hydrocarbon chains of variable length (for reversed phase SPE), quaternary ammonium or amino groups (for anion exchange), and sulfonic acid or carboxyl groups (for cation exchange).

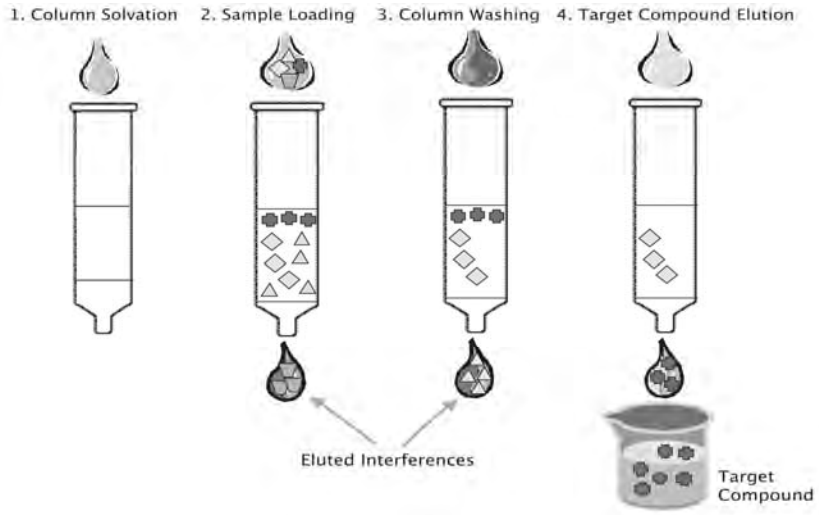


Figure 2.2 Steps in a SPE extraction

A selection of solid phase extraction cartridges, available in many sizes, shapes, and types of stationary phase. A typical solid phase extraction involves four basic steps. First, the cartridge is equilibrated with a non-polar or slightly polar solvent, which wets the surface and penetrates the bonded phase. Then water, or buffer of the same composition as the sample, is typically washed through the column to wet the silica surface. The sample is then added to the cartridge. As the sample passes through the stationary phase, the analytes in the sample will interact and remain on the sorbent while the solvent, salts, and other impurities pass through the cartridge. After the

sample is loaded, the cartridge is washed with buffer or solvent to remove further impurities. Then, the analyte is eluted with a non-polar solvent or a buffer of the appropriate pH.

Normal Phase SPE

A stationary phase of polar functionally bonded silicas with short carbons chains frequently makes up the solid phase. This stationary phase will adsorb polar molecules which can be collected with a more polar solvent.

Reversed phase SPE

Reversed phase SPE separates analytes based on their polarity. The stationary phase of a reversed phase SPE cartridge is derivatized with hydrocarbon chains, which retain compounds of mid to low polarity due to the hydrophobic effect. The analyte can be eluted by washing the cartridge with a non-polar solvent, which disrupts the interaction of the analyte and the stationary phase. A stationary phase of silicon with carbon chains is commonly used. Relying on mainly non-polar, hydrophobic interactions, only non-polar or very weakly polar compounds will adsorb to the surface.

Ion exchange SPE

Ion exchange sorbents separate analytes based on electrostatic interactions between the analyte of interest and the positively charged groups on the stationary phase. For ion exchange to occur, both the stationary phase and sample must be at a pH where both are charged.

Anion exchange

Anion exchange sorbents are derivatized with positively charged functional groups that interact and retain negatively charged anions, such as acids. Strong anion exchange sorbents containing quaternary ammonium groups that have a permanent positive charge in aqueous solutions, and weak anion exchange sorbents use amine groups which are charged when the pH is below about 9. Strong anion exchange sorbents are useful because any strongly acidic impurities in the sample will bind to

the sorbent and usually will not be eluted with the analyte of interest; to recover a strong acid a weak anion exchange cartridge should be used. To elute the analyte from either the strong or weak sorbent, the stationary phase is washed with a solvent that neutralizes the charge of either the analyte, the stationary phase, or both. Once the charge is neutralized, the electrostatic interaction between the analyte and the stationary phase no longer exists and the analyte will elute from the cartridge.

Cation Exchange

Cation exchange sorbents are derivatized with functional groups that interact and retain positively charged cations, such as bases. Strong cation exchange sorbents contain aliphatic sulfonic acid groups that are always negatively charged in aqueous solution, and weak cation exchange sorbents contain aliphatic carboxylic acids, which are charged when the pH is above about 5. Strong cation exchange sorbents are useful because any strong basic impurities in the sample will bind to the sorbent and usually will not be eluted with the analyte of interest; to recover a strong base a weak cation exchange cartridge should be used. To elute the analyte from either the strong or weak sorbent, the stationary phase is washed with a solvent that neutralizes ionic interaction between the analyte and the stationary phase.

2.10 Super critical fluid extraction (SFE)

Supercritical Fluid Extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids. SFE can be used as a sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product or collect a desired product. Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. Extraction conditions for supercritical CO_2 are above the critical temperature of 31°C and critical pressure of 74 bar. The addition of modifiers may slightly alter this. The discussion below will mainly refer to extraction with CO_2, except where specified.

2.11 QuEChERS Technique

This instalment of "Sample Prep Perspectives" describes a new extraction technique called QuEChERS (standing for quick, easy, cheap, effective and safe and is pronounced "catchers") for the sample preparation of pesticides in foods and agricultural samples.

The process involves two simple steps. First, the homogenized samples are extracted and partitioned using an organic solvent and salt solution. Then, the supernatant is further extracted and cleaned using a dispersive solid phase extraction (dSPE) technique.

QuEChERS is a sample preparation approach entailing solvent extraction of high-moisture samples with acetonitrile, ethyl acetate, or acetone and partitioning with magnesium sulphate alone or in combination with other salts followed by clean up using dispersive solid-phase extraction (d-SPE). It is a technique which depends on analytes, matrices, instrumentation and analyst preferences explained through a flow diagram and Figure 2.3 which is how this simple procedure is performed. Basically, the sample is first extracted with a water-miscible solvent (for example, acetonitrile) in the presence of high amounts of salts (for example, sodium chloride and magnesium sulphate) and buffering agents (for example, citrate) to induce liquid separation and stabilize acidic and basic labile pesticides, respectively. Upon shaking and centrifugation, an aliquot of the organic phase is subjected to further clean up using dispersive SPE (adding small amounts of bulk SPE packing sorbents to the extract). After sample clean up, the mixture is centrifuged and the resulting supernatant can be analysed directly or can be subjected to a concentration and solvent exchange step if necessary.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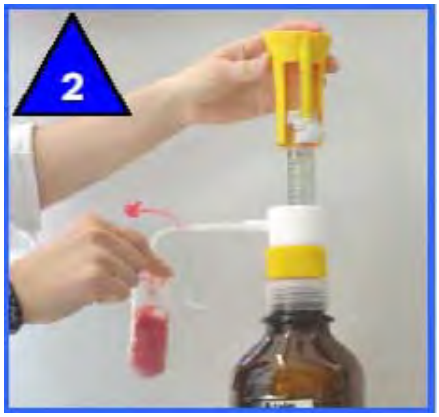
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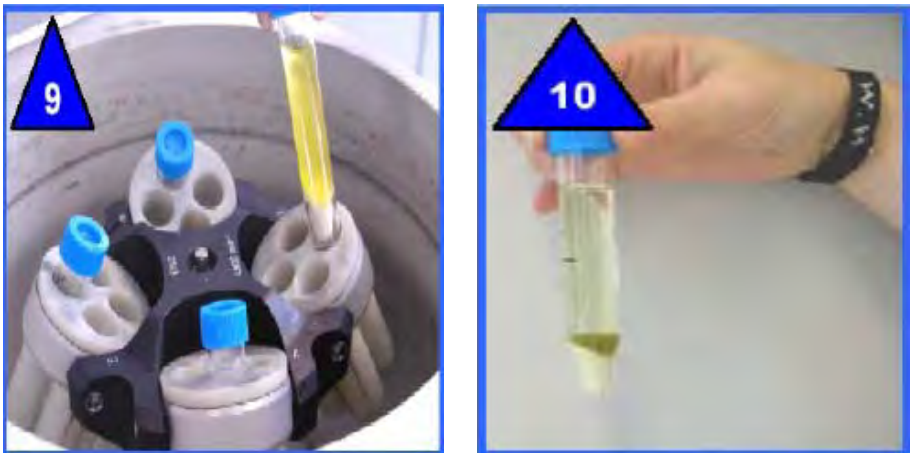

Figure 2.3 Extraction QuEChERS procedure (64)

2.4.1 Parameters affecting performing QuEChERS extraction.

2.4.1.1 Sample comminution

The sample mass (10–15 g) used in the QuEChERS technique is reduced compared with more traditional extraction approaches, it is of utmost importance to ensure that the original sample, that is typically kilograms, is extremely homogeneous. Thus, a powerful chopping device is recommended to homogenize the sample to maximize surface area and to ensure better extraction efficiencies. Such a homogenization procedure will ensure that the 10–15-g subsample is representative of the original. To prevent loss of the more volatile pesticides, the use of dry ice during the homogenization step is highly recommended.

2.4.1.2 Extraction–Partitioning

Although other nonhalogenated solvents such as acetone and ethyl acetate may be used, acetonitrile is the recommended solvent for QuEChERS because, upon the addition of salts, it is separated more easily from water than acetone. Ethyl acetate has the advantage of partial miscibility with water but it co-extracts lipids and waxes, obtains lower recoveries for acid–base pesticides, and provides less cleanup in dispersive-SPE. Acetonitrile extracts less of the lipophilic materials. However,

samples with high sugar content, acetonitrile, and water can form two phases. (64) Compared with acetone, the use of acetonitrile allows the better removal of residual water with magnesium sulfate. It is compatible with HPLC mobile phases and GC applications, although it tends to give a large solvent expansion volume during GC vaporization, interferes with nitrogen-specific GC detectors, and is less volatile than the other common organic solvents, thus making evaporative concentration steps more time consuming

2.4.1.3 Addition of Salts

The purpose of salt addition is to induce phase separation. The salting-out effect also influences analyte partition, which is dependent upon the solvent used for extraction (Step 3). The concentration of salt can influence the percentage of water in the organic phase and can adjust its "polarity." In QuEChERS, acetonitrile alone often is sufficient to perform excellent extraction efficiency without the need to add nonpolar cosolvents that dilute the extract and make the extracts too nonpolar. By using deuterated solvents and nuclear magnetic resonance, Anastassiades and colleagues (64) investigated the effect of various salt additions on recovery and other extraction parameters. They studied the effect of polarity differences between the two immiscible layers. The use of magnesium sulfate as a drying salt to reduce the water phase helped to improve recoveries by promoting partitioning of the pesticides into the organic layer. To bind a significant fraction of water, the amount of magnesium sulfate exceeded saturation concentration. The supplemental use of sodium chloride helps to control the polarity of the extraction solvents and thus influences the degree of matrix cleanup of the QuEChERS method but too much of this salt will reduce the organic layer's ability to partition polar pesticides.

In some instances, the pH of the extraction must be controlled. Most, but not all, pesticides are more stable at lower pH. For certain problematic pesticides, such as those that are strongly protonated at low pH, the extraction system must be buffered in the range of pH 2–7 for successful extractions. (65) Of course, the pH at which the extraction is performed also can influence the coextraction of matrix compounds and pesticide stability.

2.4.1.4 Internal Standard Addition

To minimize error generation in the multiple steps of the QuEChERS method, an internal standard is often added to the process. For most of the development work, the original authors (1) used tri-phenylphosphate, which had the right properties to undergo quantitative extraction for low fat matrices. A more complete study of various internal standards was undertaken by Anastassiades (3), who recommended the use of more than one internal standard as quality control measures to enable recognition of errors due to mispipetting or discrimination during partitioning or cleanup. In most cases, the internal standard is employed at an early stage of the analytical procedure. However, in the case of samples with high fat content, excessive fat can form an additional layer into which analytes can partition. In the presence of elevated fat amounts (for example, > 0.3 g of fat/10 mL of acetonitrile), it was recommended to employ the internal standard at the end of the procedure (assuming the volume of the organic phase is exactly 10 mL).

2.4.1.5 Dispersive solid-phase extraction

Traditionally, SPE cleanup used plastic cartridges containing various amounts of sorbent material. In dispersive solid-phase extraction, an aliquot of sample extract (for example, 1 mL) is added to a vial containing a small amount of SPE sorbent (50 mg of primary secondary amine, PSA) and the mixture is shaken or mixed on a vortex mixer to evenly distribute the SPE material and facilitate the cleanup process. The sorbent is then separated by centrifugation and an aliquot of the supernatant is subjected to analysis. The sorbent is chosen to retain matrix components and not the analytes of interest. In some cases, other sorbents or mixed sorbents can be used. For samples with high fat, PSA mixed with a C18 sorbent is recommended while for samples with moderate and high levels of chlorophyll and carotinoids (for example, carrots, romaine lettuce), PSA mixed with graphitized carbon black at various ratios of sorbents is used. Although the addition of graphitized carbon black helps with the partial removal of chlorophyll, there is an accompanying

partial loss of certain structurally planar analytes, so these processes in a balancing act.

Dispersive solid-phase extraction is similar in some respects to matrix solid-phase dispersion developed by Barker (65, 67-68), but in this case, the sorbent is added to an aliquot of the extract rather than to the original solid sample as in matrix solid-phase extraction. In dispersive solid-phase extraction, a smaller amount of sorbent is used because only an aliquot of the sample is subjected to the cleanup. Compared with SPE, dispersive solid-phase extraction takes less time and uses less labor and lower amounts of solvent. One need not worry about channeling, analyte or matrix breakthrough, or preconditioning of SPE cartridges. Just as a drying agent is sometimes added to the top of an SPE cartridge, magnesium sulfate is added simultaneously with the SPE sorbent to remove much of the excess water and improve analyte partitioning to provide better cleanup.

2.4.1.6 Add Acetic Acid and "Analyte Protectants"

This optional step is found to be most useful for pesticides that are unstable at intermediate pH values and for analytes that might tail or breakdown on the capillary GC column interior surfaces, on sorbed nonvolatile compounds from previous injection, on the inlet liner or on the precolumn (guard column). In this case, analyte protectants are added to the extracts before GC. The protectant compounds are chosen so that they do not interfere with the separation of the pesticides of interest yet will cut down on interactions of these pesticides with active groups in the GC flow stream. Thorough studies were devoted to selecting the appropriate analyte protectants (69-70), and a combination of sorbitol, gulonolactone, and ethylglycerol were found to cover the entire range of pesticides. The hydroxyl groups of these protectants interacted with active sites on the chromatographic column and in the flowstream and enhanced the pesticide analyte response. The results demonstrated that errors in GC analysis caused by matrix effects also were reduced dramatically with the help of analyte protectants. Of course, with LC and LC–MS, the protectants are not required.

2.4.1.7 Analysis

Often, the sample aliquot from Step 6 can be injected directly into a GC or HPLC system without further workup. For example, for LC–MS analysis, it might be necessary to add formic acid to provide better MS sensitivity or for GC–MS analysis, and if the instrument is not equipped with a programmable temperature vaporizer, evaporation of the supernatant with reconstitution in toluene might be needed.

Primary and secondary amine exchange material (PSA)

Primary and secondary amine exchange material (PSA) is the base sorbent used for dSPE cleanup of QuEChERS fruit and vegetable extracts because it removes many organic acids and sugars that might act as instrumental interferences. sugars, fatty acids, organic acids, and anthocyanine pigments

C18: removes nonpolar interferences

Carbon: removes pigments, sterols, and nonpolar interferences

$MgSO_4$: removes excess water

2.5 Matrix solid phase dispersion (MSPD)

Matrix solid-phase dispersion is an analytical technique for the preparation and extraction of solid and viscous samples. The technique uses bonded-phase solid supports as an abrasive to produce disruption of sample architecture and a bound solvent to aid complete sample disruption during the sample blending process. The sample disperses over the surface of the bonded phase-support material to provide a new mixed phase for isolating analytes from various sample matrices. This review discusses the factors that affect the use of matrix solid-phase dispersion and provides a bibliography of its applications for the extraction and analysis of a range of compounds

Matrix solid phase dispersion (MSPD) has found particular application as an analytical process for the preparation, extraction and fractionation of solid, semi-solid

and/or highly viscous biological samples. Its simplicity and flexibility have been cited as contributing to it being chosen over more classical methods for these purposes. Indeed, MSPD is based on several simple principles of chemistry and physics, involving forces applied to the sample by mechanical blending to produce complete sample disruption and the interactions of the sample matrix with a solid support bonded-phase (SPE) or the surface chemistry of other solid support materials.

In MSPD conception, the blending of a bonded-phase solid support material with a biological sample is seen as acting as both an abrasive, producing shearing and grinding force that induces disruption of the sample architecture, and as a “bound” solvent that assists in accomplishing complete sample disruption and dispersion. In this manner, the sample is dispersed over the surface of the bonded-phase support material, producing, through hydrophobic and hydrophilic interactions of the various components, a unique mixed-character phase for conducting target analyte isolation. Indeed, blended samples (muscle tissue and ODS-silica support) have been examined by scanning electron microscopy (SEM) and show that sample architecture is completely disrupted and that sample matrix components are, apparently, evenly distributed over the surface of the bonded phase/ support.

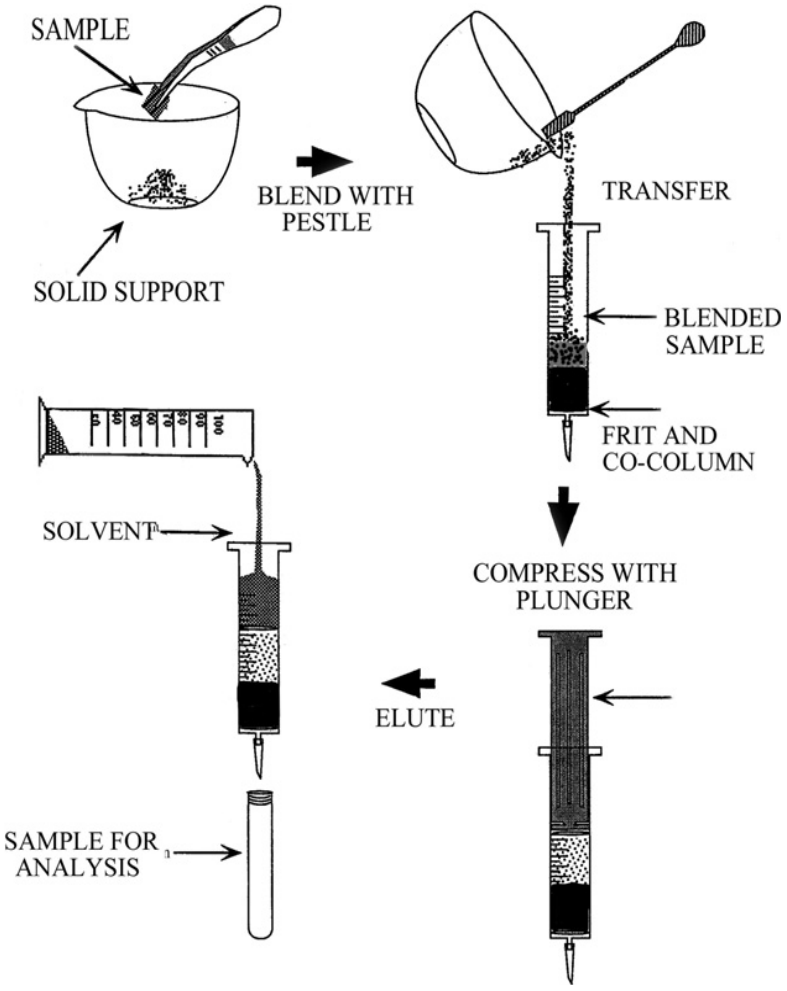

Figure 2.4 Steps in a MSPD extraction (71)

2.5.1 Parameters affecting performing a MSPD extraction

2.5.1.1 Solid support

2.5.1.1.1 A particle size diameter

As expected, very small particle sizes (3–10 μm) lead to extended solvent elution times and the need for excessive pressures or vacuum to obtain adequate flow. A blend of silicas possessing a range of particle sizes (40–100 μm) works quite well and such materials also tend to be less expensive.

2.5.1.1.2 Non-end-capped vs. end-capped materials

Materials having a range of carbon loading (8–18%)

2.5.1.1.3 Character of the bonded-phase

Depending on the polarity of the phase chosen, rather dramatic effects on the results may be observed. Applications requiring a lipophilic bonded-phase may use C18 and C8 materials interchangeably.

2.5.1.1.4 The underivatized silica or other solid supports

Use of non modified or underivatized solids, such as sand, to blend samples do not work in exactly the same manner as originally described for bonded-phase solid supports, such as ODS. However, the same basic principles will apply; abrasion and sample disruption will occur during the blending process. However, further disruption of the sample and component dispersion will only occur to the degree that the components interact with the chemical characteristics of the particular surface and each other. All surfaces have a definable chemistry and many substances, including a variety of minerals, may well serve to enhance isolation of specific compounds or classes of compounds and may even be blended together to form unique interactions to accomplish desired results. To date, silica-based support materials (derivatized silica, silica gel, sand, Florisil) have been almost exclusively reported for use in MSPD. The further use and effect of synthetic polymer-based solid supports and of granular minerals is a subject for further study, particularly of supports that possess unique surface and/or pore chemistries, such as hydrophobic interaction supports. For silica-based materials, however, studies have shown that the pore size is of minor importance in MSPD. This effect could vary with the sample and should, nonetheless, be considered.

2.5.1.1.5 The ratio of sample to solid support material

Most protocols use lipophilic bonded-phase (C18, C8) materials, blending 2.0 g of solid support with 0.5 g of sample. This ratio is dependent on the

application and must be examined as a major variable during method development. Both smaller and greater ratios have been used successfully

2.5.1.1.6 Chemical modification of the matrix or matrix solid support blend

Addition of chelating agents, acids, bases, etc. at the time of blending affect the distribution and elution of target analytes from the sample. The elution profile of matrix components is likewise affected.

2.5.1.2 The Elution solvent

2.5.1.2.1 Elution solvents and the sequence of their application to a column

The elution solvent sequence attempts to isolate the analyte or further clean the column of interfering substances with each solvent step. MSPD columns permit isolation of different polarity analytes or entire chemical classes of compounds in a single solvent or in differing polarity solvents passed through the column, making MSPD amenable to conducting multiresidue isolation and analysis on a single sample, on MSPD free of hazardous solvents and are even less expensive to perform. Preconditioning of the support materials used for any MSPD application enhances analyte recovery and speeds the process of sample blending and dispersal. This is due to the breaking of surface tension differences that may exist between the sample and bonded-phase solid support. As with SPE, washing or rinsing the solid support materials also eliminates contaminants from the final eluates.(71)

2.5.1.2.2 The elution volume

It has been observed that for an 8 ml elution of a 2 g MSPD column blended with 0.5 g of sample that target analytes usually elute in the first 4 ml, approximately one column volume. This will vary for each application and should be examined to reduce the use of solvent and the unintended coelution of potential interferences. Miniaturization of the MSPD technique, using smaller sample sizes and proportionately less support or solvent.

2.6 Gas Chromotography

Gas chromatography (GC), is based on a partition equilibrium of analyte between a solid stationary phase (often a liquid silicone-based material) and a mobile gas (most often Helium). Gas chromatography (GC), also sometimes known as Gas-Liquid chromatography, (GLC), is a separation technique in which the mobile phase is a carrier gas, usually an inert gas such as helium . The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass called a column. It is widely used in analytical chemistry; though the high temperatures used in GC make it unsuitable for high molecular weight biopolymers or proteins (heat will denature them), frequently encountered in biochemistry, it is well suited for use in the petrochemical, environmental monitoring, and industrial chemical fields. It is also used extensively in chemistry research.

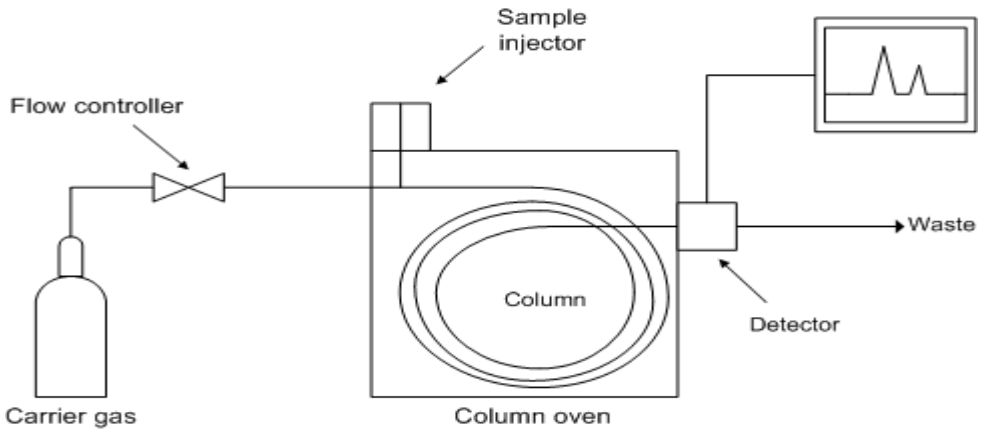

Figure 2.5 Schematic diagram of GC system

GC is a common type of chromatography used in analytic chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture. The gaseous compounds being analyzed interact with the walls of the column, which is coated with different stationary phases. This causes each compound

to elute at a different time, known as the *retention time* of the compound. The comparison of retention times is what gives GC its analytical usefulness.The instrument used to perform gas chromatography is called a *gas chromatograph*.

2.6.1 The gas source

The carrier gas must be pure. Contaminants may react with the sample or the column, create spurious peaks, load the detector and raise baselines, and so on. A high-purity gas with traps for water, hydrocarbons and oxygen is recommended.

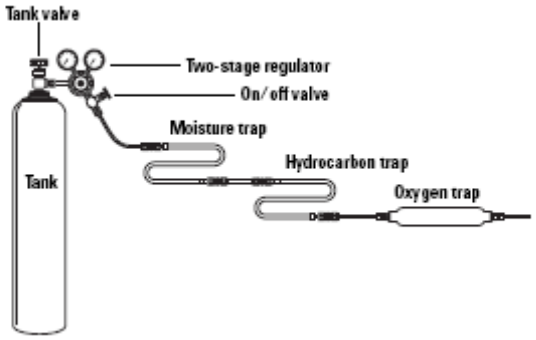

Figure 2.6 The gas source

Typical carrier gases include helium, nitrogen, argon, hydrogen and air. Which gas to use is usually determined by the detector being used, for example, a DID requires helium as the carrier gas. When analyzing gas samples, however, the carrier is sometimes selected based on the sample's matrix, for example, when analyzing a mixture in argon, an argon carrier is preferred, because the argon in the sample does not show up on the chromatogram. Safety and availability can also influence carrier selection, for example, hydrogen is flammable, and high-purity helium can be difficult to obtain in some areas of the world.

The purity of the carrier gas is also frequently determined by the detector, though the level of sensitivity needed can also play a significant role. Typically, purities of 99.995% or higher are used. Trade names for typical purities include "Zero Grade," "Ultra-High Purity (UHP) Grade," "4.5 Grade" and "5.0 Grade."

The carrier gas flow rate affects the analysis in the same way that temperature does (see above). The higher the flow rate the faster the analysis, but the lower the separation between analytes. Selecting the flow rate is therefore the same compromise between the level of separation and length of analysis as selecting the column temperature.

With GCs made before the 1990s, carrier flow rate was controlled indirectly by controlling the carrier inlet pressure, or "column head pressure." The actual flow rate was measured at the outlet of the column or the detector with an electronic flow meter, or a bubble flow meter, and could be an involved, time consuming, and frustrating process. The pressure setting was not able to be varied during the run, and thus the flow was essentially constant during the analysis.

Many modern GCs, however, electronically measure the flow rate, and electronically control the carrier gas pressure to set the flow rate. Consequently, carrier pressures and flow rates can be adjusted during the run, creating pressure/flow programs similar to temperature programs.

2.6.2 Inlets

The inlet introduces the vaporized sample into the carrier gas stream. The most common inlets are injection ports and sampling valves.

2.6.2.1 Autosample

The autosampler provides the means to introduce a sample automatically into the inlets. Manual insertion of the sample is possible but is no longer common. Automatic insertion provides better reproducibility and time-optimization.

Different kinds of auto samplers exist. Auto samplers can be classified in relation to sample capacity (auto-injectors vs. auto samplers, where auto-injectors can work a small number of samples), to robotic technologies (XYZ robot vs. rotating robot – the most common)

2.6.2.2 Injection ports

Handle gas or liquid samples. Often heated to vaporize liquid samples. Liquid or gas syringes are used to insert the sample through a septum into the carrier gas stream.

The injector can be used in one of two modes; split or split less. The injector contains a heated chamber containing a glass liner into which the sample is injected through the septum. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vapourises to form a mixture of carrier gas, vapourised solvent and vapourised solutes. A proportion of this mixture passes onto the column, but most exit through the split outlet. The septum purge outlet prevents septum bleed components from entering the column.

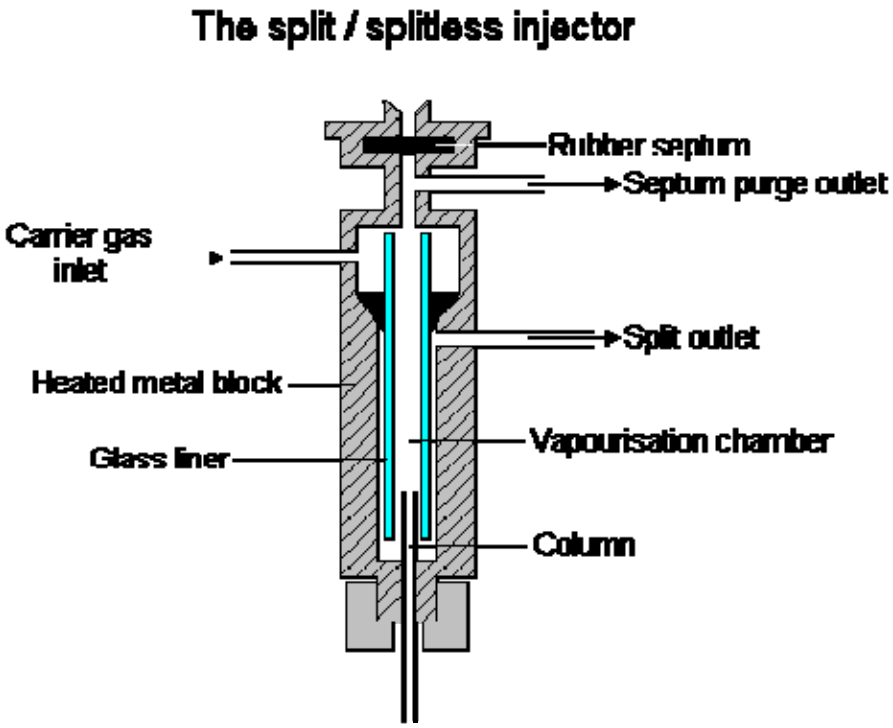

Figure 2.7 Injection port

2.6.2.3 S/SL (Split/Splitless) injector

The split/split less port, used with capillary columns, has two operating modes. A sample is introduced into a heated small chamber via a syringe through a *septum* - the heat facilitates volatilization of the sample and sample matrix. The carrier gas then either sweeps the entirety (*split less* mode) or a portion (*split* mode) of the sample into the column. In split mode, a part of the sample/carrier gas mixture in the injection chamber is exhausted through the *split vent*. Split injection is preferred when working with samples with high analyte concentrations (>0.1%) whereas split less injection is best suited for trace analysis with low amounts of analytes. (<0.01%)

2.6.2.3.1 Split mode

Capillary columns have low sample capacities. Very small sample sizes, usually much less than a microliter, must be used to avoid overloading the column. It is very difficult to handle such small sample sizes. The split mode provides a way to inject a normal-size sample, vaporize it, and then transfer only part of it to the column for analysis. The rest is vented to waste.

The split valve is open and remains that way. The sample is injected into the liner, where it vaporizes. The vaporized sample divides between the column (high flow resistance) and the split vent (adjustable flow resistance).

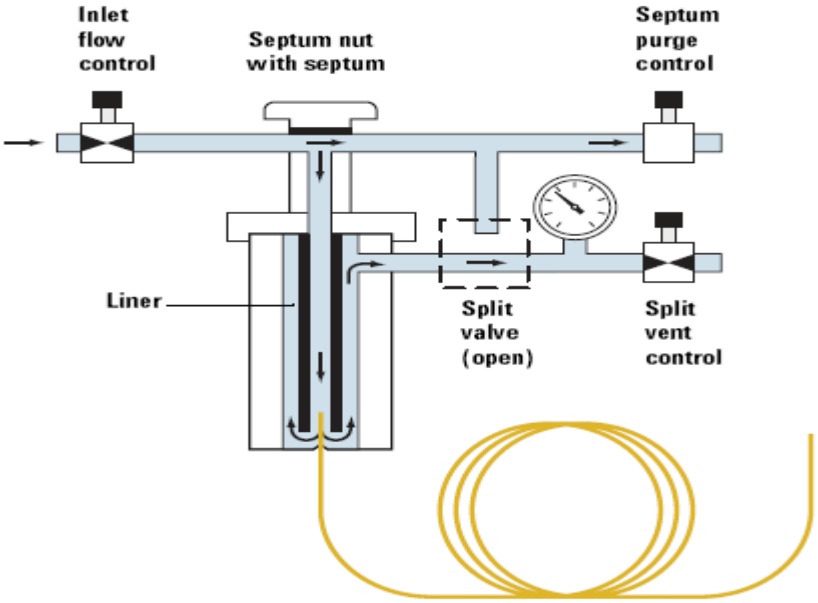

Figure 2.8 A typical split/split less port in *split mode*

2.6.2.3.2 Split less mode

This mode is particularly well suited to low concentration samples. It traps the sample at the head of the column while venting residual solvent vapor in the inlet to waste.

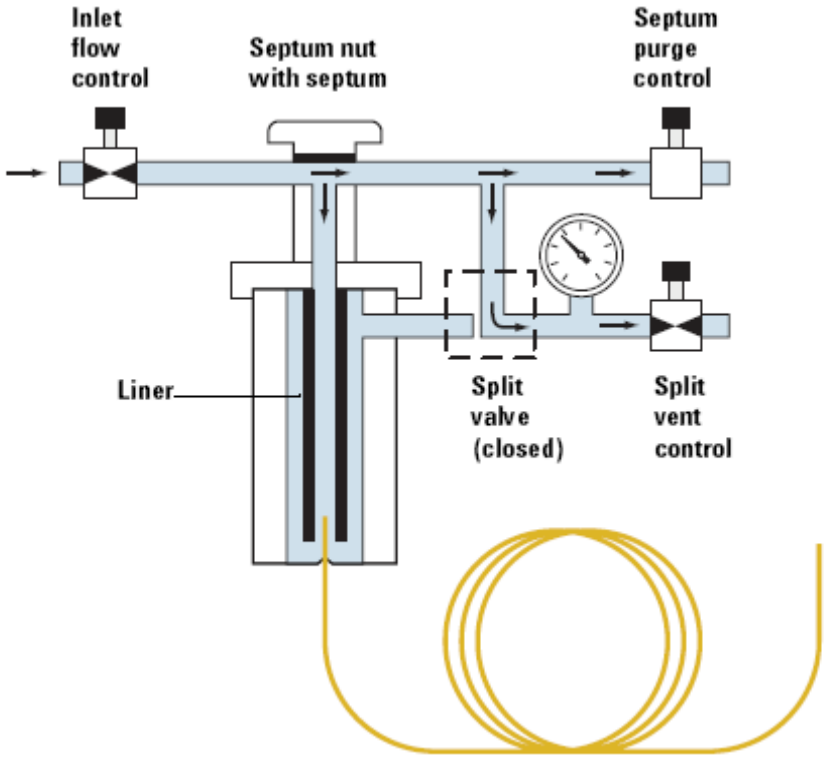

Figure 2.9 The flows at injection in the *splitless* mode

Two steps are involved:

1. Sample injection

Close the split valve. The carrier flow divides between the septum purge and the column. The pressure at the head of the column, and therefore the flow through it, is set by the split vent control. Inject the sample. The solvent, the major component, creates a saturated zone at the head of the column which traps the sample components. This mode is particularly well suited to low concentration samples. It traps the sample at the head of the column while venting residual solvent vapor in the inlet to waste.

2. Inlet purge
3. After the sample has been trapped on the column, open the split valve. The residual vapor in the inlet, now mostly solvent, is swept out of the vent. The flows are now the same as in the split mode. Raise the oven temperature to begin moving the components through the column. This mode works better for components

with boiling points higher than that of the solvent. The solvent peak will be large. The oven temperature profile is used to separate peaks of interest from the solvent.

2.6.2.4 PTV injector

Temperature-programmed sample introduction was first described by Vogt in 1979. Originally Vogt developed the technique as a method for the introduction of large sample volumes (up to 250 µL) in capillary GC. Vogt introduced the sample into the liner at a controlled injection rate. The temperature of the liner chosen was slightly below the boiling point of the solvent. The low-boiling solvent was continuously evaporated and vented through the split line. Based on this technique, Poy developed the Programmed Temperature Vaporizing injector; PTV. By introducing the sample at a low initial liner temperature many of the disadvantages of the classic hot injection techniques could be circumvented.

Programmed temperature vaporizing injectors (PTV) have been shown to be eminently suited for large volume sample introduction in capillary gas chromatography. Large volume injection can be applied in trace analysis to improve analyte detectability. Very often it can replace an off-line evaporation step carried out to concentrate a diluted sample extract. In a recent paper we demonstrated that, for sample volumes up to 150 pl, the procedure for large volume injection is very simple when using PTV injectors equipped with liners with internal diameters larger than ***ca*** 2.5 mm .With the split valve open and at a liner temperature below the solvent boiling point the sample can be rapidly injected, either manually or with an auto sampler equipped with a large volume syringe. The solvent is vented via the split exit while the analytes are retained in the liner. After the solvent elimination step the analytes are transferred to the column in the split less mode. For large volume sampling with PTV injectors the liner has to be packed in order to retain the liquid sample after injection. In the above mentioned paper silylated glass wool was used as packing material. With liners packed with glass wool, reliable results can be obtained for thermostable compounds such as polycyclic aromatic hydrocarbons and polychlorobiphenyls. For thermolabile compounds, and also for polar analytes, interaction with active sites on the glass wool surface may lead to degradation or adsorption of the analytes in the liner, as is also known from (PTV) split split less

injection. The aim of this work is to find alternatives for glass wool as packing material for use in large volume sampling with PTV injectors. Aspects that will be discussed are the sample volume that is retained by the packed liner, and the inertness and thermo stability of the packing material.

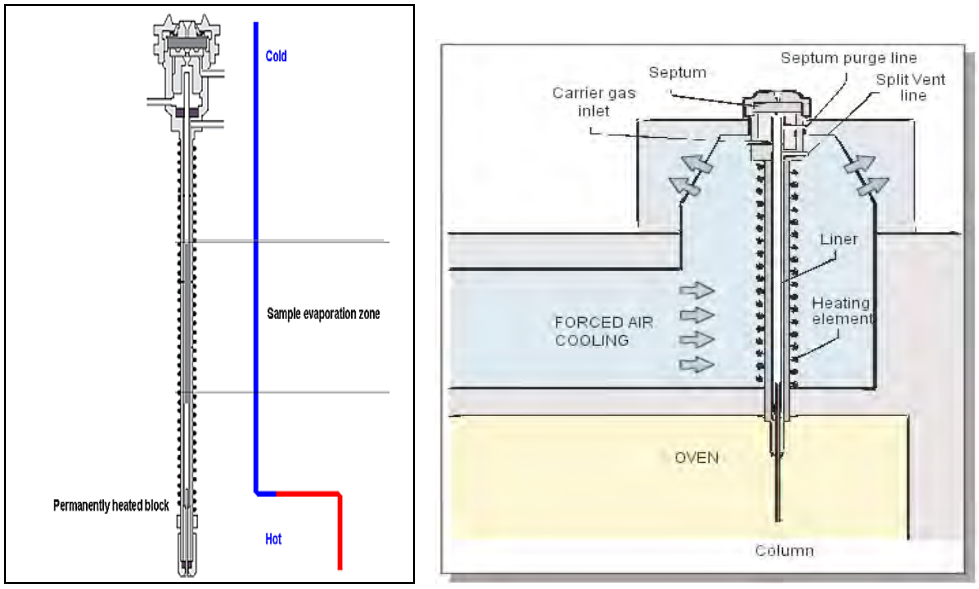

Figure 2.10 The PTV injector

2.6.2.4.1 Inlet Temperature

Liquid samples require a heated inlet. The temperature must be high enough to vaporize the sample but not so high that degradation occurs.

Hot enough Start with the solvent boiling point and examine the peaks. If they are all about the same shape (the sizes will differ), the inlet is probably hot enough. If the later peaks show excess broadening, raise the inlet temperature about 10°C to see if the shapes improve.

Too hot If you have more peaks than components and if they are poorly formed, suspect degradation problems. Degradation in the inlet creates peaks whose size depends strongly on inlet temperature. To detect this, make a second analysis at a slightly lower temperature. Compare the peak sizes; any significant change could indicate degradation in the inlet

2.6.3 Columns

The separation happens here. Because the column type is selected by the user, many different analyses can be performed using the same equipment. Most separations are highly temperature-dependent, so the column is placed in a well-controlled oven.

The column(s) in a GC are contained in an oven, the temperature of which is precisely controlled electronically. (When discussing the "temperature of the column," an analyst is technically referring to the temperature of the column oven. The distinction, however, is not important and will not subsequently be made in this article.)

The rate at which a sample passes through the column is directly proportional to the temperature of the column. The higher the column temperature, the faster the sample moves through the column. However, the faster a sample moves through the column, the less it interacts with the stationary phase, and the less the analytes are separated.

In general, the column temperature is selected to compromise between the length of the analysis and the level of separation.

A method which holds the column at the same temperature for the entire analysis is called "isothermal." Most methods, however, increase the column temperature during the analysis, the initial temperature, rate of temperature increase (the temperature "ramp") and final temperature is called the "**temperature program**."

A temperature program allows analytes that elute early in the analysis to separate adequately, while shortening the time it takes for late-eluting analytes to pass through the column.

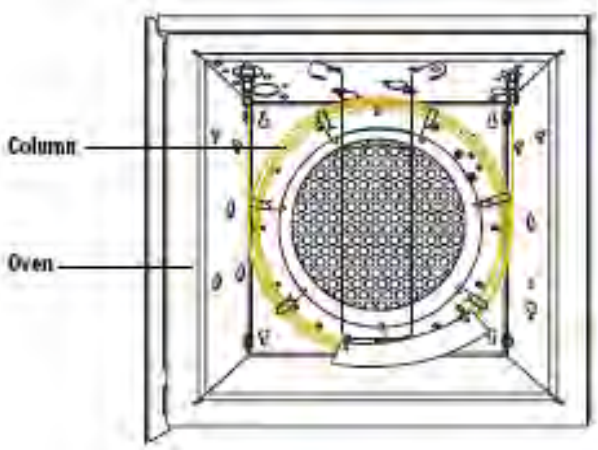

Figure 2.11 The column oven

2.6.3.1 Packed columns

In a packed column, the stationary phase is coated on a finely-divided inert material to maximize its area and minimize its thickness. The coated material is then packed into a metal, glass, or plastic tube. Most metal packed columns are either 1/8- or 1/4-inch outside diameter. Glass columns are generally 1/4-inch outside diameter, but the inside diameter varies to produce the equivalent of the two metal column sizes. Packed columns have high sample capacity, a necessity with older, less sensitive detectors. However, with modern high-sensitivity detectors, this advantage has vanished. Packed columns are still useful for gas samples, but capillary columns offer better resolution for most liquid samples.

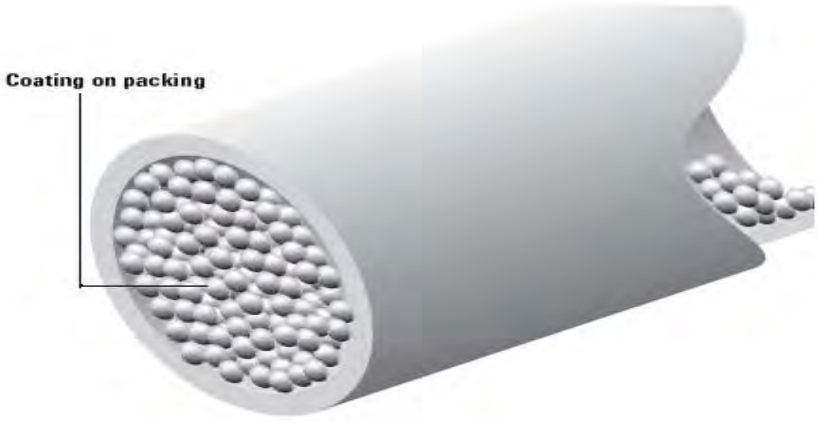

Figure 2.12 A packed column

2.6.3.2 Capillary columns

A capillary column is an open tube with the stationary phase coated on its inside surface. There is no packing. The columns have a very small internal diameter, to the order of a few tenths of millimeters, and lengths between 25–60 meters are common. The inner column walls are coated with the active materials (WCOT columns), some columns are quasi solid filled with many parallel micropores (PLOT columns). Most capillary columns are made of fused-silica (FSOT columns) with a polyimide outer coating. These columns are flexible, so a very long column can be wound into a small coil. Capillary columns produce very narrow peaks. This allows the separation of very complex mixtures. For example, a typical automobile fuel yields between 400 and 500 peaks. These columns, when made with fused silica tubing, are very inert. Difficult samples such as mercaptans, which tail severely on metal or glass columns, separate to the baseline on such columns. Capillary columns require smaller samples than packed columns. These columns range from about 0.1 to 0.5 mm inside diameter. A typical column length is 30 m.

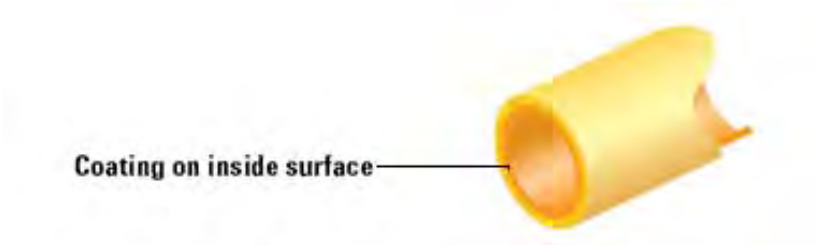

Figure 2.13 A capillary column

2.6.3.3 Column Temperature

The stationary phase (coating) in the column has a preferred temperature range.

- The *minimum* temperature is usually a melting point. Below this, you are doing gas/solid chromatography; above it, you are performing gas/liquid chromatography. Results can be quite different.

- The *maximum* temperature is usually related to a boiling or degradation point. Columns are mounted in a temperature-controlled oven because separations are highly temperature dependent.

2.6.3.3.1 Isothermal oven

This is the simplest way to run the oven. The oven remains at the same temperature throughout the analysis. It has *advantages*:

- The oven is always ready for a sample analysis.
- There is no recovery time between analyses.

2.6.3.3.2 Programmed oven

The oven temperature changes, usually upward, during the analysis. The *advantages* are analysis time is reduced and peak shapes are constant throughout the run, making detection and measurement easier.

The *disadvantages* are components are subjected to higher temperatures than with an isothermal oven. This could cause degradation of sensitive components.

- The oven must cool to the starting temperature between runs. This cancels part of the time gained.

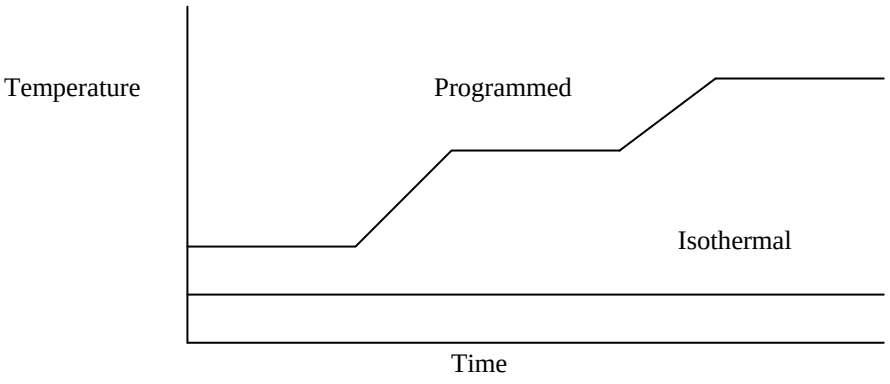

Figure 2.14 The oven temperature can be isothermal or programmed

2.6.4 Detectors

The gas stream from the column, which contains the separated components, passes through a detector. The output from the detector becomes the chromatogram.

Front column ⟶ Detector ⟶ Chromatogram

Figure 2.15 The detector

2.6.4.1 Electron Capture Detector (ECD)

The Electron Capture Detector (ECD) uses radioactive isotope, usually 63Ni, in the detector cell emits beta particles. These collide with carrier gas to create showers of low-energy free electrons. Two electrodes and a polarizing voltage collect the electrons as a current. Some molecules can capture low-energy electrons to form negative ions. When such a molecule enters the cell, some of the electrons are captured and the collected current decreases. After processing, this signal creates the chromatogram. The electron capture detector has found wide use in environmental work because of its very high sensitivity to halogen-containing components, which include most herbicides and pesticides. The ECD is as sensitive as the FID but has a limited dynamic range and finds its greatest application in analysis of halogenated compounds.

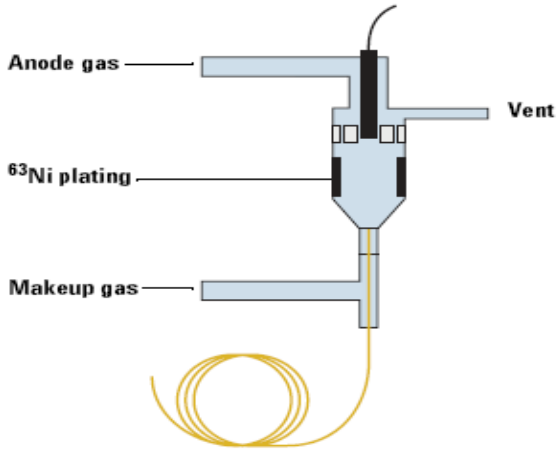

Figure 2.16 Electron capture detector

2.6.4.2 Flame Photometric GC Detector

The reason to use more than one kind of detector for gas chromatography is to achieve selective and/or highly sensitive detection of specific compounds encountered in particular chromatographic analyses. The determination of sulfur or phosphorus containing compounds is the job of the flame photometric detector (FPD). This device uses the chemiluminescent reactions of these compounds in a hydrogen/air flame as a source of analytical information that is relatively specific for substances containing these two kinds of atoms. The emitting species for sulfur compounds is excited S_2. The lambda max for emission of excited S_2 is approximately 394 nm. The emitter for phosphorus compounds in the flame is excited HPO (lambda max = doublet 510-526 nm). In order to selectively detect one or the other family of compounds as it elutes from the GC column, an interference filter is used between the flame and the photomultiplier tube (PMT) to isolate the appropriate emission band. The drawback here being that the filter must be exchanged between chromatographic runs if the other family of compounds is to be detected.

In addition to the instrumental requirements for 1) a combustion chamber to house the flame, 2) gas lines for hydrogen (fuel) and air (oxidant), and 3) an exhaust chimney to remove combustion products, the final component necessary for this instrument is a thermal (bandpass) filter to isolate only the visible and UV radiation emitted by the flame. Without this the large amounts of infrared radiation emitted by the flame's combustion reaction would heat up the PMT and increase its background signal. The PMT is also physically insulated from the combustion chamber by using poorly (thermally) conducting metals to attach the PMT housing, filters, etc.

The physical arrangement of these components is as follows: flame (combustion) chamber with exhaust, permenant thermal filter (two IR filters in some commercial designs), a removable phosphorus or sulfur selective filter, and finally the PMT.

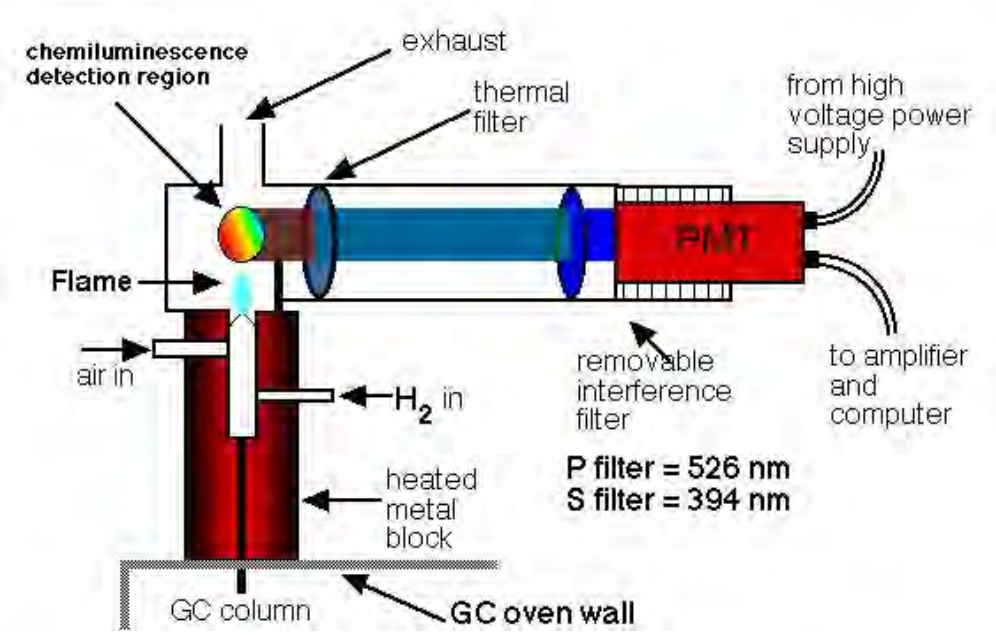

Figure 2.17 The Flame Photometric Detector

2.7 Three Way Splitter

The splitter divides the effluent from a column among three different detectors. The detectors can be operated at different pressures, that is, any mix of the following can be used:

- **Atmospheric pressure**

  FID (flame ionization detector)

  TCD (thermal conductivity detector)

  NPD (nitrogen phosphorus detector)

  ECD (electron capture detector)

  FPD (flame photometric detector)

- **Below atmospheric pressure**

  MSD (mass selective detector)

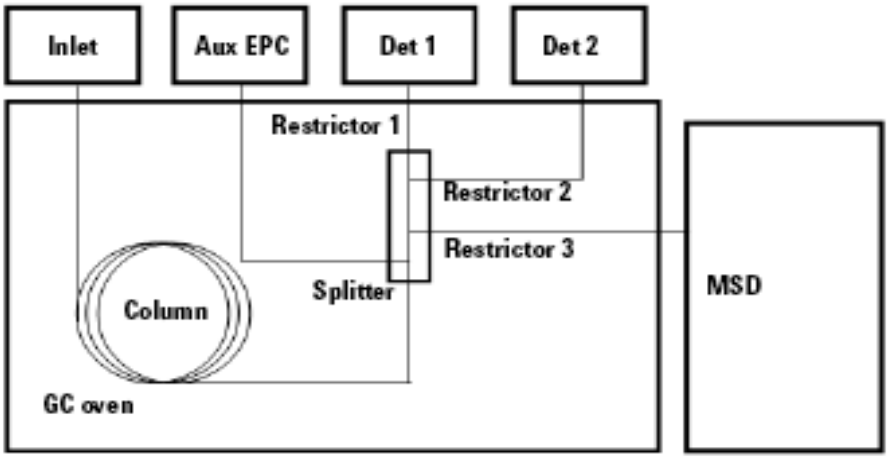

Figure 2.18 The plumbing configuration for the splitter

The column flow mixes with the makeup flow in the splitter. This mixture then flows through lengths of uncoated, deactivated, fused-silica tubing to each detector. These tubes act as flow restrictors. While the flows through the restrictors change with oven temperature, the *ratio* of the flows at any temperature is constant.

Metal ferrules

The splitter uses metal column ferrules, which eliminate air leakage into the sample stream. Unlike polyimide, metal ferrules do not loosen upon thermal cycling of the oven. They also do not outgas contaminants or shed particles (like graphite) that can result in chromatographic problems.

Micro fluidic plate

The splitting hardware is based on micro fluidic plate technology. This allows very low dead volume connections between the column end and the three detector restrictor tubes. The thin metal plate has fast thermal response and is mounted solidly on the oven wall for ease of use. The interior plate surfaces are deactivated to prevent adsorption of active compounds. Figure 2.19 shows the micro fluidic plate.

The splitter uses a source of makeup gas supplied by electronic pneumatics control (EPC). This maintains the splitter at a known and constant pressure. Constant pressure allows easier splitting to vacuum detectors like the MSD. It simplifies choice

of splitter parameters, allowing all aspects of the chromatographic setup to be calculated. Constant pressure makeup allows the column to be run in constant flow mode while still maintaining a constant split ratio between three detectors of different operating pressures such as the FPD and the MSD. Because the EPC pressure can be time programmed, useful operations like back flushing unwanted heavy materials from the column and changing columns in MSD systems without venting are possible.

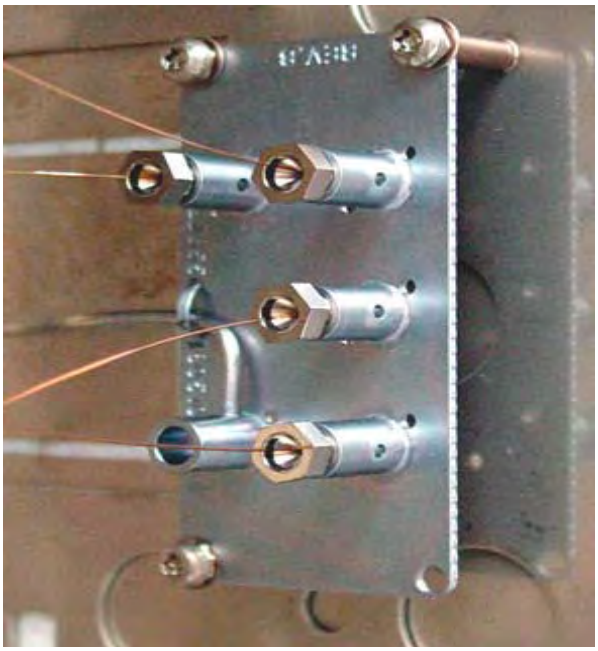

Figure 2.19 The micro fluidic plate

Constant pressure operation

The splitter uses a source of makeup gas supplied by electronic pneumatics control (EPC). This maintains the splitter at a known and constant pressure. Constant pressure allows easier splitting to vacuum detectors like the MSD. It simplifies choice of splitter parameters, allowing all aspects of the chromatographic setup to be calculated. Constant pressure makeup allows the column to be run in constant flow mode while still maintaining a constant split ratio between three detectors of different operating pressures such as the FPD and the MSD. Because the EPC pressure can be time programmed, useful operations like back flushing unwanted heavy materials from the column and changing columns in MSD systems without venting are possible.

2.8 Mass Spectrometry (MS)

MS is an analytical technique for the determination of the elemental composition of a sample or molecule. It is also used for elucidating the chemical structures of molecules, such as peptides and other chemical compounds. The MS principle consists of ionizing chemical compounds to generate charged molecules or molecule fragments and measurement of their mass-to-charge ratios.

A mass spectrometer creates charged particles (ions) from molecules. It then analyzes those ions to provide information about the molecular weight of the compound and its chemical structure. There are many types of mass spectrometers and sample introduction techniques which allow a wide range of analyses.

Mass spectrometers use the difference in mass-to-charge ratio (m/z) of ionized atoms or molecules to separate them from each other. Mass spectrometry is therefore useful for quantitation of atoms or molecules and also for determining chemical and structural information about molecules. Molecules have distinctive fragmentation patterns that provide structural information to identify structural components.

Mass spectrometry is one of the most important analytical tools, in order to obtain information about the chemical composition and abundance of isotopes. A mass spectrometer produces ions from the substance, separates them according to their mass to charge ratio (m/z), and records the relative abundance of each ionic species present.The three major components of a MS instrument are ion source, mass analyzer, and detector. Figure 2.20 shows a schematic diagram of the mass spectrometry process.

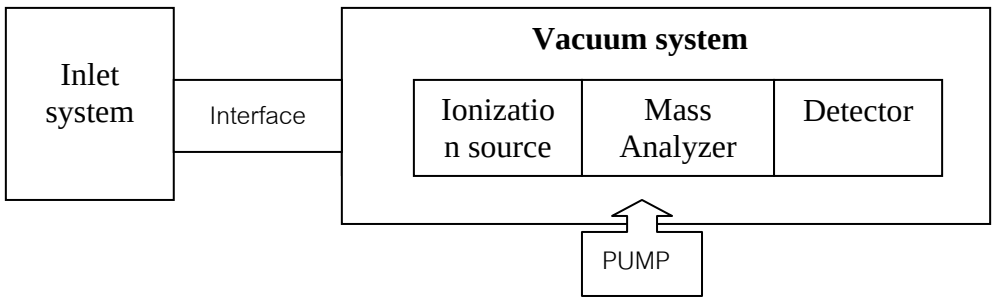

Figure 2.20 Schematic diagram of GC/MS system

2.8.1 Interface

The pressure incompatibility problem between GC and MS was solved in several ways. The earliest approach, dating from the late 1950s, simply split a small fraction of the gas chromatographic effluent into the mass spectrometer (1). Depending on the pumping speed of the mass spectrometer, about 1 to 5% of the GC effluent was split off into the mass spectrometer, venting the remaining 95 to 99% of the analytes into the atmosphere. It was soon recognized that this was not the best way to maintain the high sensitivity of the two techniques, and improved GC-MS interfaces were designed (2). These interfaces reduced the pressure of the GC effluent from about 760 torr to l0–6 to 10–5 torr, but at the same time, they passed all (or most) of the analyte molecules from the GC into the mass spectrometer. These interfaces were no longer just GC carrier gas splitters, but carrier gas separators; that is, they separated the carrier gas from the organic analytes and actually increased the concentration of the organic compounds in the carrier gas stream. The most important commercial GC carrier gas separator is called the jet separator.

In practice, most GC-MS interfacing is now done by simply inserting the capillary column directly into the ion source. Fig. 2.21 is a diagram of one such system. The fused silica column runs through a 1/16-in.-diameter tube directly into the ion source. Other gases, such as methane for chemical ionization, are brought into the ion source by a T joint around the capillary column. One of the other two lines into the ion source is used for a thermocouple vacuum gauge tube so that the pressure in the ion source can be roughly measured. The remaining line into the ion source is for the delivery of the mass spectrometer calibration standard, perfluorotributylamine. Most joints are welded together to avoid leaks when this inlet system is thermally cycled or vented. The only removable (Swagelok) fitting is at the junction of the GC column and the far end of the inlet tube (marked with an asterisk in Fig. 2.21. This fitting uses Vespel ferrules. Once the ferrules are on the GC column and it is in the ion source, it is desirable to cut off a few centimeters of the column, if possible. This eliminates the possibility of fine particles partially occluding the end of the column. If the end of the column cannot be placed directly in the ion source, the material in the GC-MS interface becomes important. The interface is held at 250 to 280 °C; thus, it should not include a reactive metal (such as copper). In some interfaces, glass-lined

stainless steel tubing has been used, even though this tubing is difficult to bend properly.

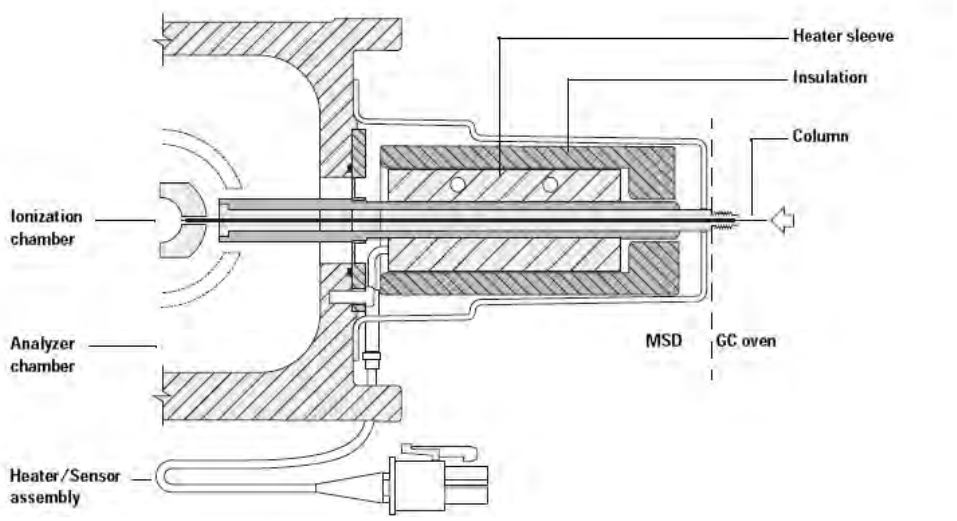

Figure 2.21 The GC/MSD interface

2.8.2 Ionization source

An **ion source** is an electro-magnetic device that is used to create charged particles. These are used primarily to form ions for mass spectrometers, optical emission spectrometers, particle accelerators, ion implanters and ion engines.

After the molecules travel the length of the column, pass through the transfer line and enter into the mass spectrometer they are ionized by various methods with typically only one method being used at any given time. Once the sample is fragmented it will then be detected, usually by an electron multiplier diode, which essentially turns the ionized mass fragment into an electrical signal that is then detected.

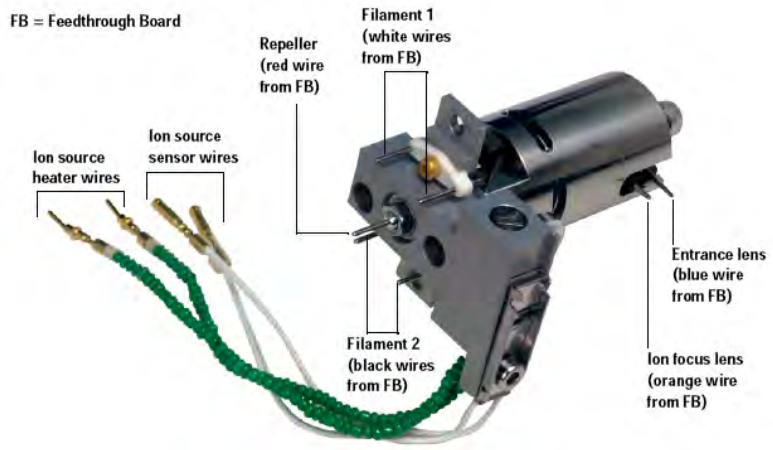

Figure 2.22 Ion source

2.8.2.1 Electron Ionization

Electron ionization (EI**)** (formerly known as **electron impact**) is an ionization method in which energetic electrons interact with gas phase atoms or molecules to produce ions. This technique is widely used in mass spectrometry, particularly for gases and volatile organic molecules. EI systems ionize sample molecules by bombarding them with electrons. The ions, including fragments, are drawn into the quadrupole analyzer where they are separated by their mass-to-charge (*m/z*) ratios and detected.

By far the most common and perhaps standard form of ionization is electron ionization (EI). The molecules enter into the MS (the source is a quadrupole or the ion trap itself in an ion trap MS) where they are bombarded with free electrons emitted from a filament, not much unlike the filament one would find in a standard light bulb. The electrons bombard the molecules, causing the molecule to fragment in a characteristic and reproducible way. This "hard ionization" technique results in the creation of more fragments of low mass to charge ratio (m/z) and few, if any, molecules approaching the molecular mass unit. Hard ionization is considered by mass spectroscopists as the employ of molecular electron bombardment, whereas "soft ionization" is charge by molecular collision with an introduced gas. The molecular fragmentation pattern is dependant upon the electron energy applied to the

system, typically 70 eV (electron Volts). The use of 70 eV facilitates comparison of generated spectra with National Institute of Standard (NIST-USA) library of spectra applying algorithmic matching programs and the use of methods of analysis written by many method standardization agencies.

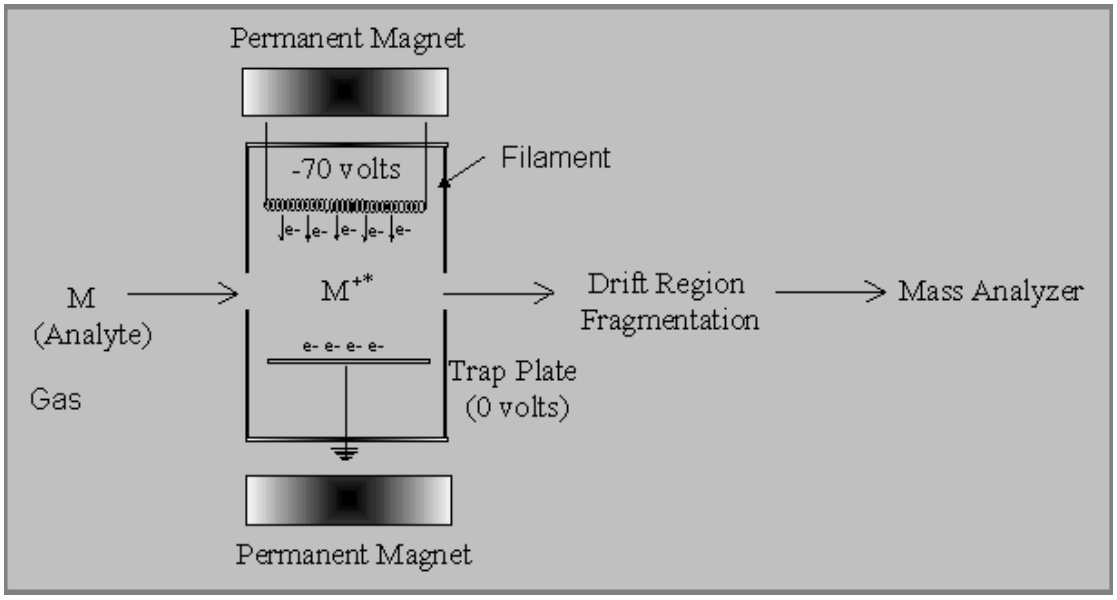

Figure 2.23 Electron Ionization

2.8.2.2 Chemical Ionization

Chemical ionization (CI) is an ionization technique used in mass spectrometry.[1][2][3] Chemical ionization is a lower energy process than electron ionization. The lower energy yields less fragmentation, and usually a simpler spectrum. A typical CI spectra has an easily identifiable molecular ion.

CI systems use a reagent gas as an intermediate between the electrons and the sample. CI is more gentle than direct electron bombardment.In chemical ionization a reagent gas, typically methane or ammonia is introduced into the mass spectrometer. Depending on the technique (positive CI or negative CI) chosen, this reagent gas will interact with the electrons and analyte and cause a 'soft' ionization of the molecule of interest. A softer ionization fragments the molecule to a lower degree than the hard ionization of EI. One of the main benefits of using chemical ionization

is that a mass fragment **closely corresponding to the molecular weight of the analyte of interest is produced.**

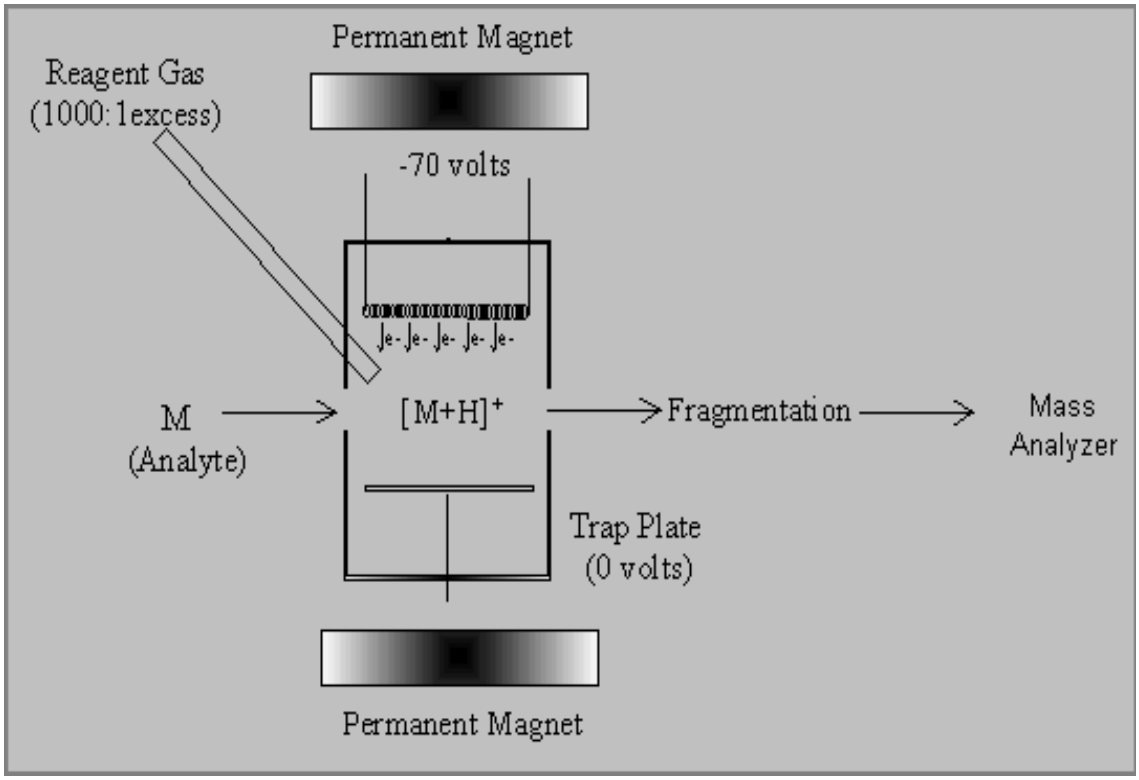

Figure 2.24 Chemical Ionization

Mechanism

In a CI experiment, ions are produced through the collision of the analyte with ions of a reagent gas that are present in the ion source. Some common reagent gases include: methane, ammonia, and isobutane. Inside the ion source, the reagent gas is present in large excess compared to the analyte. Electrons entering the source will preferentially ionize the reagent gas. The resultant collisions with other reagent gas molecules will create an ionization plasma. Positive and negative ions of the analyte are formed by reactions with this plasma.

Positive Chemical Ionization

In Positive Chemical Ionization (PCI) the reagent gas interacts with the target molecule, most often with a proton exchange. This produces the species in relatively high amounts.

Negative Chemical Ionization

In Negative Chemical Ionization (NCI) the reagent gas decreases the impact of the free electrons on the target analyte. This decreased energy typically leaves the fragment in great supply.

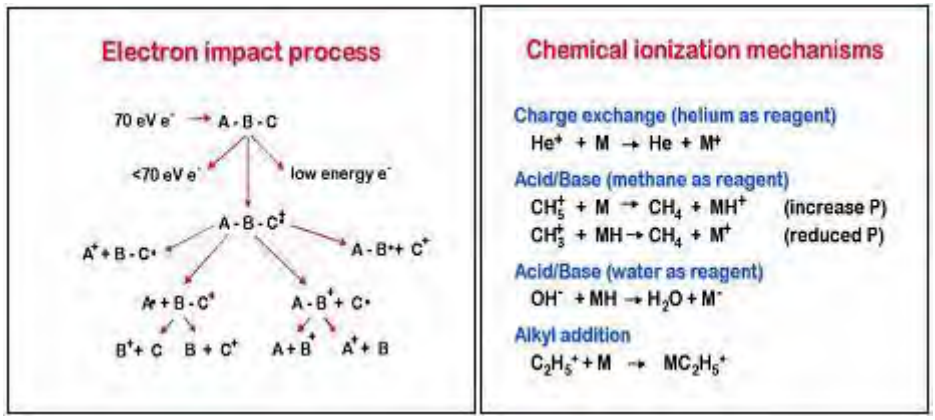

Figure 2.25 The process Ionization

2.8.3 Mass Analyzer

The analyzer is the heart of the MSD. It ionizes the sample, filters the ions, and detects them. The sample components exiting the GC column flow into the ion source. In the ion source, the sample molecules are ionized and fragmented. The resulting ions are repelled from the ion source into the quadrupole mass filter. The mass filter allows selected ions to pass through the filter and strike the detector. The detector generates a signal current proportional to the number of ions striking it. The analyzer is attached to the vacuum side of the side plate. The side plate is hinged for easy access. The ion source and the mass filter are independently heated. Each is mounted inside a radiator for correct heat distribution.

Molecular ions and fragment ions are accelerated by manipulation of the charged particles through the mass spectrometer. Uncharged molecules and fragments are pumped away. The quadrupole mass analyzer in this example uses positive (+) and negative (-) voltages to control the path of the ions. Ions travel down the path based on their mass to charge ratio (m/z). EI ionization produces singly

charged particles, so the charge (z) is one. Therefore an ion's path will depend on its mass. If the (+) and (-) rods shown in the mass spectrometer schematic were ‘fixed' at a particular rf/dc voltage ratio, then one particular m/z would travel the successful path shown by the solid line to the detector. However, voltages are not fixed, but are scanned so that ever increasing masses can find a successful path through the rods to the detector.

2.8.3.1 Quadrupole mass analyzer

The quadrupole mass analyzer is one type of mass analyzer used in mass spectrometry. As the name implies, it consists of 4 circular rods, set parallel to each other. In a quadrupole mass spectrometer (acronym *QMS*) the quadrupole is the component of the instrument responsible for filtering sample ions, based on their mass-to-charge ratio (*m/z*). Ions are separated in a quadrupole based on the stability of their trajectories in the oscillating electric fields that are applied to the rods. The quadrupole consists of four parallel metal rods. Each opposing rod pair is connected together electrically, and a radio frequency (RF) voltage is applied between one pair of rods and the other. A direct current voltage is then superimposed on the RF voltage. Ions travel down the quadrupole between the rods. Only ions of a certain mass-to-charge ratio *m/z* will reach the detector for a given ratio of voltages: other ions have unstable trajectories and will collide with the rods. This permits selection of an ion with a particular *m/z* or allows the operator to scan for a range of *m/z*-values by continuously varying the applied voltage. Ideally the rods are hyperbolic. Circular rods with a specific ratio of rod diameter-to-spacing provide an easier-to-manufacture adequate approximation to hyperbolas. Small variations in the ratio have large effects on resolution and peak shape. Different manufacturers choose slightly different ratios to fine-tune operating characteristics in context of anticipated application requirements. In recent decades some manufacturers have produced quadrupole mass spectrometers with true hyperbolic rods.

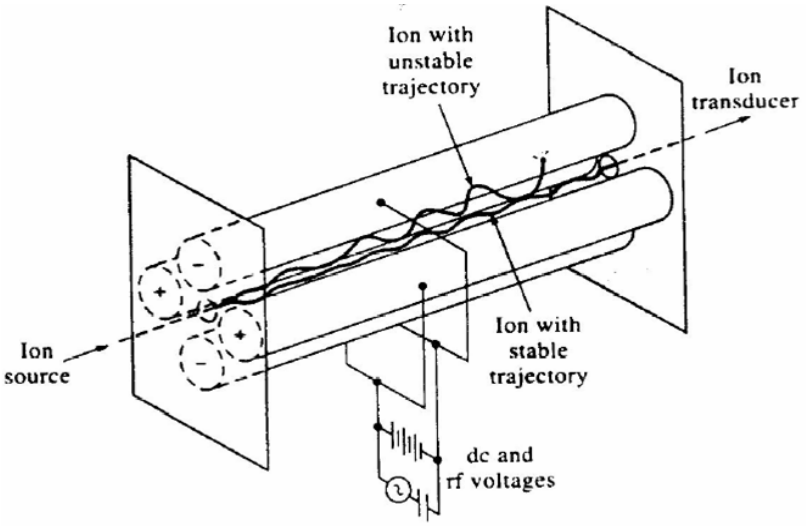

Figure 2.26 Quadrupole mass analyzer

2.8.4 Mass Detector

The detector is used to measure the ions leaving from the mass analyzer by converting ions into an electrical current or other forms of signal, processing and recording into mass spectrum. A detector is selected by speed, dynamic range, gain, and geometry. Most detectors currently used to amplify the ion signal are the electron multiplier tube (Figure 2.28) and the photo multiplier tube (Figure 2.28). The electron multiplier tube offers electrons from the surface of the tube for analyte ions. The entrance of the tube is held with potential charge opposite from the analyte ions. Analyte ions are attracted to the entrance of the tube and collide with the tube surface, then the inner surface coated with electron-emissive material releases electrons. These electrons are accelerated to hit another portion of the tube by electrostatic force and the surface loses more electrons in every collision. Amplified electrons are counted by an electrical circuit and displayed as signal intensity. The photo multiplier tube comprises a photocathode and a series of dynodes. In the high voltage tube, incident photon strikes the photo cathode and emits electrons due to the photoelectric effect. These electrons are accelerated towards a series of additional electrodes called dynodes. At the dynodes, the amount of electrons is increased at every collision. This creates an amplified signal that is finally collected and measured at the anode.

Typically, some type of electron multiplier is used, though other detectors including Faraday cups and ion-to-photon detectors are also used. Because the number of ions leaving the mass analyzer at a particular instant is typically quite small, considerable amplification is often necessary to get a signal. Microchannel plate detectors are commonly used in modern commercial instruments. The detector in the MSD analyzer is a high energy conversion dynode (HED) coupled to an electron multiplier (EM). The detector is located at the exit end of the quadrupole mass filter. It receives the ions that have passed through the mass filter. The detector generates an electronic signal proportional to the number of ions striking it. The detector has three main components: the detector ion focus, the HED and the EM horn

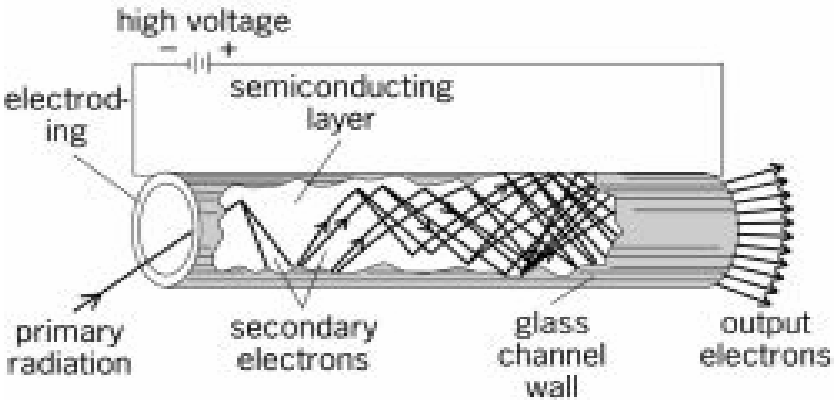

Figure 2.27 Electron multiplier tube

2.8.5 Analysis

A mass spectrometer is typically utilized in one of two ways: Full Scan or Selective Ion Monitoring (SIM). The typical GC/MS instrument is capable of performing both functions either individually or concomitantly, depending on the setup of the particular instrument.

2.8.5.1 Full scan MS

When collecting data in the full scan mode, a target range of mass fragments is determined and put into the instrument's method. An example of a typical broad range of mass fragments to monitor would be *m/z* 50 to *m/z* 400. The determination of what range to use is largely dictated by what one anticipates being in the sample while being cognizant of the solvent and other possible interferences. An

MS should not be set to look for mass fragments too low or else one may detect air (found as *m/z* 28 due to nitrogen), carbon dioxide (*m/z* 44) or other possible interferences. Additionally if one is to use a large scan range then sensitivity of the instrument is decreased due to performing fewer scans per second since each scan will have to detect a wide range of mass fragments.

Full scan is useful in determining unknown compounds in a sample. It provides more information than SIM when it comes to confirming or resolving compounds in a sample. During instrument method development it may be common to first analyze test solutions in full scan mode to determine the retention time and the mass fragment fingerprint before moving to a SIM instrument method.

2.8.5.2 Selected ion monitoring

In selected ion monitoring (SIM) certain ion fragments are entered into the instrument method and only those mass fragments are detected by the mass spectrometer. The advantages of SIM are that the detection limit is lower since the instrument is only looking at a small number of fragments (e.g. three fragments) during each scan. More scans can take place each second. Since only a few mass fragments of interest are being monitored, matrix interferences are typically lower. To additionally confirm the likelihood of a potentially positive result, it is relatively important to be sure that the ion ratios of the various mass fragments are comparable to a known reference standard.

CHAPTER III

EXPERIMENT

3.1 Instrumental and Apparatus

3.1.1 Gas Chromatography model 7890A with Triple Axis Detector : micro-Electro Capture Detector (μ-ECD), MS 5975C inert XL Mass Selective Detector (MSD), Flame Photometric Detector (FPD) GC System with Programmable Temperature Vaporizing (PTV) Injector and 7673 B autosampler : Agilent Technologies

3.1.2 GC Column: HP-5MS (5% phenyl methyl siloxane,25m.X 0.25mm, 0.25 μm film thickness)

3.1.3 Balance: model XS4002S, Mettler-Toledo, Inc., OH, Switzerland.

3.1.4 Homogenizer: model Ultra-Turrax T25 basic, Becthai Bangkok Equipment & Chemical.

3.1.5 Rotary Evaporator: model R215, Buchi, Becthai Ltd.

3.1.6 Vortex: model vortex - genie 2, Scientific Industries, Inc.

3.1.7 Centrifuge: model sorvall biofuge stratos, Utech Products,Inc., NY, USA.

3.1.8 Nitrogen - Evaporator: model N - EVAP 12 position 5085, Sithiporn Associate.

3.1.9 Microwave : model R294, Sharp Co., LTD

3.1.10 Ultrasonicate: model crest575d, Crest Ultrasonic corporation, NY, USA.

3.1.11 Water bath: model J-BS 3D, Panapolytech Co.,LTd.

3.1.12 Freezer: model Up-Right Freezer, Zest-Med Co., LTD.

3.1.13 Refrigerator model: SBC-2DA

3.1.14 Microsyringes, 100-µL, Hamilton, Bonaduz, Switzerland.

3.1.15 Micropipettes, 2-20 µL, 50-200 µL, and 200-1000 µL, Gilson, Inc., Middleton,USA.

3.1.16 Micropipette tips, 200 µL and 1000 µL, Gilson Inc., Middleton, USA.

3.1.17 GC amber vials, 2 ml with PTFE cap, Agilent Technologies, CA, USA.

3.1.18 Mortar and Pestle

3.1.19 Cylinder, 100 Ml

3.1.20 PTFE Tube, 50 Ml

3.1.21 Flat bottom flask, 100 Ml

3.1.22 Micropipette, 2-20 µL,50-200 µL and 200-1000 µL, Gilson, Inc., Middleton, USA.

3.1.23 Micropipette tips, 2-20 µL, and 200-1000 µL, Gilson, Inc., Middleton, USA.

3.1.24 Volumetric flasks, 5.00 mL, 10.00 mL, 25.00 mL, 50.00 mL, 100.00 mL

3.1.25 Duran flask, 250 mL

3.1.26 Beakers, 10 mL, 50 mL, 100 mL, 250 mL, and 1000 mL.

3.1.27 Graduated cylinders, 25mL and 100mL.

3.1.28 Spatulas

3.1.29 Droppers

3.1.30 Weighting boats

3.1.31 Glass syringe 20 mL

All experimental glass ware was cleaned with detergents, dried in an oven and rinsed with acetone before used.

3.2 Chemicals

3.2.1 Standard compounds

alpha-Hexachlorocyclohexane (α-HCH), beta- Hexachlorocyclohexane (β-HCH), γ-Hexachlorocyclohexane (γ-HCH), delta-Hexachlorocyclohexane (δ-HCH), Heptachlor, Aldrin, Dicofol, Heptachlor epoxide, γ-Chlordane, alpha-Endosulfan (α-Endosulfan), p,p-DDE, Dieldrin, Endrin, β-Endosulfan (Endosulfan β), p,p-DDD, o,p-DDT, Endosulfan sulfate, p,p-DDT, Methoxychlor , Bifenthrin, lamda Cyhalothrin, Permethrin, Cyfluthrin, Cypermethrin, Fenvalerate and Deltamethrin all were purchased from Dr.Ehrenstorfer (Agsburg,Germany) with purity of 97.5, 98.4, 98.5, 99.5, 99.5, 98.0, 99, 99.5, 99.0, 98.5, 95, 99,99, 98.5, 99, 98, 99.5, 98.5, 98, 97.5,98, 97, 98, 94, 98.5 and 99 respectively.

3.2.2 Organic solvents

Acetone, methylene chloride, acetonitrile and n-hexane were ultra-residues grade, were purchased from J.T. Baker Chemical Company (Deverter, Holland).

3.2.3 Reagents and Other chemical

Extra pure silica gel (60-230 mesh), florisil (60 – 100 mesh), sodium chloride (NaCl) and sodium sulfate anhydrous (Na_2SO_4) which were ultra residues grade were obtained from J.T. Baker Chemical Company (Deverter, Holland). Octadecylsilyl (ODS)-derivatized silica (C18) bulk sorbent was purchased from Anaserwis (Baranowo, Poland) Analytical grade charcoal (Activated carbon, decolorizing) was supplied from Sigma–Aldrich (St. Louis, MO, USA). SPE columns packed with 1000 mg Florisil were purchased from Water (made in Ireland). Primary Secondary Amine (cleanert PSA) 40-60 µm , magnesium sulfate ($MgSO_4$) was obtained from Agela

Technologies**,** di sodium hydrogen citrate 1,5-hydrate ($C_6H_6Na_2O_7$ 1,5 H_2O) and trisodium citrate ($Na_3C_6H_5O_7.2H_2O$) analytical grade were purchased from Merck (Darmstadt, Germany)

3.3 Preparation of standard solutions

3.3.1 Preparation of stock standard solutions

Each standard solution containing 1000 µg /mL in hexane was prepared by weighing 10.0 mg of each single standard in 10.00 mL volumetric flasks with n-hexane. All stock standard solutions were stored in closed vials with Teflon screw cap at 4 ºC in a refrigerator until used.

3.3.2 Preparation of mixture standard solutions

A 10 µg/mL of mixture of standard solution of organochlorine and pyrethroid pesticides was prepared by pipetting 100 µL of 1000 µg/mL of each single standard stock solution into a 10.00 mL volumetric flask and diluting with n-hexane. The mixture standard solution was kept in closed vials with Teflon screw cap and prepared daily.

3.3.3 Preparation of mixture standard solutions for Calibration Curve

The mixture of standard solution of organochlorine and pyrethroid pesticides 10 µg/mL was prepared by pipetting the standard mixture solutions 10 µg/mL and diluting them to 5.0 mL with n- hexane in 5 mL amber volumetric flask. The volume of standard mixture solution had to be put into pipettes of each concentration as shown in Table 3.1

Table 3.1 The measuring volume of mixed standard organochlorine and pyrethroid pesticides solutions and final concentration

Concentration of Organochlorine and Pyrethroid pesticides solution (mg/L)	The volume of standard mixture solution (µL)
0.005	5
0.010	10
0.050	50
0.10	100
0.20	200
0.50	500

3.4 GC Optimization

In this proposal, the analysis was performed by GC/MS System with a three way splitter added the end of the column. The column effluent could be splited three ways to micro-Electron Capture Detector (µ-ECD), Flame Photometric Detector (FPD) and MS 5975C inert XL Mass Selective Detector (MSD). The splitter system is therefore to three signals from a single injection. : Agilent Technologies Thailand Ltd.

First, The standard solutions of Organochlorine, Pyrethroid and Organophosphate were mixed and injected into GC/MS system with a three way splitter. A FPD detector was employed to determine organophosphate pesticides and the µ- ECD detector was used to determine Organochlorine and Pyrethroid pesticides due to for this technique do not support for confirmatary. GC/MS conditions were employed to confirm the qualitative work, and mass spectrometer conditions were shown in Table 4.3. Therefore all these developing of the Sample preparation technique were not suitable to analyse organophosphate pesticides in these samples. It was inconvenient to extract and run with GC so the experiment was conducted on the

GC/MS system; all the subsequence runs were performed with micro-Electron Capture Detector (μ-ECD) and Mass Selective Detector (MSD).

At the first optimization, the standard solutions of Organochlorine and Pyrethroid were mixed and injected into GC/MS system with a three way splitter. The split less was employed injection mode for analysis Organochlorine and Pyrethroid pesticides and the GC conditions were recorded as shown in Table 4.1.The split less mode could be applied for mixing standard pesticides solution, because these techniques were not suitable for spiked sample solution.. Therefore, all of my subsequent experiments were performed under the GC conditions by using PTV solvent vent mode as injection mode and the GC conditions were shown in Table 4.2

The mixture of 26 standard organochlorine and pyrethroid pesticide solutions and spiked sample were injected according to the GC conditions in Table 4.1 (split less mode) and 4.2 (PTV Solvent vent mode), that was within the suitable GC conditions for separation of each compound. The chromatogram of 26 organochlorine and pyrethroid pesticides at 50 ng /mL was shown in Figure 4.1-4.4.

3.4.1 The study of Selectivity of GC

The selectivity of two conditions determined by the retention time of each peak under the suitable GC conditions in Table 4.1 and 4.2. Resolution could be determined by observing the baseline separation that was the best separation and would give resolution of more than 1.5 as described in Table 4.5 (split less mode) and 4.6 (PTV Solvent vent mode).

$$\text{Resolution} = \frac{t_{R2} - t_{R1}}{Wh_{1/2}1 + Wh_{1/2}2}$$

3.4.2 The study of Standard calibration curves

Standard calibration curves were prepared with hexane at various concentrations. The standard solution concentrations were in the range of 5.00.005 - 0.5 mg/L. Each concentration was studied in three replicates. The calibration curves were plotted as concentration over the peak area of each analyte. Each point was the average of three replicates runs. Summary of value of slope, Retention Time, Intercept and Coefficient (R^2) of each pesticides in mixed 26 standard organochlorine and pyrethroid pesticides by the condition in table 4.2 ,was shown in Table 4.7 and the calibration curves was shown in APPENDIX.

3.5 The developing of the Sample preparation technique

At the first time, we would used the standard method for a onion (63) for sample preparation and quantitative analysis of organochlorine and pyrethroid pesticide in garlic sample. It was believed that the chemical substance in onion as same as garlic it could be to analysis in it. The purpose was to find out if the method could be used with Garlic samples and how much in terms of mean percentage recovery and the interfering peaks. The comparison of results was based on the interfering peaks, background noises in chromatogram and in mean percentage recovery with GC condition in Table 4.2 The results were show in Figure 4.5-4.10

3.5.1 The study of the Pre-treatment with Microwave and Liquid Liquid Extraction (LLE) method

In 2008, herbicides in onions that were not garlic were analyzed based on preventing formation of sulfur-containing compounds in onions by microwave and extracted with acetonitrile and cleaned by solid-phase extraction. Recoveries were measured from 69.2% to105.0% (63) Thus, we would used this method for sample preparation and quantitative analysis of organochlorine and pyrethroid pesticide in the garlic sample. The comparison of results gained through the use of microwave with that without the use of microwave was considered on the interfering peaks,

background noises in chromatogram and in mean percentage recovery with GC condition in Table 4.2 The results were shown in Table 4.8 and Figure 4.5.

The process of Pre-treatment with Microwave and Liquid Liquid Extractionwas performed as follows:

3.5.1.1 Ground garlic cloves sample 25 g were weighed into to a duran bottle

3.5.1.2 The sample was heated in a microwave at 800 watt for 30 s, then rapidly cooled in cold -water.

3.5.1.3 Fifteen grams of sodium chloride and 100 mL of acetonitrile were filled into duran bottle and extracted with a homogenizer for 1 min.

3.5.1.4 After extraction, the extraction solution was left to separated for 30 min

3.5.1.5 The 50 mL upper layer was evaporated on a rotary evaporator to near dryness for clean-up.

3.5.1.6 Ten milliliters acetone/n-hexane (1:9 v/v) was added to condition the dry SPE column.

3.5.1.7 The florisil cartridge for SPE was loaded with the extract after being activated and eluted with 10 mL mixture of acetone/n-hexane (1:9 v/v).

3.5.1.8 The eluate was reduced to about 1 mL with a gentle nitrogen stream and then made up to 5 mL with n-hexane for analysis.

The results of the Pre-treatment with Microwave and liquid liquid extraction (LLE) are shown in Table 4.8 and Figure 4.5.

3.5.2 The study of the Pre-treatment with water bath and liquid liquid extraction (LLE) method

From the result in section 3.5.1, it was apparent that this method was not possible. In this section, it was further developed so that it could be most suitable to

use with Garlic samples and could effectively eliminate matrics. The use of microwave was adjusted so that it created the least volatile interferences, and most parameters were studied so that an effective extracting method could be developed. The process was changed from microwave to water bath because when we used water bath, the sample that contained organochlorine and pyrethroid will be softer volatilized than that microwaved . Furthermore, we studied by comparing sample with microwave before extraction. Before the extraction process, the mixed 26 standard organochlorine and pyrethroid pesticide solutions were added with spiking level of 0.01 mg/kg. The comparison of results gained through the use of water bath compared with the results without the use of water bath was considered based on the interfering peaks, background noises in chromatogram and in mean percentage recovery with GC condition in Table 4.2 The results were shown in Table 4.9 and Figure 4.6.

The process of Pre-treatment with water bath and liquid liquid extraction was performed as follows:

3.5.2.1 Ground garlic cloves sample 5 g were weighed into to a duran bottle.

3.5.2.2 The bottle of sample was placed on a water bath which heated at 50 ºC for 30 min

3.5.2.3 10 g of sodium chloride, 50 mL of acetone and 40 mL of dichloromethane were filled into a Duran bottle and extracted with a homogenizer for 2 min.

3.5.2.4 After extraction, the 50 mL supernatant solution was transferred to 100 mL flat bottom flask and evaporated in a rotary evaporator to near dryness.

3.5.2.5 The extraction solution was adjusted to volume 5 mL.

3.5.2.6 The 2 mL of extraction solution was cleaned up with 1g of florisil cartridge.

3.5.2.7 Preconditioned florisil cartridge with 5 mL *n*-hexane.

3.5.2.8 15.0 mL of *n*-hexane–DCM (1:1, v/v) was used to elute the pesticide residues from the cartridge.

3.5.2.9 The eluents were collected and concentrated to dryness under N_2 flow

3.5.2.10 The analyse were dissolved in 2.0 mL *n*-hexane for GC analysis.

3.5.3 The study of QuEChERS technique

QuEChERS is a new technique for Multiresidue Analysis of Pesticides in Foods and Agricultural Samples. It was believed that various sorbent in Dispersive solid-phase extraction (SPE) were adsorb matrix components it could help decrease interferences in sample. The developed method was investigated in terms of mean percentage recovery and the interfering peaks. The comparison of results gained through the use QuEChERS technique with that various Dispersive SPE sorbent the use of clean up was considered on the interference peaks, background noises in chromatogram and in mean percentage recovery with GC condition in Table 4.2 The results were show in Table 4.10 and Figure 4.7-4.8.

From the result in section 3.5.3, it was can not achieve for the method.

The process of QuEChERS technique was performed as follows:

3.5.3.1 Ground garlic cloves sample 5 g was transferred to a 50 mL PTFE tube.

3.5.3.2 10 mL of acetonitrile, 4 g of magnesium sulfate anhydrous, 1g of sodium chloride, 1g of tri sodium citrate dehydrate and 0.5 g of disodium hydrogen citrate hydrate were filled into the PTFE tube, and extracted with and shaked vigorously for 1 min

3.5.3.3 The mixture solution was centrifuged at 3000 rpm for 5 min.

3.5.3.4 The supernatant solution was transferred to a test tube

3.5.3.5 Fifty milligrams of dispersive SPE and 300 mg of MgSO4 were filled into the PTFE tube . PSA, florisil, silica gel, C18, graphite carbon black and Al_2O_3 were investigated as dispersive SPE in two replicates.

3.5.3.6 The mixture solution was centrifuged at 3000 rpm for 3 min.

3.5.3.7 The mixture solution was placed in a refrigerator overnight.

3.5.3.8 The upper layer supernatant solution was transferred to a test for GC analysis.

3.5.4 The study of MSPD technique

In 2008, a simple and effective extraction method based on matrix solid-phase dispersion (MSPD) was developed to determine 8 pesticides such as dimethoate, malathion, lufenuron, carbofuran, 3-hydroxycarbofuran, thiabendazole, difenoconazole and trichlorfon in coconut pulp , C18 as dispersant sorbent , Florisil 1 g. as clean-up sorbent and acetonitrile saturated with *n*-hexane as eluting solvent Quantified by GC/MS in SIM mode. Recoveries were measured from 70.1% to 98.7% with the relative standard deviations (RSD) below 10.7%. (72) Thus, we would use this method as a basis for sample preparation and quantitative analysis of organochlorine and pyethroid pesticides in garlic sample the procedure of the basis method is as follows Figure 3.1

The process of MSPD technique was performed as follows:

3.5.4.1 Five grams of grind garlic cloves sample was placed into a mortar.

3.5.4.2 The sample was blended gently with 5 g of florisil.

3.5.4.3 Forty milligrams of charcoals was filled into the sample and blended the sample mixture against.

3.5.4.4 Then the homogeneous mixture was transferred into a glass cartridge under laid with a glass filter paper (Whatman GF/A).

3.5.4.5 The florisil cartridge was preconditioned with 5 mL of *n*-hexane.

3.5.4.6 The sample mixture glass cartridge was connected to a Florisil cartridge.

3.5.4.7 Thirty milliliters of *n*-hexane was introduced to elute the pesticides from the cartridge directly.

3.5.4.8 Five milliliters additional eluent was adopted to wash the mortar and pestle, and then transferred into the cartridge.

3.5.4.9 The eluents was collected and evaporated in a rotary evaporator to near dryness.

3.5.4.10 The extractant was dissolved in 7 mL *n*-hexane.

3.5.4.11 The extraction solution was concentrated to 1 mL with N_2 flow and placed into a refrigerator at under -18˚C for 5 hours.

3.5.4.12 The upper layer supernatant solution was transferred to a GC vial and analyzed with gas chromatography mass spectrometry system.

The results of MSPD technique are shown in Table 4.11 and and Figure 4.9 and 4.10.

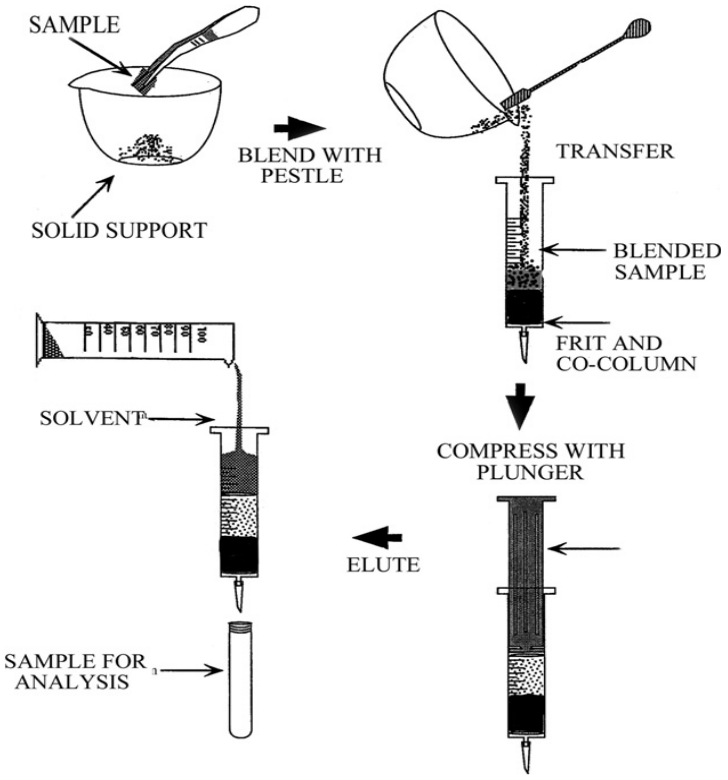

Figure 3.1 Steps in a MSPD extraction.(71)

3.6 Matrix Solid Phase Dispersion (MSPD) optimization

From the result of previous section, MSPD method offered a better condition for extraction. From the result in Table 4.11 , % recovery of Organochlorine and Pyrethroid pesticide as δ-BHC, Dicofol, Hept.Epoxide, α-Endosulfan, α-Chlordane, Dieldrin, Endrin, Cypermethrin and Deltamethrin were lower than 60 % .Therefore, in this section , it was further developed so under experimental conditions, a critical step for extraction of pesticide residues by MSPD was the characterized of solid support and elution solvent.

Parameters affecting MSPD procedure such as solid support type, type and volume of elution solvent and were investigated as can seen in Figure 3.1**.** The results are displayed as mean percentage recoveries and matrix cleanup.

3.6.1 The procedure of solid support type optimization

In this section, sorbent type was the first factor considered since it could have effects on the extraction efficiency. The sulfur compound interferences in garlic were a polar compound of phenolic and steroidal. (1) The florisil and silica gel are polar sorbent and were investigated in terms of mean percentage recovery and the interfering peaks.

The process of solid support type optimization in MSPD was performed as follows:

3.6.1.1 Five grams of ground garlic cloves sample was placed into a mortar.

3.6.1.2 The sample was blended gently with 5 g of solid support, Florisil, silica gel and C18 were investigated as solid supports in two replicates.

3.6.1.3 Forty milligrams of charcoal was filled into the sample and blended against the sample mixture.

3.6.1.4 Then the homogeneous mixture was transferred into a glass cartridge under laid with a glass filter paper (Whatman GF/A).

3.6.1.5 The florisil cartridge was preconditioned with 5mL of *n*-hexane.

3.6.1.6 The sample mixture glass cartridge was connected to a Florisil cartridge.

3.6.1.7 Thirty milliliters of *n*-hexane was introduced to elute the pesticides from the cartridge directly.

3.6.1.8 Five milliliters additional eluent was used to wash the mortar and pestle, and then transferred into the cartridge.

3.6.1.9 The eluents were collected and evaporated in a rotary evaporator to near dryness.

3.6.1.10 The extractant was dissolved in 7 mL *n*-hexane.

3.6.1.11 The extraction solution was concentrated to 1 mL with N_2 flow and placed into a refrigerator under -18˚C for 5 hours.

3.6.1.12 The upper layer supernatant solution was transferred to a GC vial and analyzed with gas chromatography mass spectrometry system.

The results of solid support type optimization are shown in Table 4.12.

3.6.2 The procedure of solid support composition optimization

From the result in section 3.6.1, more than one could either be chosen for the procedure. In this section, the mixed solid support was studied. It was believed that when the value of florisil: silica gel increased it could help disrupt interferences in sample and yield better % recover.

The process of solid support composition in MSPD was performed as follows:

3.6.2.1 Five grams of ground garlic cloves sample was placed into a mortar.

3.6.2.2 The sample was blended gently with 5 g of mixture of solid support: 5g mixture of Florisil and silica gel were studied in 3 compositions. Each composition was studied with two replicates.

Composition I : ratio 1:1 Florisil - silica gel

Composition II : ratio 1:4 Florisil - silica gel

Composition III : ratio 4:1 Florisil - silica gel

3.6.2.3 Forty milligrams of charcoal was filled into the sample and blended against the sample mixture.

3.6.2.4 Then the homogeneous mixture was transferred into a glass cartridge under laid with a glass filter paper (Whatman GF/A).

3.6.2.5 The Florisil cartridge was preconditioned with 5ml of *n*-hexane.

3.6.2.6 The sample mixture glass cartridge was connected to a Florisil cartridge.

3.6.2.7 Thirty milliliters of *n*-hexane was introduced to elute the pesticides from the cartridge directly.

3.6.2.8 Five milliliters additional eluent was adopted to wash the mortar and pestle, and then transferred into the cartridge.

3.6.2.9 The eluents were collected and evaporated in a rotary evaporator to near dryness.

3.6.2.10 The analyte was dissolved in 7 mL *n*-hexane.

3.6.2.11 The extraction solution was concentrated to 1 mL with N_2 flow and placed into a refrigerator at -18˚C for 5 hours.

3.6.2.12 The upper layer supernatant solution was transferred to a GC vial and analyzed with gas chromatography mass spectrometry system.

The results of solid support type optimization are shown in Table 4.13.

3.6.3 The procedure of type of elution solvent optimization.

In this section, once the sorbent was identified, the method was further optimized by fine-tuning the elution strength of the solvent. It was further noticed that the type of elution solvent was the continuous factor considered since it could influence the method extraction efficiency. The non-polar solvent as n-hexane, and dichloromethane were considered as solvent elution types**.** Comparing the interference peaks and background noises in chromatogram and the mean percentage recovery with GC condition in Table 4.2.

The process of type of elution solvent in MSPD was performed as follows:

3.6.3.1 Five grams of ground garlic cloves sample was placed into a mortar.

3.6.3.2 The sample was blended gently with 5 g of florisil and silica gel ratio1:1

3.6.3.3 Forty mg of charcoals was put into the sample and blended into the sample mixture against.

3.6.3.4 Then the homogeneous mixture was transferred into a glass cartridge underlaid with a glass filter paper (Whatman GF/A).

3.6.3.5 The Florisil cartridge was preconditioned with 5mL of *n*-hexane.

3.6.3.6 The sample mixture glass cartridge was connected to a Florisil cartridge .

3.6.3.7 Thirty milliliters of elution solvent was introduced to elute the pesticides from the cartridge directly. Dichloromethane and n-hexane were investigated as elution solvents in two replicates.

3.6.3.8 Five milliliters additional eluent was adopted to wash the mortar and pestle, and then transferred into the cartridge.

3.6.3.9 The eluents was collected and evaporated in a rotary evaporator to near dryness.

3.6.3.10 The analyte was dissolved in 7 mL *n*-hexane.

3.6.3.11 The extraction solution was concentrated to 1 mL with N_2 flow and placed into refrigerator under -18˚C for 5 hours.

3.6.3.12 The upper layer supernatant solution was transferred to a GC vial and analyzed with gas chromatography mass spectrometry system.

The results of elution solvent type optimization are shown in Table 4.14 and Figure 4.11-4.13.

3.6.4 The procedure of volume of elution solvent optimization

From the result in section 3.6.3, one can achieve the best elution solvent for the method. In this section, the volume of elution solvent was studied. It was believed that when the value of n-hexane increased it could help elute more interest substances and yield better % recovery

The process of volume of elution solvent optimization in MSPD was performed as follows:

3.6.4.1 Five grams of ground garlic cloves sample was placed into a mortar.

3.6.4.2 The sample was blended gently with 5 g of florisil and silica gel ratio1:1

3.6.4.3 Forty milligrams of charcoals was filled into the sample and blended into the sample mixture.

3.6.4.4 Then the homogeneous mixture was transferred into a glass cartridge underlaid with a glass filter paper (Whatman GF/A).

3.6.4.5 The Florisil cartridge was preconditioned with 5 mL of *n*-hexane.

3.6.4.6 The sample mixture glass cartridge was connected to a Florisil cartridge .

3.6.4.7 The various volumes of elution solvent were introduced to elute the pesticides from the cartridge directly. 15 mL, 30 mL and 45 mL were investigated as volume of elution solvents in two replicates.

3.6.4.8 Five milliliters additional eluent was adopted to wash the mortar and pestle, and then transferred into the cartridge.

3.6.4.9 The eluents was collected and evaporated in a rotary evaporator to near dryness.

3.6.4.10 The analyte was dissolved in 7 mL *n*-hexane.

3.6.4.11 The extraction solution was concentrated to 1 mL with N_2 flow.

3.6.4.12 The extraction solution was concentrated to 1 mL with N_2 flow and placed into a refrigerator at -18°C for 5 hours.

3.6.4.13 The upper layer supernatant solution was transferred to a GC vial and analyzed with gas chromatography mass spectrometry system.

The results of elution solvent type optimization are shown in Table 4.15 and Figure 4.14.

3.6.5 The procedure of ratio of sample to solid support material optimization.

In this section, ratio of sample to solid support sorbent type was the last factor considered since it could have effects on the extraction efficiency and interference peaks. Comparing the interference peaks and background noises in chromatogram and the mean percentage recovery with GC condition in Table 4.2.

3.6.5.1 The various ratio of sample to solid support material were placed into a mortar and studied in 3 compositions. Each composition was studied with two replicates.

Ratio I : 0.5:1 sample 2.5 g - solid support material 5.0 g

Ratio II : 1:1 sample 5.0 g - solid support material 5.0 g

Ratio III : 1:2 sample 5.0 g - solid support material 10.0 g

3.6.5.2 The various ratio of sample to solid support material were blended gently.

3.6.5.3 Forty milligrams of charcoals was filled into the sample and blended into the sample mixture.

3.6.5.4 Then the homogeneous mixture was transferred into a glass cartridge underlaid with a glass filter paper (Whatman GF/A).

3.6.5.5 The Florisil cartridge was preconditioned with 5 mL of *n*-hexane.

3.6.5.6 The sample mixture glass cartridge was connected to a Florisil cartridge .

3.6.5.7 Thirty milliliters of *n*-hexane was introduced to elute the pesticides from the cartridge directly.

3.6.5.8 Five milliliters additional eluent was adopted to wash the mortar and pestle, and then transferred into the cartridge.

3.6.5.9 The eluents was collected and evaporated in a rotary evaporator to near dryness.

3.6.5.10 The analyte was dissolved in 7 mL *n*-hexane and concentrated to 1 mL with N_2 flow.

3.6.5.11 The extraction solution was concentrated to 1 mL with N_2 flow and placed into a refrigerator at -18˚C for 5 hours.

3.6.5.12 The upper layer supernatant solution was transferred to a GC vial and analyzed with gas chromatography mass spectrometry system.

The results of elution solvent type optimization are shown in Table 4.16 and Figure 4.15-17.

3.7 Method Validation

3.7.1 Limit of detections (LODs)and limit of quantifications (LOQs)

LOD and LOQ are important in the determination process and refer to the efficiency of the method in terms of detection and quantification. While LOD refers to the method lowest concentration of analyte detected, LOQ is the lowest concentration of analyte that can be quantitatively determined. An examination was made on the method to find out what was the lowest level of substances it could detect in terms of LOD and LOQ. From chromatogram, the limits of detection were calculated as chromatographic signal (peak height) being three times higher than background noise (S/N = 3). The chromatographic signal was observed from extraction of the lowest spiked concentration of each standard (0.01 mg/L) under optimized MSPD condition in seven replicates. The limits of quantification were calculated similar to LOD, but with a signal to noise ratio of S/N = 10. Both LODs and LOQs of method are shown in Table 4.18.

3.7.2 Linearity range

Linearity of method was obtained from a standard calibration curve of twenty seven analytes. Correlation coefficient (R^2) represents the linearity of the proposed method. Under optimized MSPD conditions, the linearity was performed over a concentration range of 0.05-0.50 mg/L with three replicates of each level. The slope, y-intercept, and correlation coefficient (R^2) of twenty six organochlorine and pyrethroid pesticides are shown in Table 4.19.

3.7.3 Precision

The precision is the closeness of agreement between independent test results obtained under the same conditions. The two categories of precision are intra-assay precision and intermediate precision. The intra-assay precision is the precision derived from repeated tests on the same method with single analytical runs, while the intermediate precision is the precision acquired from repeated tests on the same method with different analytical runs or different times. In this work, precision was

determined with 26 analytes spiked at 10 μg/L with the optimized MSPD conditions in seven replicates. The extractions were performed in seven replicates in all three analytical days.

The peak area obtained was calculated in the regression equation from standard calibration curve and resulted in concentration of analyte from the method. The percent of relative standard deviations (%R.S.D) were calculated from concentration obtained in seven replicates. The %R.S.D. obtained from the results of one analytical day refers to intra-assay precision, whereas intermediate precision was reported as the %R.S.D from the results of three analytical days.

The acceptable value for %R.S.D within day was calculated from Horwitz equation *(73)*:

$$R.S.D.r = 0.67 \times 2(1-0.5\log C) \qquad \text{(Eq.11)}$$

where C is the concentration of the analyte in the sample To evaluate the intermediate precision, the two-tailed F test was employed to determine the significant difference of results obtained. The results of both intra-assay precision and intermediate precision were presented in Table 4.20-4.31.

3.7.4 Accuracy

The method accuracy refers to the closeness of agreement between the observed results from method and the true value of the analyte in the sample. Accuracy was derived from the extraction of analyte spiked under optimized MSPD parameters. In this work, two concentration levels of 0.01 ,0.05 and 0.10 mg/kg were studied and each concentration was investigated in seven replicates. The observed concentration was determined from the calculation of obtained peak area in the regression equation from standard calibration curve and the average value of seven calculated concentrations was used to represent the observed concentration. The comparison between observed concentration and spiked concentration lead to the recovery of analytes. The recoveries (%) of 26 organochlorine and pyrethroid pesticides at two spiked concentrations are presented in Table 4.32.

3.7.5 The Determination of organochlorine and pyrethroid pesticide in Garlic products from 2 Thai markets and 2 types of garlic.

Two types of garlic were purchased from 2 Thai markets. It was determined to find out the amount of organochlorine and pyrethroid pesticides according to the GC conditions in Table 4.2 and described in Table 4.33.

1. Thai garlic from maket A
2. Chinese garlic from maket A
3. Thai garlic from maket B
4. Chinese garlic from maket B

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Gas chromatographic condition

At the first time, the standard solutions of Organochlorine and Pyrethroid were mixed and injected into GC/MS system with a three way splitter. The split less method was employed to the injection mode for analysis. Organochlorine and Pyrethroid pesticides and the GC conditions were recorded as shown in Table 4.1. The split less mode could separate up to the total of 33 peaks in mixture standard pesticides solution. Therefore these techniques were not suitable for spiked sample solution. It was decided to run with GC. The interference substances and analyst were combined; furthermore it couldn't be determined and separated because the split less mode pushed up all substance into the GC system. Therefore, all of my subsequent experiments were performed using the GC conditions by using a PTV solvent vent as injection mode and the GC conditions were shown in Table 4.2.

The mixture of 26 standard organochlorine and pyrethroid pesticide solutions and spiked sample were injected according to the GC conditions in Table 4.1 (split less mode) and 4.2. (PTV Solvent vent mode) The chromatogram of 26 organochlorine and pyrethroid pesticides at 0.05 mg /L was shown in Figure 4.1, 4.2, 4.3 and 4.4.

For PTV solvent vent mode, the mixed 26 standard organochlorine and pyrethroid pesticides solution in hexane 0.050 mg/L were also separated by GC conditions as shown in Table 4.2. It could separate 33 peaks in mixture standard organochlorine and pyrethroid pesticides solution and could be separately applied for spiked sample solution. The GC condition in Table 4.2

could be used to decrease interference peaks because the low boiling point of interference substances were heated on a liner. The low-boiling interference substances were continuously evaporated and vented through the split line with the solvent.

Table 4.1 The gas chromatographic conditions using the split less mode for study of mixed standard organochlorine and pyrethroid pesticide solutions

GC Parameters	GC Condition
	(25 m 0.25mm I.D.2.5 mm film thickness)
Analytical Column	HP-5 MS (5% diphenyl, 95%dimethylpolysiloxane)
	capillary column
Restrictor Column	µECD: I.D.2.5 mm 2.018 M MSD : I.D.2.5 mm 5.483 M
Temperature Program	50 ºC , rate A 35 ºC /min to 200 ºC (5 min)
	200ºC rate B of 5 ºC /min to 250 ºC (5 min)
	250ºC rate C of 35 ºC /min to 290 ºC (10 min)
Injection Mode	Split less mode , purge time 0.75 min
Injection Volume	4µL
Injection Temperature	210 ºC
Flow Rate of carrier Gas (He)	1.44 mL /min
Flow Rate of Nitrogen gas	60 mL /min
PCM makeup supply	4.5 mL/min
Detector	micro-Electron Capture Detector (µ-ECD)
Detector Temperature	300 ºC

Table 4.2 The gas chromatographic conditions using PTV solvent vent mode for study of mixed standard organochlorine and pyrethroid pesticide solutions

GC Parameters	GC Condition
	(25 m 0.25mm I.D.2.5 mm film thickness)
Analytical Column	HP-5 MS (5% diphenyl, 95%dimethylpolysiloxane)
	capillary column
Restrictor Column	µECD: I.D.2.5 mm 2.018 M MSD : I.D.2.5 mm 5.483 M
Temperature Program	50 ºC , rate A 35 ºC /min to 200 ºC (5 min)
	200ºC rate B of 5 ºC /min to 250 ºC (5 min)
	250ºC rate C of 35 ºC /min to 290 ºC (10 min)
Injection Mode	PTV solvent vent mode
Injection Volume	4µL
Injection Temperature	50 ºC (1.1 min) rate700 ºC /min to 300 ºC
Flow Rate of carrier Gas (He)	1.44 mL /min
Flow Rate of Nitrogen gas	60 mL /min
PCM makeup supply	4.5 mL/min
Detector	micro-Electron Capture Detector (µ-ECD)
Detector Temperature	300 ºC

The mass spectrometer parameters were shown in Table 4.3. Full scan mode used for confirm purposes of organochlorine and pyrethroid pesticides.

Table 4.3 The Mass spectrometer parameter for study of mixed standard organochlorine and pyrethroid pesticides solutions

Mass Parameters	**Mass Condition**
Solvent Delay Time	3 min
Transfer line Temperature	280ºC
Ionization source temperature	300 ºC
Quardapole temperature	150 ºC
Electron impact ionization voltage	70 eV
Ionization Type	EI
Analysis	SIM mode

The confirmation information of organochlorine and pyrethroid pesticide are shown in Table 4.4. Instrument control and data acquisition and evaluation were performed with ChemStation G 1701EA Revision E. 0101 software package provided by Agilent Technologies

Table 4.4 The confirmation information of organochlorine and pyrethroid pesticides

Compound	Monitoring ion
α, β, γ, δ -BHC	219, 181, 109
Heptachlor	272, 274,237
Aldrin	263, 265, 293
Dicofol	139, 250,111
Hept.Epoxide	353,355,263
γ, α -Chlordane	373,375,272
α-Endosulfan	241,237,195
p,p'-DDE	246,318,176
Dieldrin	263,279,79
Endrin	263,317,245
p,p'-DDD	235,237,165
o,p'-DDT	235,237,165
Endosulfan sulfate	272,274,387,229
p,p'-DDT	235,237,165
Bifenthrin	181,164,166
Methoxychlor	227,228,274
Lamda Cyhalothrin	181,197,208
Permethrin	183,163,165
Cyfluthrin	163,206,227
Cypermethrin	181,163,209
Fenvalerate	167,209,125
Deltamethrin	181,253,172

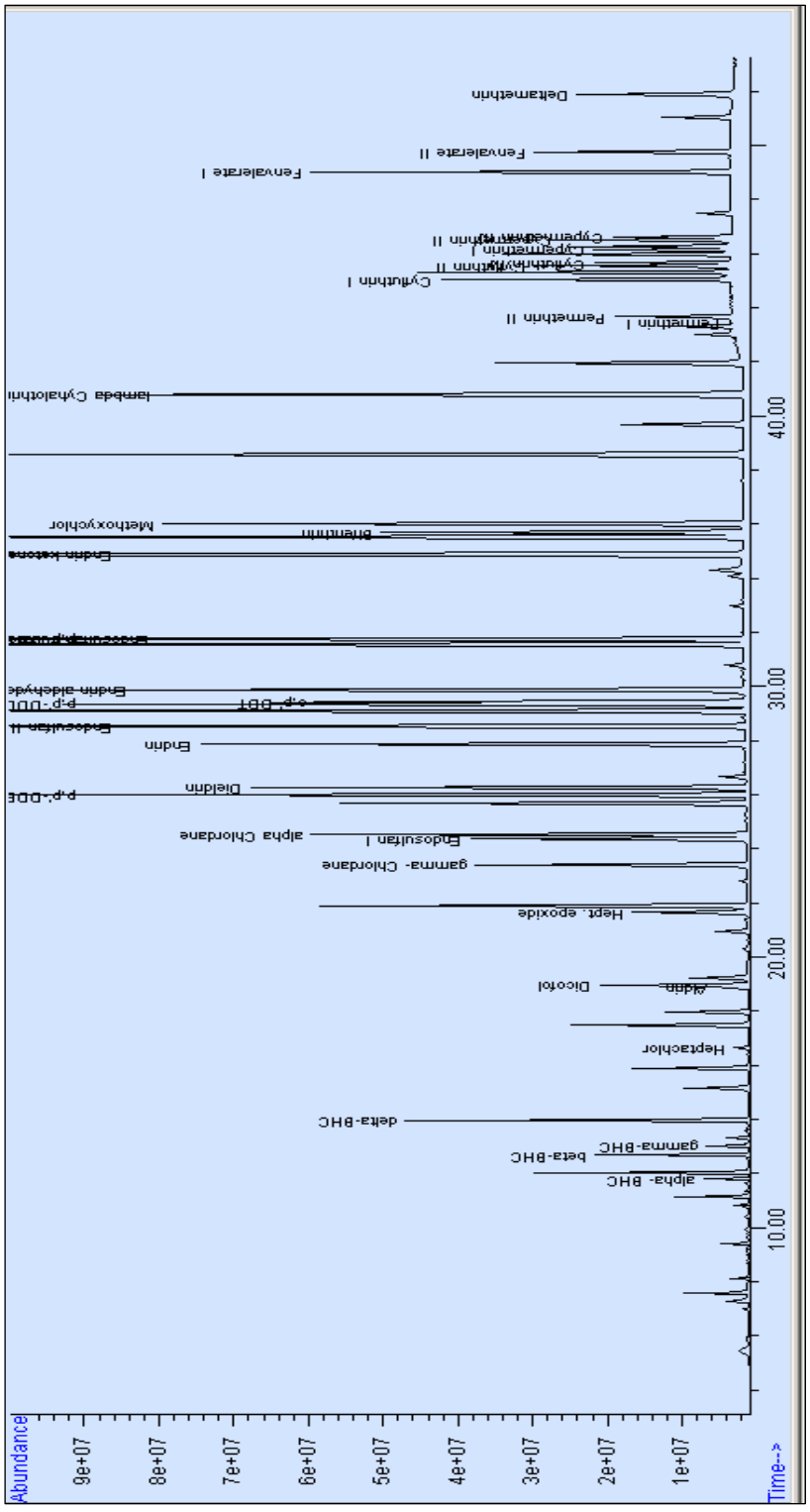

Figure 4.1 The chromatogram of mixed 26 standard organochlorine and pyerthroid pesticides in hxane 0.050 mg / L under GC conditions in Table 4.1

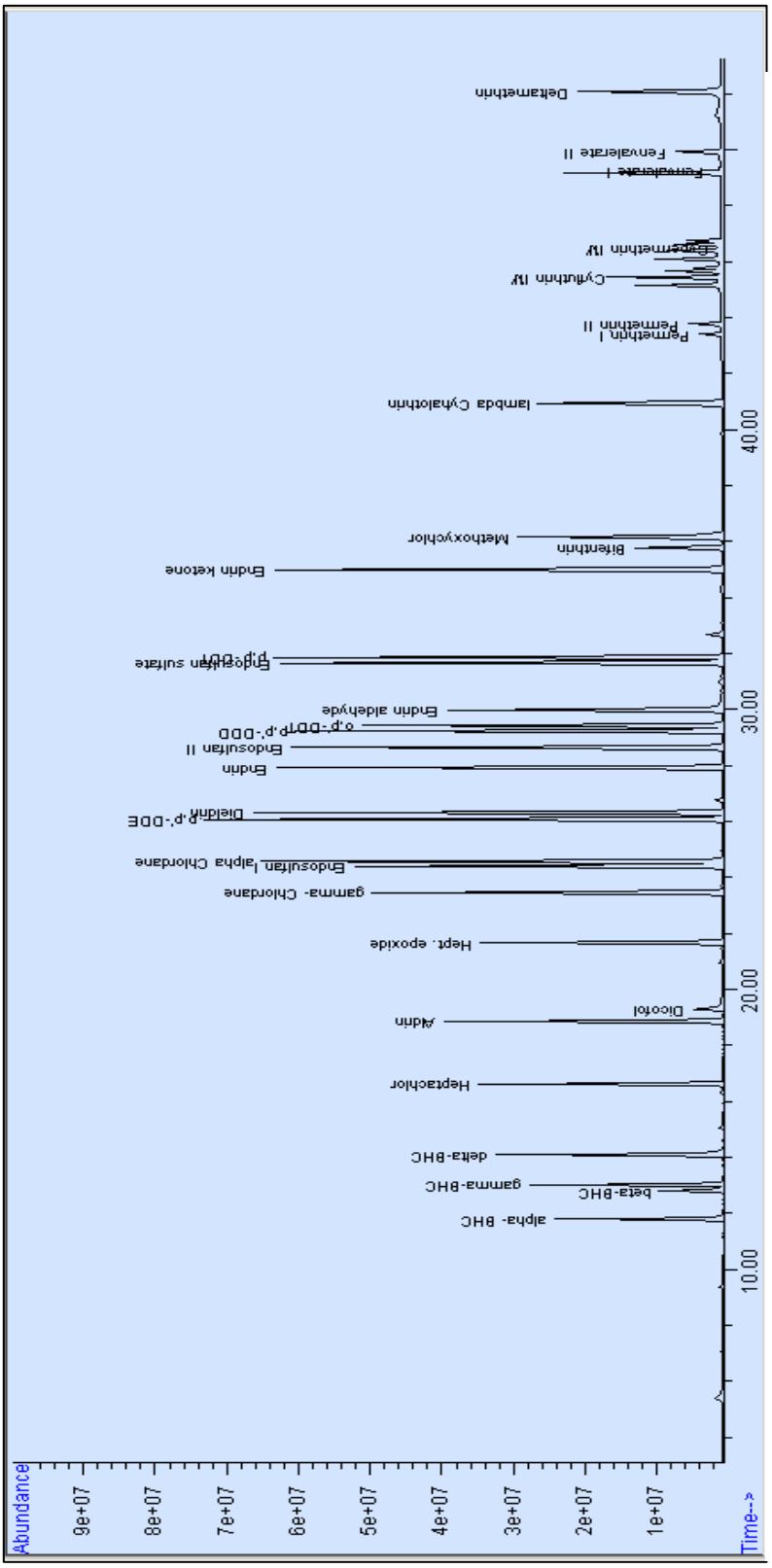

Figure 4.2 The chromatogram of mixed 26 standard organochlorine and pyerthroid pesticides in hxane 0.050 mg / L under GC conditions in Table 4.2

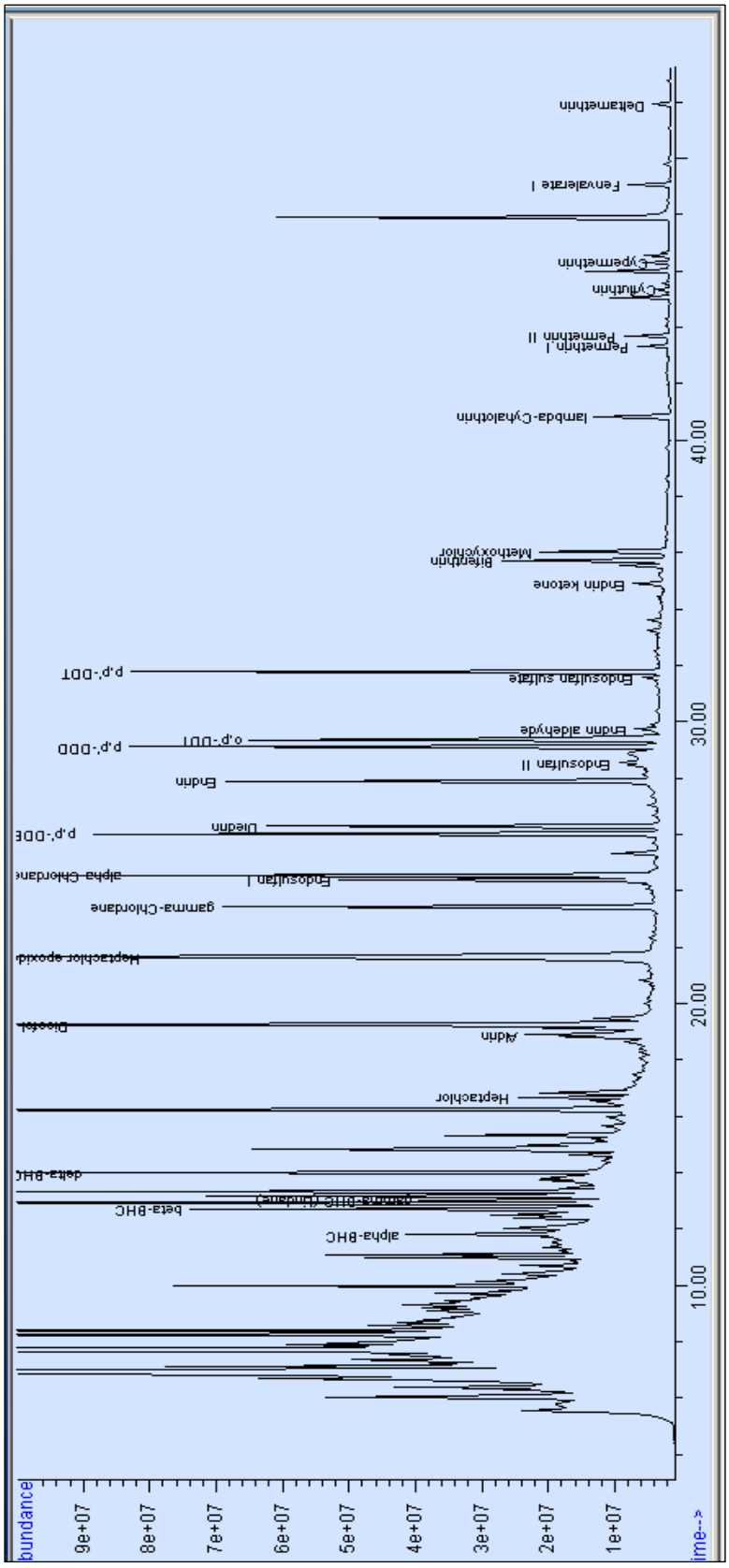

Figure 4.3 The chromatogram of spiked sample concentration level 0.01 mg / kg under GC conditions in Table 4.1

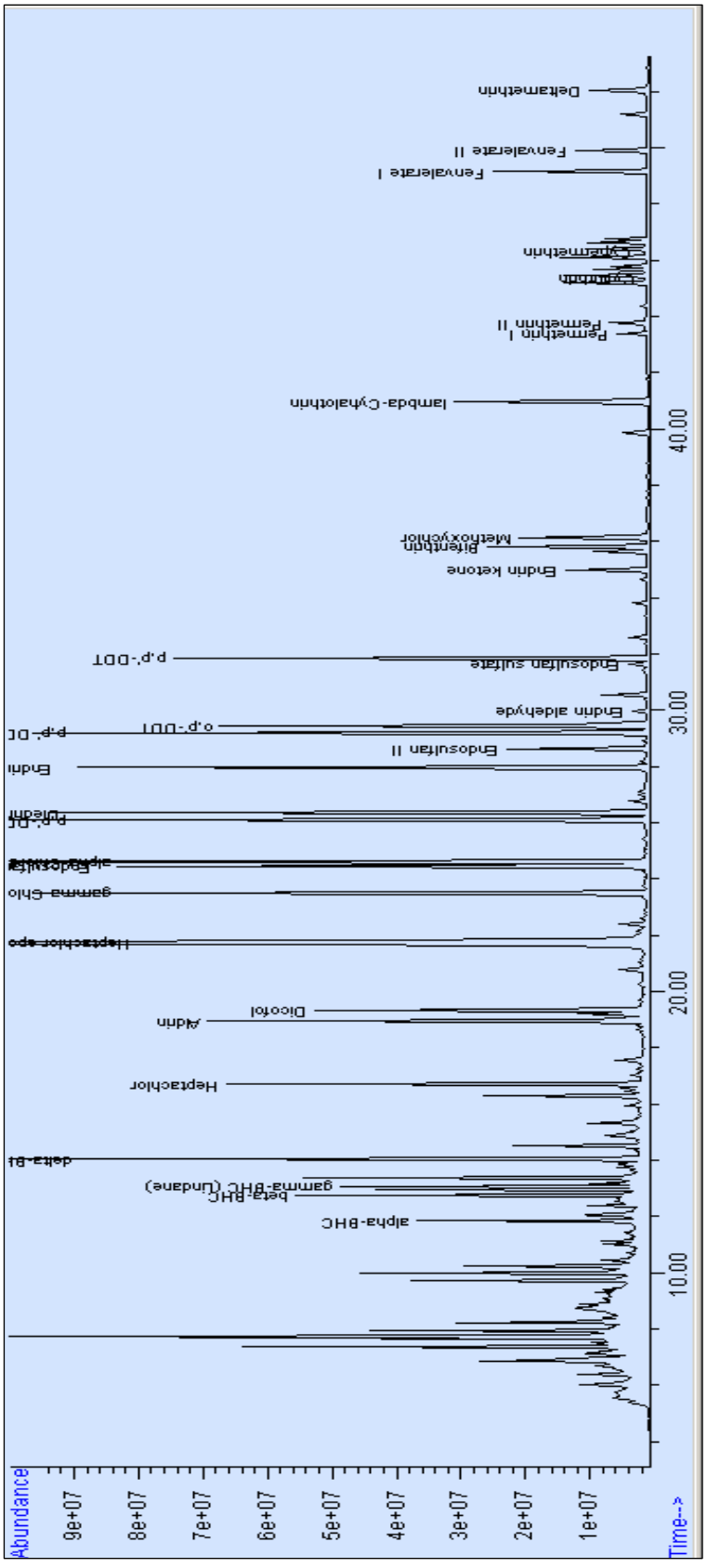

Figure 4.4 The chromatogram of spiked sample concentration level 0.010 mg / kg under GC conditions in Table 4.2

4.1.1 The Result of Selectivity of GC

The selectivity of GC conditions in Table 4.1 and 4.2 can be determined by the retention time and resolution value of the critical pair (R_S) of each peak. From Table 4.5 and 4.6, all of the interesting peaks can be separated and there is no interference between the 33 peaks of organochlorine and pyrethroid pesticides with other peaks. Concerning the resolution, all of the interesting peaks. There is a baseline resolution which is acceptable due to the fact that the resolution is greater than 1.5.

Table 4.5 Retention time and resolution of 26 organochlorine and pyrethroid pesticides under GC conditions in Table 4.1 (Split less mode)

No.	Pesticides	Retention time (min)	Resolution
1	α-BHC	11.784	-
2	β-BHC	12.799	10.23
3	γ-BHC	13.013	2.03
4	δ-BHC	14.083	10.96
5	Heptachlor	16.621	25.78
6	Aldrin	18.857	21.48
7	Dicofol	19.298	3.57
8	Hept.Epoxide	21.668	24.36
9	γ-Chlordane	23.458	15.03
10	α-Endosulfan	24.401	7.47
11	α-Chlordane	24.567	1.5
12	p,p'-DDE	26.079	12.81
13	Dieldrin	26.312	2.51
14	Endrin	27.925	14.15
15	p,p'-DDD	29.228	5.41
16	o,p'-DDT	29.42	1.71
17	Endosulfan sulfate	31.664	14.01
18	p,p'-DDT	31.864	1.83
19	Bifenthrin	35.78	6.18
20	Methoxychlor	36.159	2.35
21	Cyhalothrin	40.945	32.87
22	Permethrin I	43.402	19.73
	Permethrin II	43.774	4.01
23	Cyfluthrin I	45.154	33.45
	Cyfluthrin II	45.434	8.6
	Cyfluthrin III	45.659	1.5
	Cyfluthrin IV	45.779	1.3
24	Cypermethrin I	46.087	9.6
	Cypermethrin II	46.396	11.8
	Cypermethrin III	46.626	2.3
	Cypermethrin IV	46.745	1.4
25	Fenvalerate I	49.171	22.87
	Fenvalerate II	49.924	6.32
26	Deltamethrin	52.073	16.03

Table 4.6 Retention time and resolution of 26 organochlorine and pyrethroid pesticides under GC conditions in Table 4.2 (PTV Solvent vent mode)

No.	Pesticides	Retention time (min)	Resolution
1	α-BHC	11.824	-
2	β-BHC	12.789	10.66
3	γ-BHC	13.013	2.23
4	δ-BHC	14.086	11.16
5	Heptachlor	16.631	26.08
6	Aldrin	18.861	22.48
7	Dicofol	19.298	3.47
8	Hept.Epoxide	21.67	24.96
9	γ-Chlordane	23.458	15.13
10	α-Endosulfan	24.401	7.97
11	α-Chlordane	24.567	1.5
12	p,p'-DDE	26.081	12.81
13	Dieldrin	26.312	2.04
14	Endrin	27.925	14.17
15	p,p'-DDD	29.228	5.1
16	o,p'-DDT	29.42	1.71
17	Endosulfan	31.664	14.08
18	p,p'-DDT	31.864	1.83
19	Bifenthrin	35.78	6.18
20	Methoxychlor	36.159	2.95
21	Cyhalothrin	40.945	32.87
22	Permethrin I	43.402	19.73
	Permethrin II	43.774	4.21
23	Cyfluthrin I	45.166	32.16
	Cyfluthrin II	45.514	7.9
	Cyfluthrin III	45.697	1.5
	Cyfluthrin IV	45.821	1.2
24	Cypermethrin I	46.124	8.5
	Cypermethrin II	46.416	10.2
	Cypermethrin III	46.626	2.6
	Cypermethrin IV	46.783	1.5
25	Fenvalerate I	49.242	21.45
	Fenvalerate II	49.986	6.52
26	Deltamethrin	52.11	15.83

4.1.2 The result of Standard calibration curves

The mixed 26 standard organochlorine and pyrethroid pesticides solutions covered the concentration range of 0.005-0.50 mg/L. The data for the calibration curve were plotted by peak height versus concentration at the GC conditions in Table 4.2. The 6 points calibration curve is shown in Appendix and the result of slope value summary, intercept and correlation coefficient are shown in Table 4.7.

From Table 4.7 the data of the 6 points calibration curve can be acceptable for quantitation because the correlative coefficient (R^2) was greater than 0.995.

Table 4.7 Summary of Value of Slope, Intercept and Correlation Coefficient (R^2) of each pesticide in mixed 26 standards organochlorine and pyrethoid pesticides with the range of 0.005-0.50 mg/L by the GC condition in Table 4.2

No.	Pesticides	Slope	Intercept	R^2
1	α-BHC	1884179.82	6248.78	0.9981
2	β-BHC	656103.00	4175.60	0.9976
3	γ-BHC	1675144.26	5154.95	0.9977
4	δ-BHC	1782259.87	8237.12	0.9976
5	Heptachlor	1376891.42	6854.588	0.9975
6	Aldrin	2073674.44	17357.89	0.9972
7	Dicofol	188048.00	2835.40	0.9953
8	Hept.Epoxide	1012397.42	7816.77	0.9966
9	γ-Chlordane	1284402.77	4402.71	0.9973
10	α-Endosulfan	1348592.52	4190.01	0.9969
11	α-Chlordane	1857023.38	- 1276.58	0.9951
12	p,p'-DDE	1495631.72	1780.587	0.9990
13	Dieldrin	1596095.92	1038.34	0.9961
14	Endrin	1411515.886	-3708.65	0.9950
15	p,p'-DDD	1380485.25	-524.818	0.9950
16	o,p'-DDT	878802.00	-4135.2	0.9964
17	Endosulfan sulfate	1219465.97	-1513.552	0.9950
18	p,p'-DDT	1042272.88	-8315.28	0.9991
19	Bifenthrin	309612.00	1092.30	0.9972
20	Methoxychlor	564966.00	-2923.40	0.9984
21	Cyhalothrin	1119592.13	-1469.89	0.9976
22	Permethrin	151366.00	945.9	0.9957
23	Cyfluthrin	921111.00	-2851.5	0.9972
24	Cypermethrin	746011	-63.401	0.9972
25	Fenvalerate	722005	-340.07	0.9962
26	Deltamethrin	842614	-1133.60	0.9953

4.2 The developing of the sample preparation technique

4.2.1 The study of the pre-treatment with microwave and liquid liquid extraction (LLE) method.

Following from the first method (63), samples have to be pretreated with microwave before using. In this section, considering the background noise and interfering peaks of the chromatogram of spiked sample with and without the microwave and GC conditions in Table 4.2, as shown in Figure 4.5 there was no difference in the background noise and interfering peaks in the chromatogram. Moreover, the spiked sample with the microwave has lower interfering peaks than that without the microwave, because this method was not suitable for quantitation work.

Furthermore, from Table 4.8, almost % recoveries of some organochlorine and pyrethroid pesticide of the spiked sample with the microwave are lower than those of the spiked sample without the microwave. Using the microwave procedure is ineffective in the removal of sulfur-containing compounds in the matrix sample. The interesting peak of organochlorine and pyrethroid pesticides directly affected disappearance. Thus, for spiking level of 0.01 mg/kg, it can be detected in some peaks but we can’t quantify all the compounds.

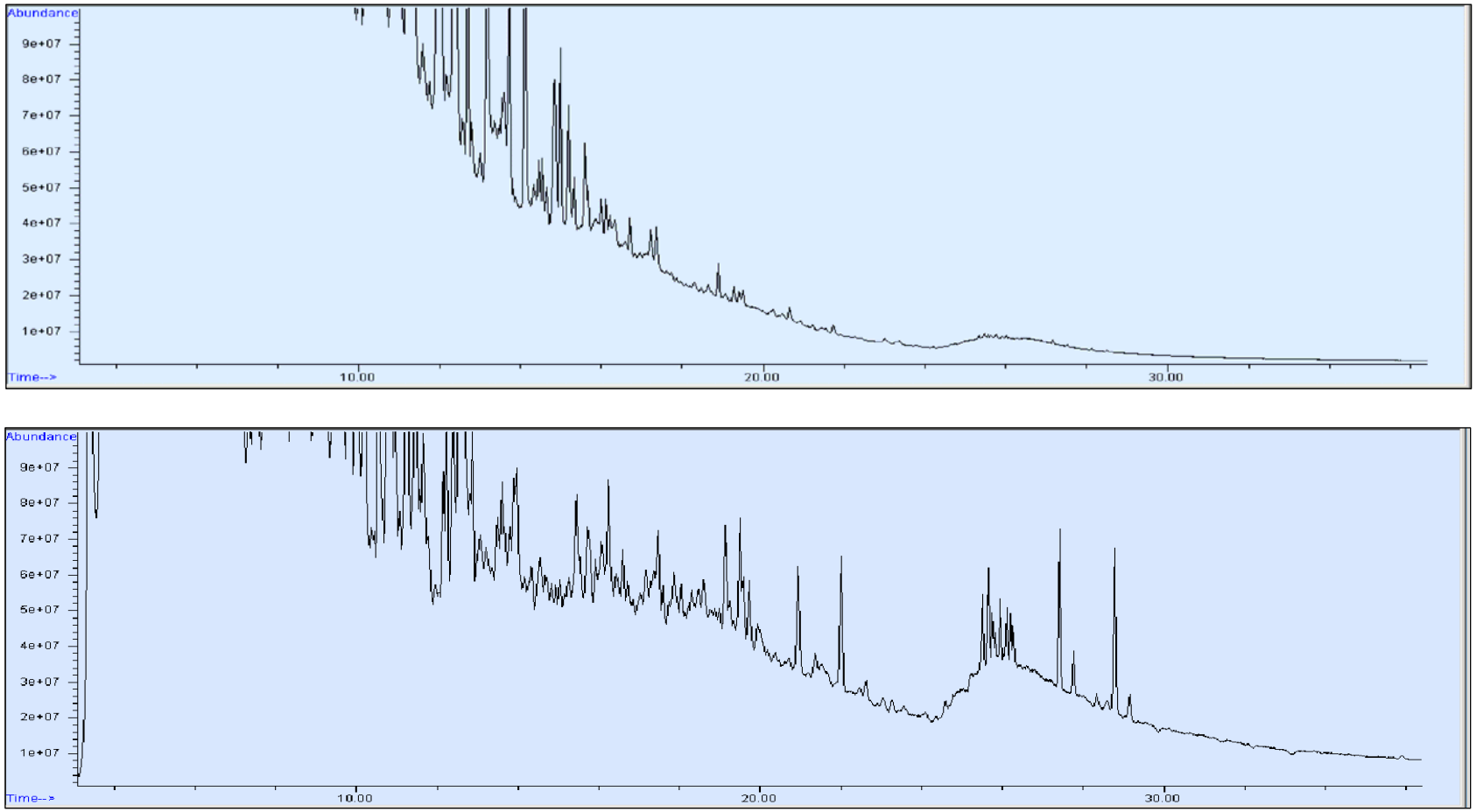

Figure 4.5 The chromatogram of spiked sample with- without microwave at spiking level of 0.01 mg/kg under GC condition in Table 4.2

Table 4.8 % Recovery of spiked sample with and without microwave at spiking level of0.01 mg/kg

No.	Pesticide	% Recovery of spiked sample	
		without microwave	with microwave
1	α-BHC	0	0
2	β-BHC	0	0
3	γ-BHC	0	0
4	δ-BHC	0	0
5	Heptachlor	0	0
6	Aldrin	0	0
7	Dicofol	0	0
8	Hept.Epoxide	0	0
9	γ-Chlordane	0	0
10	α-Endosulfan	0	0
11	α-Chlordane	0	0
12	p,p'-DDE	0	0
13	Dieldrin	0	0
14	Endrin	0	0
15	p,p'-DDD	30	0
16	o,p'-DDT	41	0
17	Endosulfan sulfate	40	0
18	p,p'-DDT	0	0
19	Bifenthrin	32	0
20	Methoxychlor	0	0
21	Cyhalothrin	32	0
22	Permethrin	50	0
23	Cyfluthrin	75	0
24	Cypermethrin	65	0
25	Fenvalerate	70	0
26	Deltamethrin	88	0

4.2.2 The study of the pre-treatment with water bath and liquid liquid extraction (LLE) method.

From the previous result in section 4.2.1, it couldn't be achieved using this method. In this section, it was further developed so that it could be most suitable to use with Garlic samples and could effectively eliminate matrices. The process was changed from microwave to water bath and GC conditions in Table 4.2. It was believed that when we used water bath sample that contained organochlorine and pyrethroid will be softer volatilized than that microwaved. The chromatogra as shown in Figure 4.6. There was different background noise and interfering peaks. Moreover, the spiked sample with the water bath has lower interfering peaks and background noise than that without the water bath because this method was not suitable for both qualitative and quantitation work.

Furthermore, from Table 4.9, almost % recoveries all of organochlorine and pyrethroid pesticide of the spiked sample with the water bath are lower than those of the spiked sample without the microwave. Using the water bath could eliminate the volatile substance interference in matrix sample and directly affect all organochlorine and pyrethroid pesticides. The % recovery of spiked sample level of 0.01 mg/kg were lose .Therefore, it couldn't be detected and quantitation of all compounds.

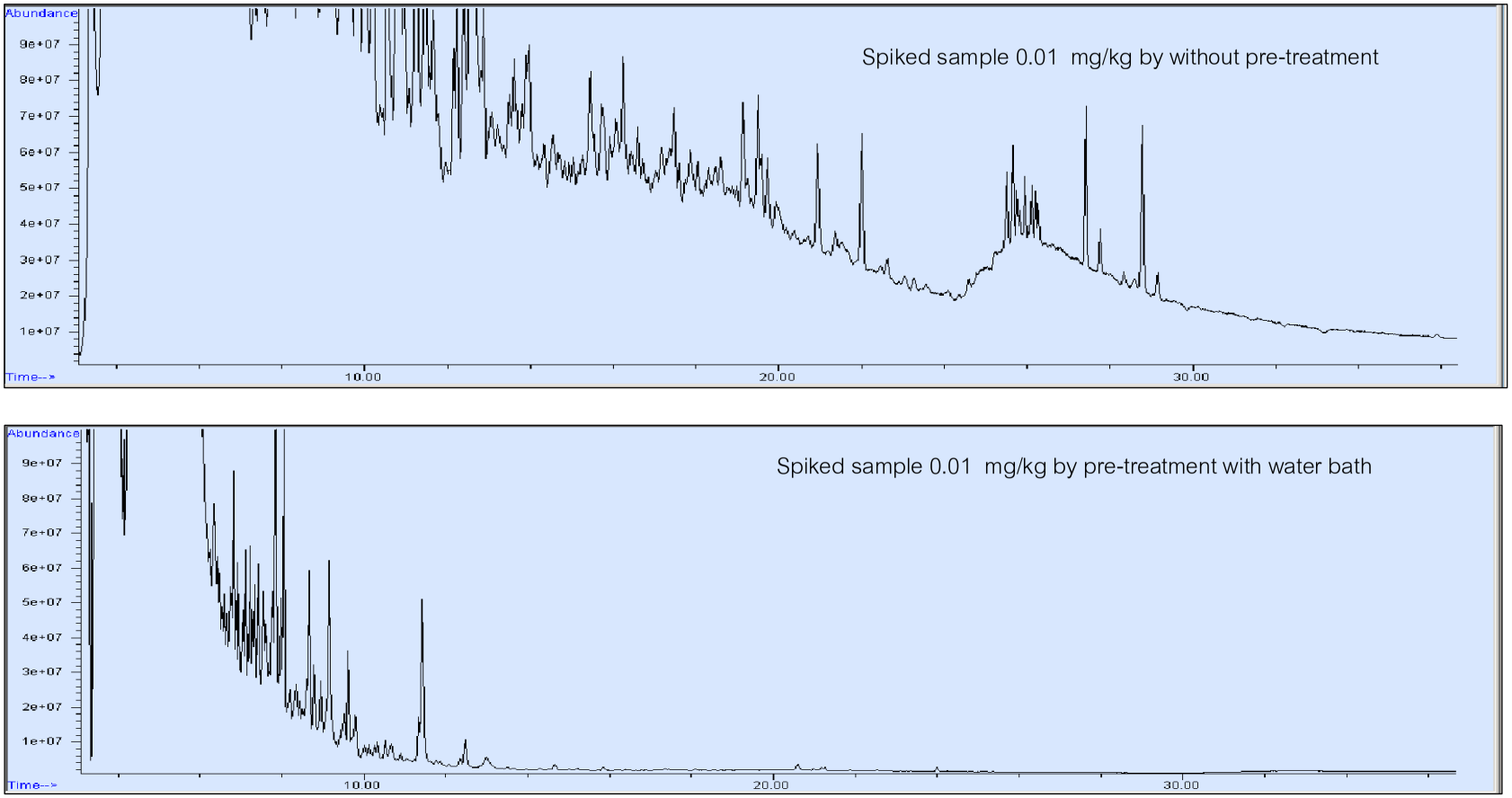

Figure 4.6 The chromatogram of spiked sample with-without water bath at spiking level of 0.01 mg/kg under GC condition in Table 4.2

Table 4.9 % Recovery of spiked sample with and without water bath at spiking level of 0.01 mg/kg

No.	Pesticide	% Recovery of spiked sample	
		without water bath	with water bath
1	α-BHC	0	0
2	β-BHC	0	0
3	γ-BHC	0	0
4	δ-BHC	0	0
5	Heptachlor	0	0
6	Aldrin	0	0
7	Dicofol	0	0
8	Hept.Epoxide	0	0
9	γ-Chlordane	0	0
10	α-Endosulfan	0	0
11	α-Chlordane	0	0
12	p,p'-DDE	0	0
13	Dieldrin	0	0
14	Endrin	0	0
15	p,p'-DDD	30	0
16	o,p'-DDT	41	0
17	Endosulfan sulfate	40	0
18	p,p'-DDT	0	0
19	Bifenthrin	32	0
20	Methoxychlor	0	0
21	Cyhalothrin	32	0
22	Permethrin	50	0
23	Cyfluthrin	75	0
24	Cypermethrin	65	0
25	Fenvalerate	70	0
26	Deltamethrin	88	0

4.2.3 The result of of QuEChERS technique.

From the previous result in section 4.2.1 and 4.2.2, this method was not suitable for the garlic sample. In this section, the QuEChERS technique and clean up with various dispersive SPE sorbents were considered. Comparing the background noise and interfering peaks with QuEChERS technique and the pre-treatment without microwave and LLE method, there were different background noise and interfering peaks between the chromatogram as shown in Figure 4.7. The spiked sample with the QuEChERS technique has lower interfering peaks and background noise than the pre-treatment with microwave and LLE method without the microwave. Therefore the various dispersive SPE sorbent in QuEChERS technique can eliminated interference in matrix sample because in figure 4.8, there were more interfering peak and those are affect to be deplete of instrument and these method was not suitable for both qualitative and quantitation work.

Moreover, the % recovery of QuEChERS methods with various dispersive SPE sorbents that were lower than the pre-treatment with microwave and LLE method caused the interested substance to be lost, the result were shown in Table 4.10

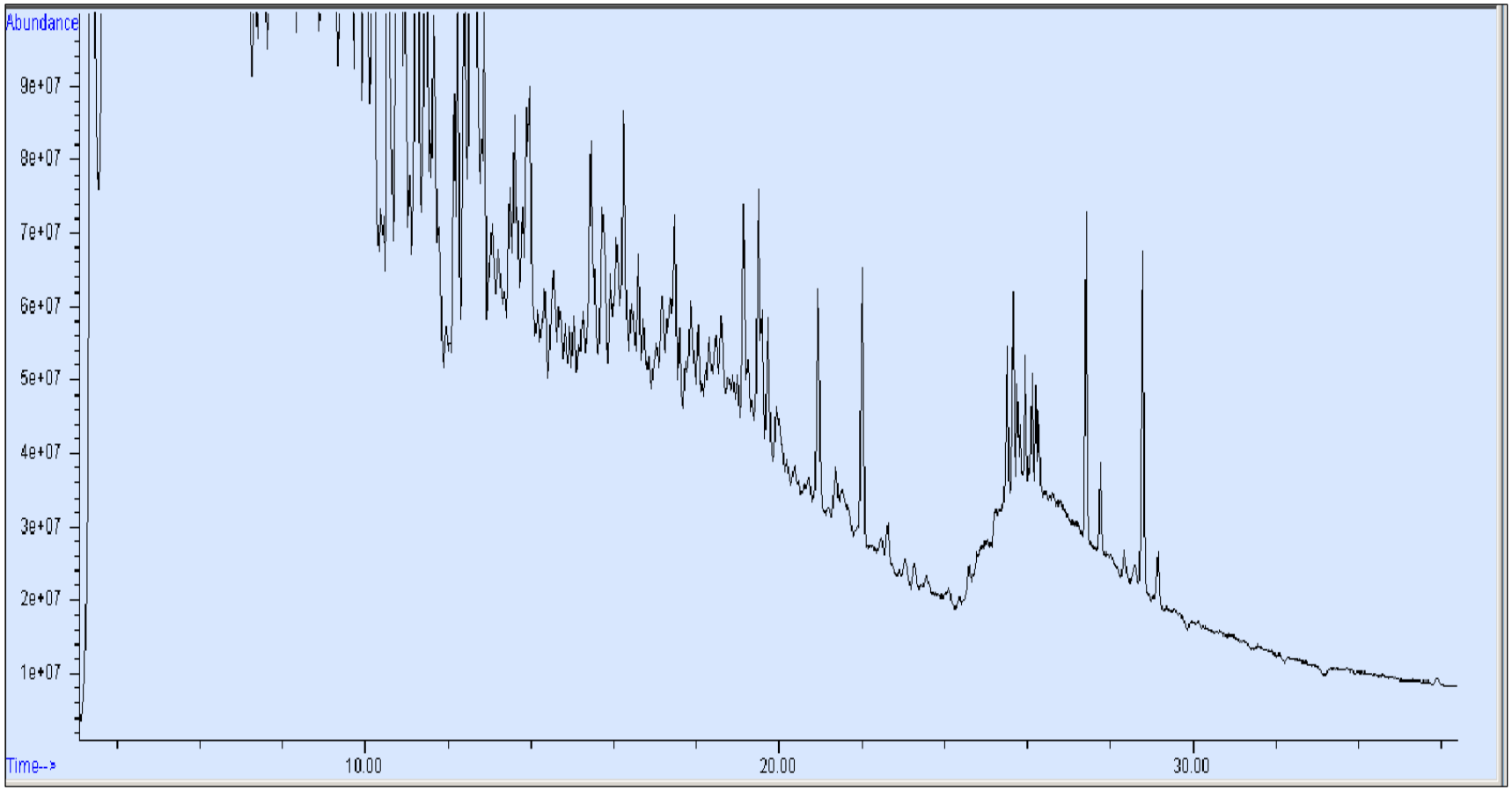

Figure 4.7 The chromatogram of spiked sample without pre-treatment at spiking level of 0.01 mg/kg under GC condition in Table 4.2

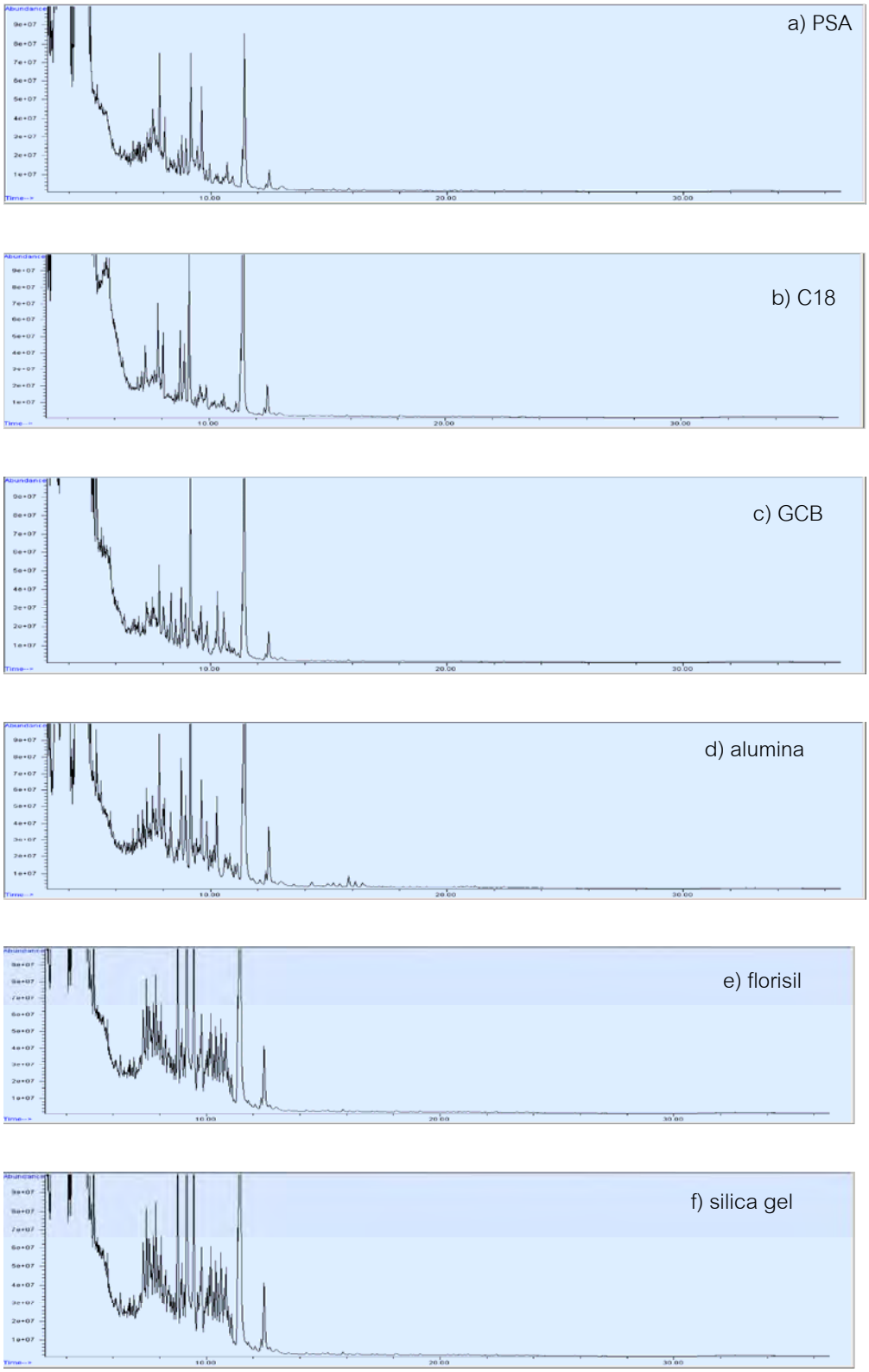

Figure 4.8 The chromatogram of spiked sample with QuEChERS method and clean up with various dispersive SPE at spiking level of 0.01 mg/kg under GC condition in Table 4.2

Table 4.10 % Recovery of spiked sample with QuEChERS method and without microwave at spiking level of 0.01 mg/kg

Pesticide	% Recovery of spiked sample						
	LLE	florisil	C18	Alumina	Silica gel	PSA	GCB
α-BHC	0	0	0	0	0	0	0
β-BHC	0	0	0	0	0	0	0
γ-BHC	0	0	0	0	0	0	0
δ-BHC	0	0	0	0	0	0	0
Heptachlor	0	0	0	0	0	0	0
Aldrin	0	0	0	0	0	0	0
Dicofol	0	0	0	0	0	0	0
Hept.Epoxide	0	0	0	0	0	0	0
γ-Chlordane	0	0	0	0	0	0	0
α-Endosulfan	0	0	0	0	0	0	0
α-Chlordane	0	0	0	0	0	0	0
p,p'-DDE	0	0	0	0	0	0	0
Dieldrin	0	0	0	0	0	0	0
Endrin	0	0	0	0	0	0	0
p,p'-DDD	30	0	0	0	0	0	0
o,p'-DDT	41	0	0	0	0	0	0
Endosulfan sulfate	40	0	0	0	0	0	0
p,p'-DDT	0	0	0	0	0	0	0
Bifenthrin	32	0	0	0	0	0	0
Methoxychlor	0	0	0	0	0	0	0
Cyhalothrin	32	0	0	0	0	0	0
Permethrin	50	0	0	0	0	0	0
Cyfluthrin	75	0	0	0	0	0	0
Cypermethrin	65	0	0	0	0	0	0
Fenvalerate	70	0	0	0	0	0	0
Deltamethrin	88	0	0	0	0	0	0

4.2.4 The study of MSPD technique.

From the previous result in section 4.2.1 - 4.2.3, it showed “can’t achieve” for the method. In this section, the use of MSPD techniques couldn’t be achieved as per GC conditions in Table 4.2. The chromatogram of spiked sample with MSPD techniques and LLE without the microwave method and GC conditions in Table 4.2, as shown in Figure 4.9, there was difference background noise and interfering peaks between the chromatogram as shown in Figure 4.10. Moreover, the spiked sample with the MSPD techniques has lower interfering peaks and cleared up than that LLE without the microwave method.

Furthermore, from Table 4.11, almost % recoveries of Organochlorine and Pyrethroid pesticide of the spiked sample with the MSPD method are larger than those of the spiked sample LLE without the microwave. Using the MSPD procedure affected the removal of sulfur-containing compounds in the matrix sample. Thus, for spiking level of 10 ng/g , it can be detected and quantitation all the compounds.

All the data suggest that standard LLE procedure and QuEChERS method were ineffective in the removal of sulfur-containing compounds from the matrix sample. MSPD procedure is much more suitable and very effective in the removal of sulfur containing compounds in garlic. Thus, we would use this method as a basis for sample preparation and quantitative analysis of organochlorine and pyethroid pesticides in garlic sample.

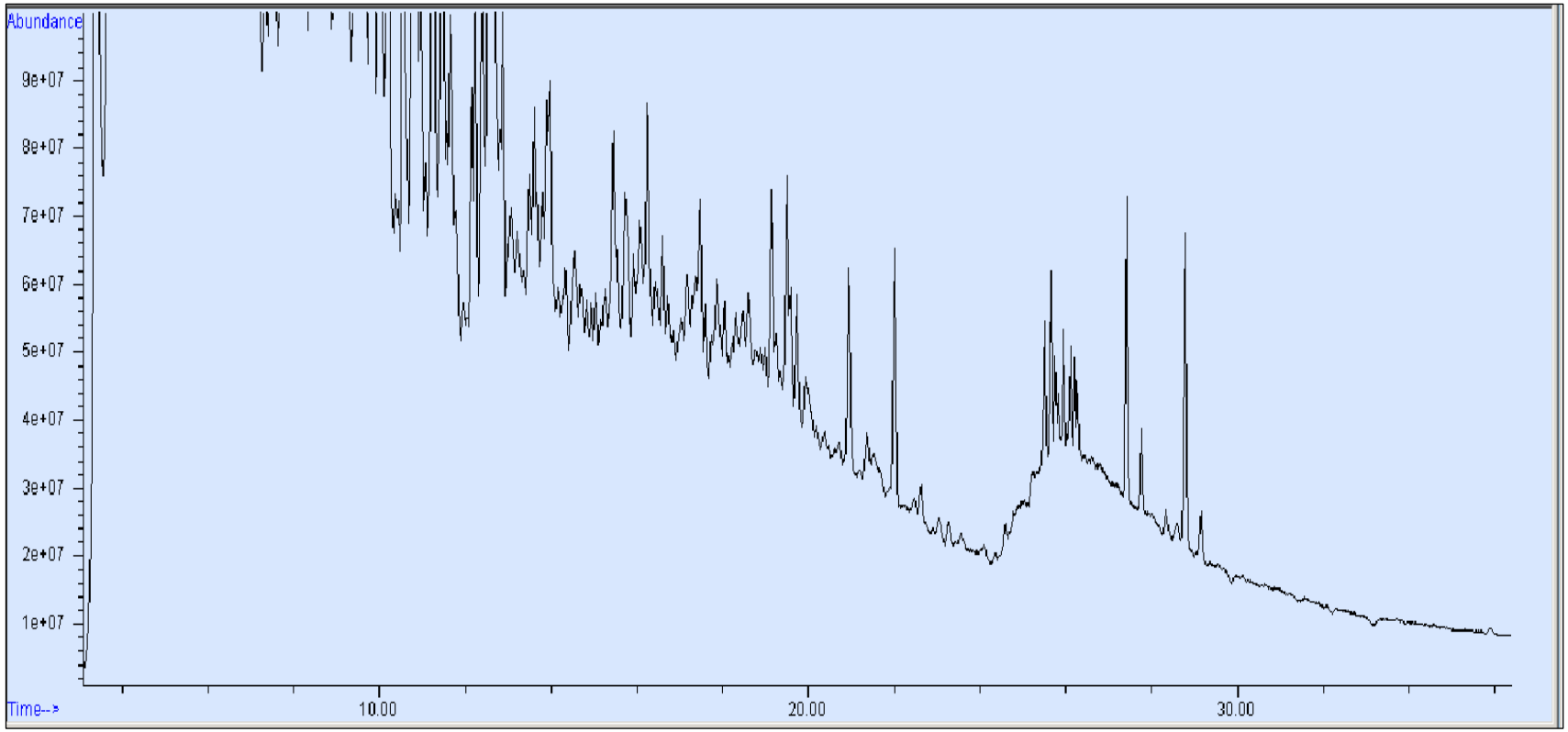

Figure 4.9 The chromatogram of spiked sample with LLE method at spiking level of 0.01 mg/kg under GC condition in Table 4.2

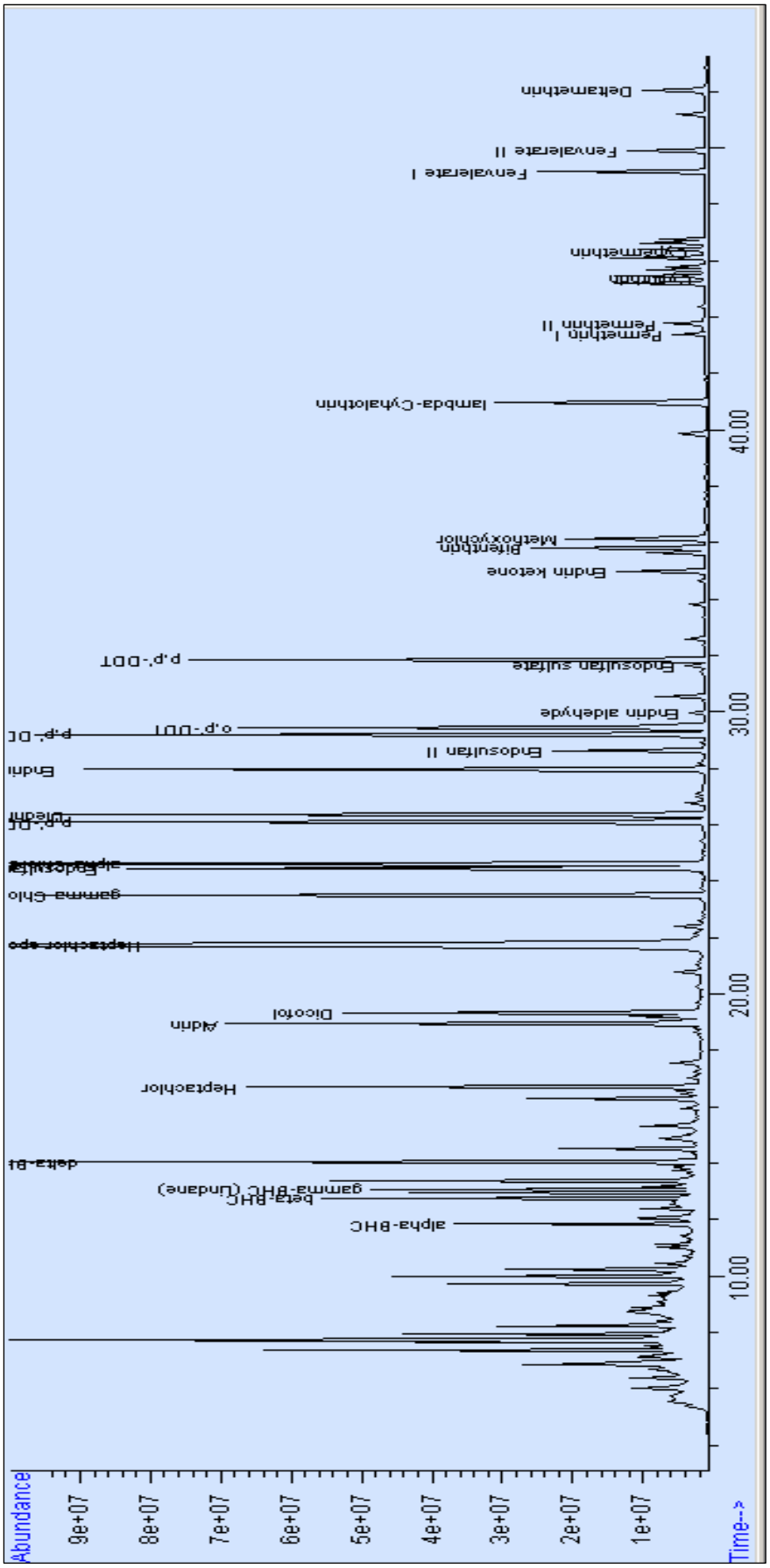

Figure 4.10 The chromatogram of spiked sample with MSPD method at spiking level of 0.01 mg/kg under GC condition in Table

Table 4.11 % Recovery of spiked sample with MSPD method and without microwave at spiking level of 0.01 mg/kg

No.	Pesticide	% Recovery of spiked sample	
		without microwave	MSPD technique
1	α-BHC	0	102
2	β-BHC	0	122
3	γ-BHC	0	72
4	δ-BHC	0	46
5	Heptachlor	0	82
6	Aldrin	0	75
7	Dicofol	0	47
8	Hept.Epoxide	0	55
9	γ-Chlordane	0	61
10	α-Endosulfan	0	55
11	α-Chlordane	0	55
12	p,p'-DDE	0	60
13	Dieldrin	0	57
14	Endrin	0	47
15	p,p'-DDD	30	79
16	o,p'-DDT	41	70
17	Endosulfan sulfate	40	81
18	p,p'-DDT	0	83
19	Bifenthrin	32	75
20	Methoxychlor	0	76
21	Cyhalothrin	32	71
22	Permethrin	50	79
23	Cyfluthrin	75	70
24	Cypermethrin	65	56
25	Fenvalerate	70	66
26	Deltamethrin	88	58

4.3 The result of MSPD optimization

From the result of the previous section, MSPD method offered a better condition for extraction. From the result in Table. 4.11 , % recovery of organochlorine and pyrethroid pesticide as δ-BHC, Dicofol, Hept.Epoxide, α-Endosulfan, α-Chlordane, Dieldrin, Endrin, Cypermethrin and Deltamethrin were lower than 60 % .Therefore, in this section , it was further developed so under experimental conditions, a critical step for extraction of pesticide residues by MSPD was the characterized of solid support and elution solvent. Parameters affecting MSPD procedure such as solid support type, type and volume of elution solvent were investigated**.** The results are displayed as background noise and mean percentage recoveries matrix cleanup.

4.3.1 The result of type of solid support optimization.

In this section, it was further developed. The type of solid support was the first factor considered since, it could influence the method extraction efficiency. The polar and non-polar solid support as florisil, silica gel and C18 were considered as a sorbent type**.** Comparing the interference peaks and background noises in chromatogram and the mean percentage recovery with GC condition in Table 4.2 the results were shown in Table 4.12 and it was found that all that Florisil siliga gel and C18 are effective in the removal of polar sulfur-containing compounds. The interferences in the matrix sample cleared up better than that LLE without the microwave method.

Furthermore, from Table 4.12, almost % recoveries are similar by using Florisil or silica gel than that of octadecylsilyl (C18). Several pesticides such as α-BHC, p,p'-DDE, p,p'-DDD , o,p'-DDT , p,p'-DDT, Methoxychlor , Cyhalothrin, Permethrin, Cyfluthrin and Cypermethrin are better recoveries by florisil than that silica gel. The recoveries pesticides of δ-BHC, α- Endosulfan, α-Chlordane, Endrin, and Deltamethrin were better by silica gel than florisil) because the interferences observed in the chromatograms originated from sulfur compounds. These compounds are reported to be mostly phenolic and steroidal *(1)* which are polar molecules. Therefore florisil and silica gel are polar sorbent and can be effectively used to extract

these OCPYs, which can decrease interferences in the sample extract. The octadecylsilyl (ODS)-derivatized silica (C18) is non polar sorbent because the % recovery was low.

Table 4.12 % Recovery of spiked sample of MSPD method with various of types of solid support at spiking level of 0.01 mg/kg

No.	Pesticide	% Recovery of spiked sample		
		florisil	Silica gel	C18
1	α-BHC	102	31	10
2	β-BHC	122	79	5
3	γ-BHC	72	70	4
4	δ-BHC	46	64	3
5	Heptachlor	82	98	1
6	Aldrin	75	62	0
7	Dicofol	47	48	0
8	Hept.Epoxide	55	68	0
9	γ-Chlordane	61	68	0
10	α-Endosulfan	55	67	0
11	α-Chlordane	55	63	0
12	p,p'-DDE	60	41	0
13	Dieldrin	57	59	0
14	Endrin	47	61	0
15	p,p'-DDD	79	40	0
16	o,p'-DDT	70	45	0
17	Endosulfan sulfate	81	65	0
18	p,p'-DDT	83	39	0
19	Bifenthrin	75	75	0
20	Methoxychlor	76	32	0
21	Cyhalothrin	71	31	0
22	Permethrin	79	50	0
23	Cyfluthrin	70	50	0
24	Cypermethrin	56	30	0
25	Fenvalerate	66	71	0
26	Deltamethrin	58	80	0

4.3.2 The result of solid support composition optimization

From the result of the previous section (Table 4.12), the wide range of analytes combined with the complex matrix sample led to a difficult extraction with a single sorbent. In this section, to improved the extraction of analytes in solid support system. About mixed solid support of florisil and silica gel of the ratio 1:1, 1:4 and 4:1 was investigated .From result in Table 4.13 and the mixed solid support of florisil and silica gel of the ratio 1:1 can be to increased the extraction efficiency. Therefore a 1:1 (w/w) mixture of Florisil : siliga gel provided optimum cleanup and was selected as the sorbent in this work.

Table 4.13 % Recovery of spiked sample of MSPD method with various of solid support compositions at spiking level of 0.01 mg/kg

No.	Pesticide	% Recovery of spiked sample		
		Ratio of Florisil : Silica gel		
		1:1	4:1	1:4
1	α-BHC	106	46	22
2	β-BHC	110	109	77
3	γ-BHC	75	90	77
4	δ-BHC	68	55	50
5	Heptachlor	90	110	108
6	Aldrin	68	102	60
7	Dicofol	60	51	55
8	Hept.Epoxide	73	65	128
9	γ-Chlordane	69	80	77
10	α-Endosulfan	80	133	110
11	α-Chlordane	74	126	110
12	p,p'-DDE	69	96	82
13	Dieldrin	66	57	42
14	Endrin	70	45	59
15	p,p'-DDD	71	137	121
16	o,p'-DDT	75	68	56
17	Endosulfan sulfate	96	59	87
18	p,p'-DDT	85	59	27
19	Bifenthrin	75	105	93
20	Methoxychlor	76	52	48
21	Cyhalothrin	69	40	75
22	Permethrin	76	119	95
23	Cyfluthrin	63	102	96
24	Cypermethrin	66	38	53
25	Fenvalerate	72	78	84
26	Deltamethrin	68	80	45

4.3.3 The result of type of elution solvent optimization

In this section, once the sorbent was identified, the method was further optimized by fine-tuning the elution strength of the solvent. It was further developed that the type of elution solvent was the continuous factor considered since it could influence the method extraction efficiency. The non-polar solvent as n-hexane, and dichloromethane were considered as solvent elution types**.** Comparing the interference peaks and background noises in chromatogram and the mean percentage recovery with GC condition in Table 4.2. the results were shown in Table 4.14 and Figure 4.11-4.13. In contrast, percent recoveries obtained when employed dichloromethane doubled the values of hexane (figure 4.12). However, the chromatogram of fortified sample eluted with DCM shows stronger interference and heavier background (figure 4.13) than the same sample that was eluted with hexane (figure 4.22). The dipole moment of DCM is larger than the value of hexane making DCM more compatible to polar compounds. Therefore, polar sulfur-containing derivatives in the matrix were eluted when employed DCM and resulted in dirty chromatogram. For this reason, hexane was chosen for this work.

Table 4.14 % Recovery of spiked sample with MSPD method and various of types of Elution solvent at spiking level of 0.01 mg/kg

No.	Pesticide	% Recovery of spiked sample	
		n-Hexane	Dichloromethane
1	α-BHC	94	904
2	β-BHC	108	742
3	γ-BHC	108	745
4	δ-BHC	117	351
5	Heptachlor	113	622
6	Aldrin	113	259
7	Dicofol	145	139
8	Hept.Epoxide	126	351
9	γ-Chlordane	131	318
10	α-Endosulfan	117	277
11	α-Chlordane	125	289
12	p,p'-DDE	103	185
13	Dieldrin	104	216
14	Endrin	107	271
15	p,p'-DDD	129	241
16	o,p'-DDT	96	163
17	Endosulfan sulfate	71	141
18	p,p'-DDT	110	147
19	Bifenthrin	99	134
20	Methoxychlor	82	148
21	Cyhalothrin	62	143
22	Permethrin	77	89
23	Cyfluthrin	94	120
24	Cypermethrin	54	175
25	Fenvalerate	86	180
26	Deltamethrin	72	142

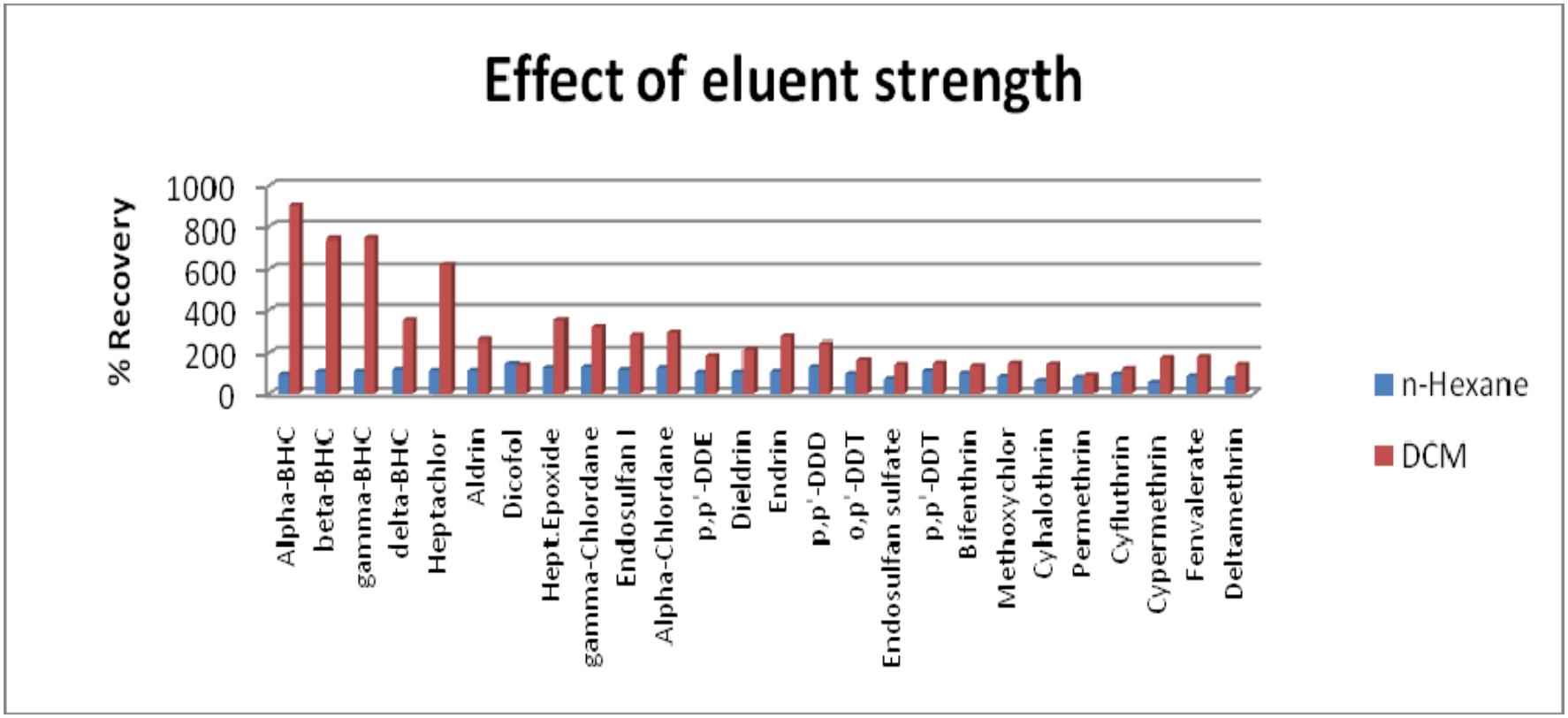

Figure 4.11 Percent recovery as a function of elution solvent

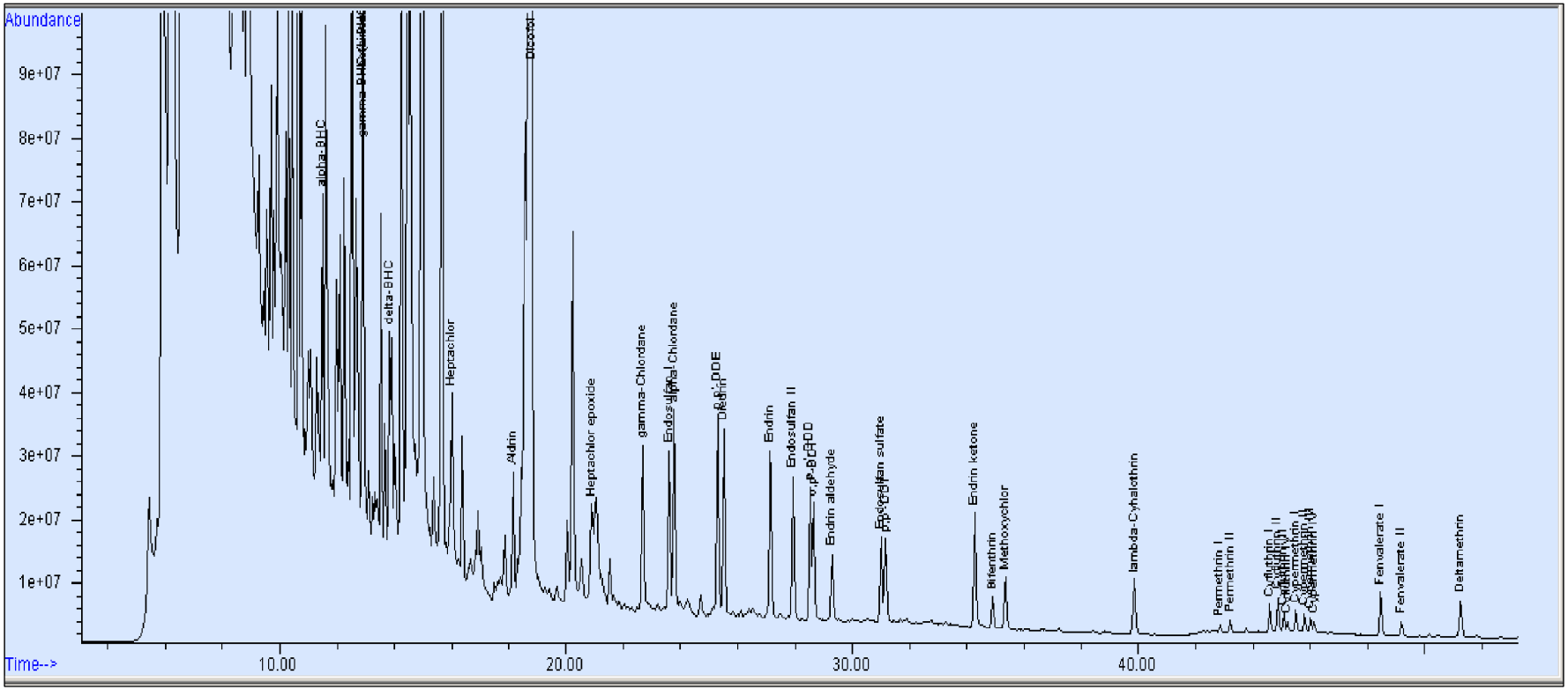

Figure 4.12 The chromatogram of spiked sample with Dichloromethane as the sorbent of MSPD method at spiking level of 0.01 mg/kg under GC condition in Table 4.2

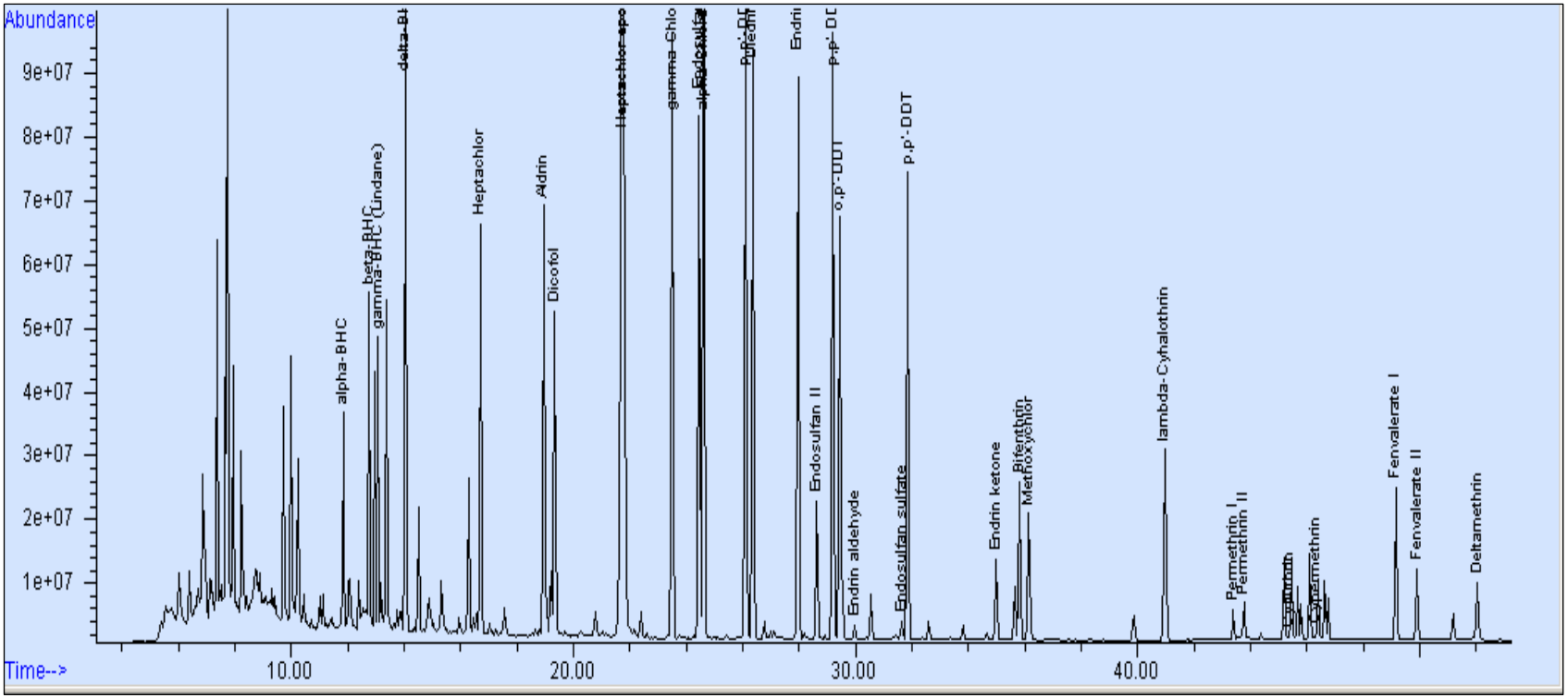

Figure 4.13 The chromatogram of spiked sample with Hexane as the sorbent of MSPD method at spiking level of 0.01 mg/kg under GC condition in Table 4.2

4.3.4 The elution volume optimization.

This section was optimized the elution volume. Because each pesticide elutes differently, it is crucial to identify the minimum solvent volume for the elution of every analyte. From the results in Table 4.15, using solvent below this volume will result in reduced recovery while using too large a volume is a waste of chemical and analysis time. Figure 4.14 compares percent recovery at 15, 30, and 45 mL of hexane. Low recoveries were obtained when only 15 mL of hexane was employed. The best recoveries were observed at 30 mL. At 45 mL, slightly better recoveries were observed along with some fault positives. However, in this work, the 30 ml was chosen to extract OCPYs in garlic because this volume was minimal for sufficiency efficiency.

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Table 4.15 % Recovery of spiked sample of MSPD method with various solid support composition at spiking level of 0.01 mg/kg

No.	Pesticide	% Recovery of spiked sample		
		Volume of Elution solvent		
		15 mL	30mL	45mL
1	α-BHC	12	63	93
2	β-BHC	19	61	81
3	γ-BHC	27	61	93
4	δ-BHC	45	69	78
5	Heptachlor	64	72	105
6	Aldrin	17	66	137
7	Dicofol	53	69	158
8	Hept.Epoxide	71	105	151
9	γ-Chlordane	50	121	146
10	α-Endosulfan	75	112	78
11	α-Chlordane	63	121	98
12	p,p'-DDE	26	91	130
13	Dieldrin	31	112	138
14	Endrin	57	116	95
15	p,p'-DDD	68	91	96
16	o,p'-DDT	64	91	95
17	Endosulfan sulfate	66	81	91
18	p,p'-DDT	10	86	84
19	Bifenthrin	0	90	93
20	Methoxychlor	0	65	77
21	Cyhalothrin	0	77	92
22	Permethrin	0	95	85
23	Cyfluthrin	0	96	84
24	Cypermethrin	0	78	71
25	Fenvalerate	0	84	69
26	Deltamethrin	0	72	65

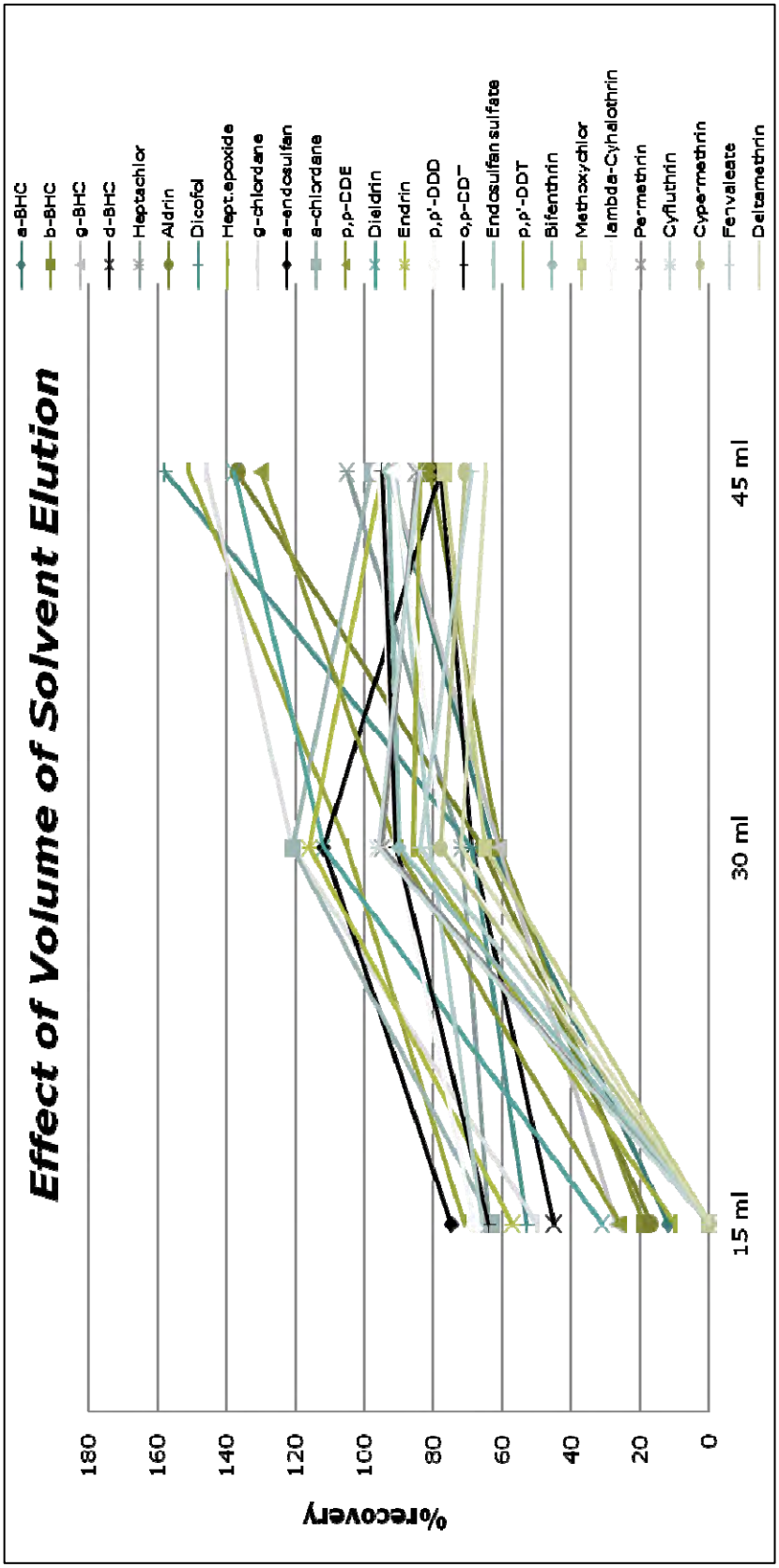

Figure 4.14 Percent recovery of OCPYs at various elution volumes 4.2

4.3.5 The ratio of sample to solid support material optimization.

This section was the last parameter to optimize ratio of sample to solid support material. About grams of sample and solid support of the ratio 0.5:1, 1:1 and 1:2 were investigated. From result in Table 4.16 and Figure 4.15-4.17, the grams of sample and solid support of the ratio 0.5:1 and 1:2 was decreased interference and background than that ratio 1:1. However, the grams of sample and solid support of the ratio 0.5:1 and 1:2 can be decreased extraction efficiency than ratio 1:1, because value of elution solvent not enough for elute analyte in matrix sample. Therefore a 1:1 (w/w) grams of sample and solid support provided optimum cleanup and was selected as the the grams of sample and solid support of the ratio 1:2 was decreased interference and background than that ratio 1:1 in this work.

Table 4.16 % Recovery of spiked sample of MSPD method with various ratio of sample to solid support material

Pesticide	% Recovery of spiked sample ratio of sample to solid support material		
	0.5:1	**1:1**	**1:2**
α-BHC	15	106	0
β-BHC	75	110	3
γ-BHC	13	75	4
δ-BHC	17	68	2
Heptachlor	20	90	5
Aldrin	24	68	0
Dicofol	18	60	2
Hept.Epoxide	15	73	2
γ-Chlordane	13	69	4
α-Endosulfan	15	80	3
α-Chlordane	16	74	5
p,p'-DDE	18	19	5
Dieldrin	14	66	0
Endrin	16	70	5
p,p'-DDD	12	71	4
o,p'-DDT	13	75	3
Endosulfan sulfate	15	96	8
p,p'-DDT	20	85	4
Bifenthrin	8	75	5
Methoxychlor	11	76	5
Cyhalothrin	20	69	4
Permethrin	16	76	3
Cyfluthrin	19	63	2
Cypermethrin	5	66	2
Fenvalerate	8	72	3
Deltamethrin	15	68	0

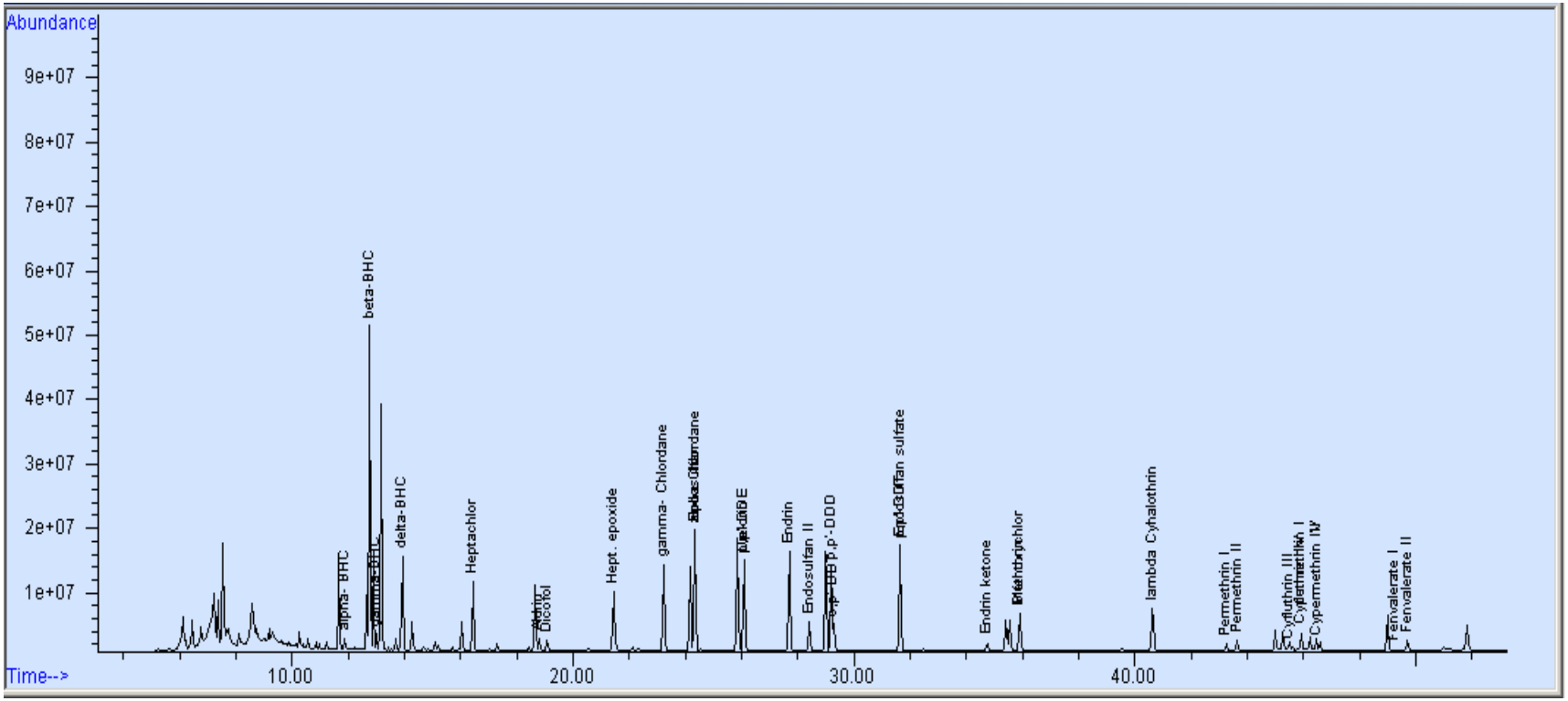

Figure 4.15 The chromatogram of spiked sample with ratio of sample to solid support material were :0.5:1 at spiking level of 0.01 mg/kg under GC condition in Table 4.2

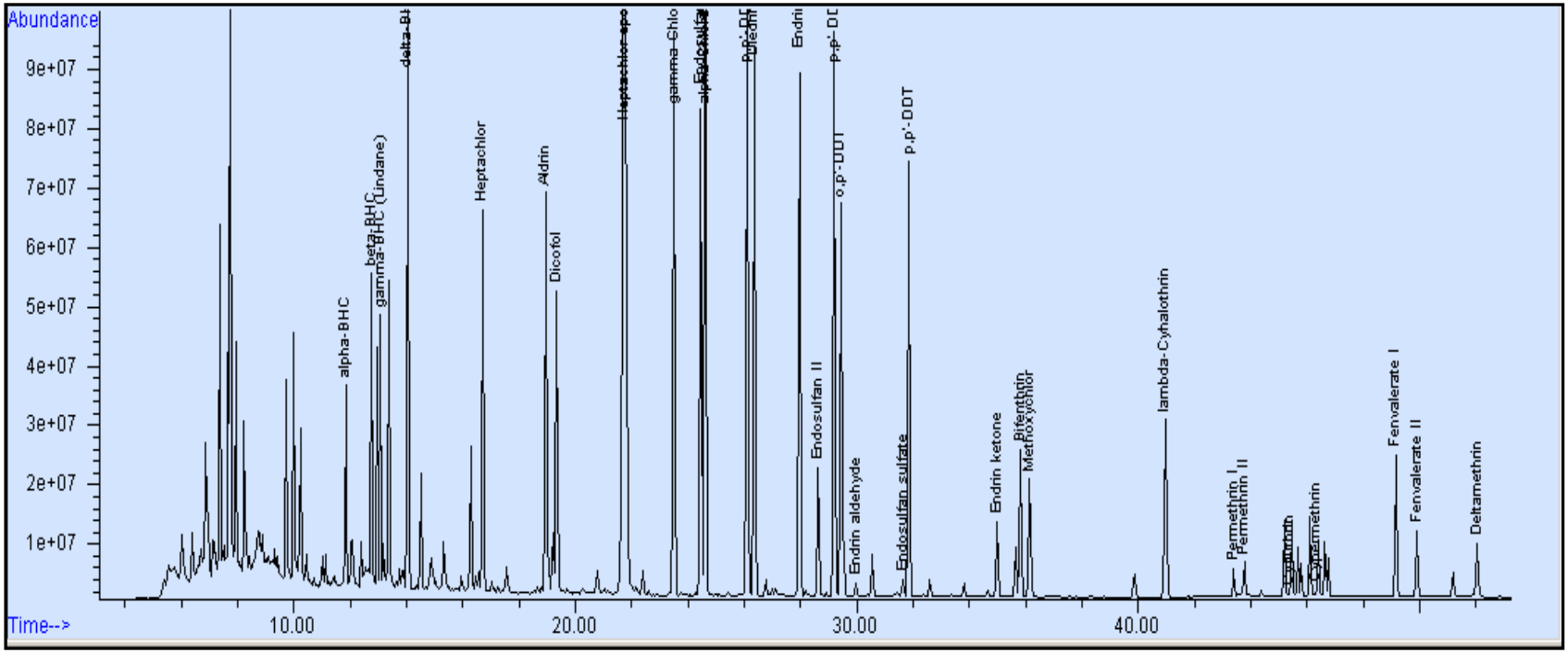

Figure 4.16 The chromatogram of spiked sample with ratio of sample to solid support material were :1:1 at spiking level of 0.01 mg/kg under GC condition in Table 4.2 ng/g

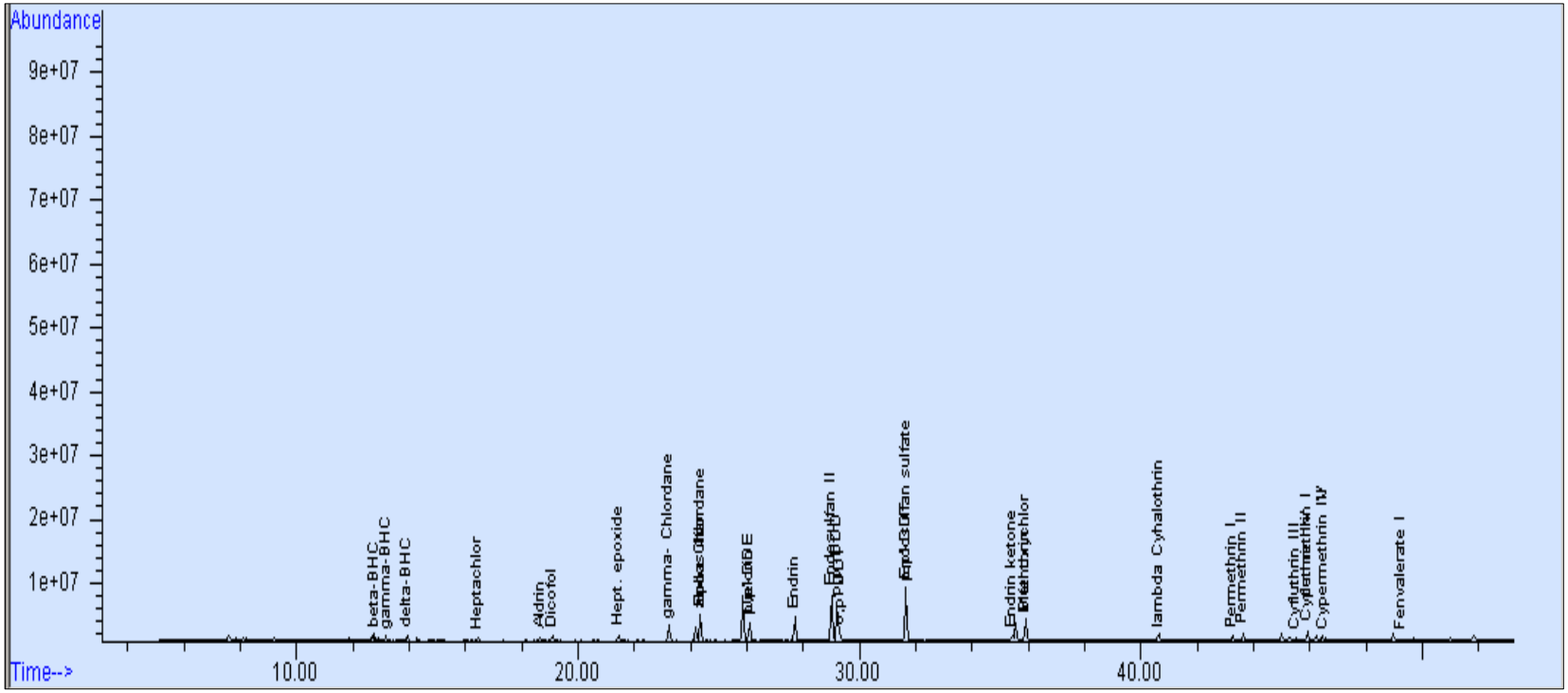

Figure 4.17 The chromatogram of spiked sample with ratio of sample to solid support material were :1:2 at spiking level of 0.01 mg/kg under GC condition in Table 4.2 ng/g

Various parameters affecting the efficiency of extracting 26 OCPY pesticides by MSPD were optimized and a summary of the optimal condition is given in Table 4.17.

Table 4.17 The optimum conditions of MSPD

MSPD parameter	Condition
type of solid support	florisil : silica gel
solid support	Ratio 1:1
type of elution solvent	n-Hexane
volume of elution solvent	30 mL
ratio of sample to solid support	1:1

4.4 Method validation

The MSPD method was validated to prove the effectiveness in the application. The method validation was done with optimized condition of MSPD in the Chinese garlic sample.

4.4.1 The result of Limit of detections (LODs)and limit of quantifications (LOQs)

The method limits of detection were calculated from chromatographic signal (peak height) at three times higher than the background noise (S/N=3). The lowest spiked concentration of each standard (0.005μg/L) under optimized MSPD condition was employed to calculate LOD and the study was done in seven replicates. In the same way, the method limits of quantification were also calculated from chromatographic signal (peak height) but estimated at ten times higher than background noise (S/N=10). Seven replicates of LOQ were studied. The method LOD

and LOQ are expressed in Table 4.18. Obviously, LOQ of the developed method complies with MRLs from EU regulations.

4.4.2 The result of Linearity range

The linearity of the method was derived from standard calibration curves of 26 OCPY pesticides with the concentration ranges of 0.005-0.50 mg/L and then Coefficient of determination (R^2)represent the method linearity. The slope, y-intercept, and correlation coefficient (R^2) are listed in Table 4.18. Coefficient of determination (R^2) ranged from 0.9951 to 0.9991. This method provided good linearity of 26 OCPY pesticides in garlic with MSPD.

Table 4.18 The LOD and LOQ of 26 OCPYs pesticides in garlic and MRLs from EU regulation (mg/kg)

Pesticide	**LOD (mg/kg)**	**LOQ (mg/kg)**	**MRLs from EU regulations (mg/kg)**
α-BHC	0.003	0.01	
β-BHC	0.003	0.01	0.01
δ-BHC	0.003	0.01	
γ-BHC	0.003	0.01	0.01
Heptachlor	0.003	0.01	0.01
Hept.Epoxide	0.003	0.01	
Dicofol	0.003	0.01	0.02
Endrin	0.003	0.01	0.01
α-Endosulfan	0.003	0.01	0.05
Endosulfan sulfate	0.003	0.01	
α-Chlordane	0.003	0.01	0.01
γ-Chlordane	0.003	0.01	
Aldrin	0.003	0.01	0.01
Dieldrin	0.003	0.01	
p,p'-DDE	0.003	0.01	
p,p'-DDD	0.003	0.01	0.05
o,p'-DDT	0.003	0.01	
p,p'-DDT	0.003	0.01	
Bifenthrin	0.003	0.01	0.05
Methoxychlor	0.003	0.01	0.01
Lambda-Cyhalothrin	0.003	0.01	0.20
Permethrin	0.004	0.01	0.05
Cyfluthrin	0.003	0.01	0.02
Cypermethrin	0.003	0.01	0.10
Fenvalerate	0.003	0.01	0.02
Deltamethrin	0.004	0.01	0.10

Table 4.19 Coefficient of determination from standard calibration curve of 26 OCPYs pesticides in MSPD

No.	Pesticide	Coefficient of determination (R^2)
1	α-BHC	0.9981
2	β-BHC	0.9976
3	γ-BHC	0.9977
4	δ-BHC	0.9976
5	Heptachlor	0.9975
6	Aldrin	0.9972
7	Dicofol	0.9953
8	Hept.Epoxide	0.9966
9	γ-Chlordane	0.9973
10	α-Endosulfan	0.9969
11	α-Chlordane	0.9951
12	p,p'-DDE	0.9990
13	Dieldrin	0.9961
14	Endrin	0.9950
15	p,p'-DDD	0.9950
16	o,p'-DDT	0.9964
17	Endosulfan sulfate	0.9950
18	p,p'-DDT	0.9991
19	Bifenthrin	0.9972
20	Methoxychlor	0.9984
21	Cyhalothrin	0.9976
22	Permethrin	0.9957
23	Cyfluthrin	0.9972
24	Cypermethrin	0.9972
25	Fenvalerate.	0.9962
26	Deltamethrin	0.9953

4.4.3 The result of Precision

The studied precision of this method was determined as intra-assay and intermediate precision. The intra-assay precision (within-day precision) was investigated in one day with seven replicates and the intermediate precision (between-day precision) was estimated from the results within three analytical days in seven replicates per day. In this work, precision was determined at 10 μg/L spiked level of the 26 analytes under optimized MSPD conditions. The percent of relative standard deviations (%R.S.D) represented the intra-assay and the intermediate precision. The % R.S.D of intra-assay precision was compared with calculated acceptable value of %R.S.D by Horwitz equation, which was 4.90% (at 10 μg/L). The % R.S.D. obtained in each day were in the range of 1.00 to 4.90 (day1), 1.70 to 4.01 (day2) and 1.29 to 3.69 (day3). The intra-assay precision of this method was acceptable because the % R.S.D. values were not larger than the calculated value from Horwitz equation and overall R.S.D. values were also satisfactory and the results are reported in Table 4.20-4.22. The % R.S.D of intermediate precision (n=21) determined the significant difference of result in three days by one single factor Anova program. The calculated F value and critical F value (P=0.05) were shown in Table4.29-4.31. Due to the calculated F values of 26 analytes were less than critical F value, the results (%R.S.D.) from three days are acceptable with no significance in difference.

Table 4.20 % Recovery of spiked sample of 26 OCPY at spiking level of 0.01 mg/kg of first day extraction

Pesticide	% Recovery							Mean ±SD	% RSD
	1	2	3	4	5	6	7		
α-BHC	87	85	83	82	89	88	86	85.71±2.56	2.99
β-BHC	88	85	83	81	86	88	85	85.14±2.99	2.99
γ-BHC	93	93	97	96	90	93	93	93.57±2.30	2.46
δ-BHC	96	94	93	92	98	91	94	94.00±2.38	2.53
Heptachlor	104	103	106	101	106	102	103	103.57±1.84	1.84
Aldrin	95	95	96	92	97	95	95	95.00±1.64	1.61
Dicofol	89	85	89	87	89	85	86	87.14±1.86	2.14
Hept.Epoxide	104	102	107	101	103	104	102	103.29±1.98	1.91
γ-Chlordane	89	88	80	89	82	89	88	86.43±3.78	4.37
α-Endosulfan	95	94	96	94	97	95	94	95.00±1.15	1.22
α-Chlordane	92	91	94	92	97	92	91	92.71±2.14	2.31
p,p'-DDE	94	93	97	95	93	94	93	94.14±1.46	1.55
Dieldrin	94	93	98	95	90	94	93	93.86±2.41	2.57
Endrin	100	102	106	102	107	105	102	103.43±2.57	2.49
p,p'-DDD	92	92	99	96	92	95	92	94.00±2.95	2.95
o,p'-DDT	94	93	91	92	98	97	93	94.00±2.58	2.75
Endosulfan sulfate	94	95	92	93	96	96	95	94.43±1.51	1.60
p,p'-DDT	95	96	93	90	99	95	96	94.86±2.79	2.95
Bifenthrin	93	93	91	93	98	98	96	94.57±2.76	2.92
Methoxychlor	89	88	86	84	82	81	83	84.71±3.59	3.59
Cyhalothrin	91	91	95	99	97	97	91	94.43±3.41	3.61
Permethrin	83	88	90	86	86	82	88	86.14±2.85	3.31
Cyfluthrin	86	91	93	98	99	96	91	93.43±4.58	4.90
Cypermethrin	95	94	94	92	95	91	94	93.57±1.51	1.62
Fenvalerate	95	95	95	94	90	92	95	93.71±1.98	2.11
Deltamethrin	95	95	94	96	97	95	95	85.71±2.56	2.99

Table 4.21 % Recovery of spiked sample of 26 OCPY at spiking level of 0.01 mg/kg of second day extraction

Pesticide	% Recovery							Mean ±SD	% RSD
	1	2	3	4	5	6	7		
α-BHC	88	82	84	85	88	90	89	86.57±2.94	3.39
β-BHC	81	89	87	85	84	86	89	86.86±3.32	3.32
γ-BHC	95	96	97	96	91	96	93	94.81±2.28	2.28
δ-BHC	97	91	92	92	99	99	96	95.14±3.61	3.61
Heptachlor	101	108	109	108	104	108	104	105.93±3.14	2.96
Aldrin	96	96	98	97	92	91	99	95.56±2.98	3.12
Dicofol	88	85	87	87	88	85	84	86.29±1.86	1.86
Hept.Epoxide	108	103	107	106	101	109	107	105.88±2.74	2.59
γ-Chlordane	88	87	89	88	82	89	87	87.14±2.41	2.77
α-Endosulfan	91	98	91	99	92	99	97	95.29±3.77	3.96
α-Chlordane	93	98	91	99	93	90	92	93.71±3.45	3.68
p,p'-DDE	97	92	95	95	99	95	93	95.14±2.34	2.46
Dieldrin	97	92	95	94	97	94	96	95.00±1.83	1.92
Endrin	103	108	104	104	107	105	106	105.33±1.79	1.70
p,p'-DDD	95	94	99	98	91	97	95	95.57±2.70	2.82
o,p'-DDT	97	92	95	93	93	94	94	94.00±1.63	1.74
Endosulfan sulfate	97	96	94	94	93	93	97	94.86±1.77	1.87
p,p'-DDT	95	98	93	99	94	92	90	94.38±3.20	3.39
Bifenthrin	91	99	94	93	97	90	96	94.29±3.25	3.45
Methoxychlor	87	83	81	82	80	88	87	83.95±3.19	3.81
Cyhalothrin	99	93	92	97	95	91	97	94.86±2.97	3.13
Permethrin	81	83	84	84	87	88	81	84.0±2.71	3.22
Cyfluthrin	91	99	97	98	99	96	93	96.20±3.01	3.13
Cypermethrin	96	93	99	90	91	95	99	94.71±3.59	3.79
Fenvalerate	94	91	95	93	91	100	100	94.86±3.80	4.01
Deltamethrin	88	82	84	85	88	90	89	86.57±2.94	4.01

Table 4.22 % Recovery of spiked sample of 26 OCPY at spiking level of 0.01 mg/kg of third day extraction

Pesticide	% Recovery							Mean ±SD	% RSD
	1	2	3	4	5	6	7		
α-BHC	88	85	87	83	89	85	88	86.37±2.10	2.43
β-BHC	89	87	88	89	88	85	82	86.86±2.54	2.93
γ-BHC	92	98	97	97	92	99	96	95.82±2.78	2.90
δ-BHC	98	96	95	98	99	97	97	97.14±1.35	1.38
Heptachlor	106	109	101	102	106	108	105	105.29±2.02	2.78
Aldrin	98	96	99	97	96	95	96	96.67±1.30	1.34
Dicofol	89	83	89	88	85	85	85	86.38±2.26	2.62
Hept.Epoxide	104	102	103	105	103	102	105	103.45±1.33	1.29
γ-Chlordane	88	88	87	82	81	83	88	86.29±3.15	3.69
α-Endosulfan	91	92	97	95	91	93	97	93.76±2.69	2.87
α-Chlordane	98	94	91	99	95	95	91	94.71±3.09	3.27
p,p'-DDE	92	98	94	92	98	97	91	94.57±3.05	3.22
Dieldrin	93	97	95	93	94	99	98	95.57±2.44	2.55
Endrin	100	105	101	107	103	103	107	103.68±2.80	2.70
p,p'-DDD	92	99	96	93	91	92	93	93.71±2.81	3.00
o,p'-DDT	92	96	91	99	94	92	94	94.00±2.77	2.95
Endosulfan sulfate	97	97	96	93	92	90	96	94.39±2.98	3.16
p,p'-DDT	99	99	95	98	93	93	94	95.86±2.73	2.85
Bifenthrin	92	96	99	98	96	91	94	95.14±2.97	3.12
Methoxychlor	84	82	83	87	89	85	82	84.59±2.59	3.06
Cyhalothrin	91	98	91	97	96	94	99	95.14±3.24	3.40
Permethrin	89	82	80	84	86	84	86	84.43±2.94	3.48
Cyfluthrin	97	94	93	95	96	94	92	94.3±1.79	1.90
Cypermethrin	93	99	98	98	92	93	91	94.86±3.34	3.52
Fenvalerate	95	99	98	98	92	97	98	96.71±2.43	2.51
Deltamethrin	98	92	93	99	97	92	91	86.37±2.10	2.43

Table 4.23 % Recovery of spiked sample of 26 OCPY at spiking level of 0.05 mg/kg of first day extraction

Pesticide	% Recovery							Mean ±SD	% RSD
	1	2	3	4	5	6	7		
α-BHC	88	87	82	85	86	85	89	86.00±2.31	2.69
β-BHC	88	88	81	83	85	85	86	85.14±2.54	2.99
γ-BHC	93	93	96	97	93	93	95	94.29±1.81	1.81
δ-BHC	91	96	92	93	94	94	96	93.71±1.89	2.02
Heptachlor	102	104	101	106	103	103	102	103.00±1.63	1.59
Aldrin	95	95	92	96	95	95	91	94.14±1.86	1.98
Dicofol	85	89	87	89	86	85	84	87.43±1.99	2.30
Hept.Epoxide	104	104	101	107	102	102	106	103.71±2.21	2.14
γ-Chlordane	89	89	89	80	88	88	85	86.86±3.34	3.84
α-Endosulfan	95	95	94	96	94	94	91	94.14±1.57	1.67
α-Chlordane	92	92	92	94	91	91	98	92.86±2.48	2.67
p,p'-DDE	94	94	95	97	93	93	96	94.57±1.51	1.60
Dieldrin	94	94	95	98	93	93	95	94.57±1.72	1.82
Endrin	105	100	102	106	102	102	104	103.00±2.08	2.02
p,p'-DDD	95	92	96	99	92	92	91	93.86±2.91	3.10
o,p'-DDT	97	94	92	91	93	93	98	94.00±2.58	2.75
Endosulfan sulfate	96	94	93	92	95	95	95	94.29±1.31	1.46
p,p'-DDT	95	95	90	93	96	96	92	93.86±2.27	2.42
Bifenthrin	98	93	93	91	96	93	90	93.43±2.76	2.95
Methoxychlor	81	89	84	86	83	88	88	85.57±2.99	3.50
Cyhalothrin	97	91	99	95	91	91	91	93.57±3.41	3.64
Permethrin	82	83	86	90	88	88	85	86.00±2.89	3.36
Cyfluthrin	96	86	98	93	91	91	95	92.86±3.98	4.28
Cypermethrin	91	95	92	94	94	94	94	93.43±1.40	1.50
Fenvalerate	92	95	94	95	95	95	91	93.86±1.68	1.79
Deltamethrin	88	87	82	85	86	85	89	86.00±2.31	2.69

Table 4.24 % Recovery of spiked sample of 26 OCPY at spiking level of 0.05 mg/kg of second day extraction

Pesticide	% Recovery							Mean ±SD	% RSD
	1	2	3	4	5	6	7		
α-BHC	85	88	82	84	89	88	90	86.57±2.94	3.39
β-BHC	85	81	89	87	89	84	84	85.57±2.94	3.43
γ-BHC	96	95	96	97	93	91	96	94.81±2.17	2.28
δ-BHC	92	97	91	92	96	99	96	94.71±3.04	3.21
Heptachlor	108	101	108	109	104	104	104	105.36±3.06	2.90
Aldrin	97	96	96	98	99	92	97	96.42±2.22	2.30
Dicofol	87	88	85	87	84	88	88	86.71±1.60	1.85
Hept.Epoxide	106	108	103	107	107	101	110	106.04±2.97	2.80
γ-Chlordane	88	88	87	89	87	82	85	86.57±2.37	2.74
α-Endosulfan	99	91	98	91	97	92	97	95.00±3.51	3.70
α-Chlordane	99	93	98	91	92	93	96	94.57±3.10	3.28
p,p'-DDE	95	97	92	95	93	99	95	95.14±2.34	2.46
Dieldrin	94	97	92	95	96	97	93	94.86±1.95	2.06
Endrin	104	103	108	104	106	107	107	105.61±1.88	1.78
p,p'-DDD	98	95	94	99	95	91	97	95.57±2.70	2.82
o,p'-DDT	93	97	92	95	94	93	93	93.86±1.68	1.79
Endosulfan sulfate	94	97	96	94	97	93	97	95.43±1.72	1.80
p,p'-DDT	99	95	98	93	90	94	95	94.81±3.02	3.19
Bifenthrin	93	91	99	94	96	97	94	94.86±2.67	2.82
Methoxychlor	82	87	83	81	87	80	89	84.14±3.48	4.14
Cyhalothrin	97	99	93	92	97	95	92	95.00±2.77	2.91
Permethrin	84	81	83	84	81	87	86	83.71±2.29	2.73
Cyfluthrin	98	91	99	97	93	99	95	96.06±3.05	3.17
Cypermethrin	90	96	93	99	99	91	94	94.57±3.60	3.81
Fenvalerate	93	94	91	95	100	91	98	94.57±3.41	3.60
Deltamethrin	85	88	82	84	89	88	90	86.57±2.94	3.39

Table 4.25 % Recovery of spiked sample of 26 OCPY at spiking level of 0.05 mg/kg of third day extraction

Pesticide	% Recovery							Mean ±SD	% RSD
	1	2	3	4	5	6	7		
α-BHC	83	88	85	87	88	89	86	86.51±2.02	2.34
β-BHC	89	89	87	88	82	88	83	86.57±2.88	3.32
γ-BHC	97	92	98	97	96	92	95	95.25±2.40	2.52
δ-BHC	98	98	96	95	97	99	93	96.57±2.07	2.14
Heptachlor	102	106	109	101	105	106	108	105.29±2.93	2.78
Aldrin	97	98	96	99	96	96	92	96.24±2.16	2.24
Dicofol	88	89	83	89	85	85	84	86.18±2.42	2.81
Hept.Epoxide	105	104	102	103	105	103	107	104.16±1.71	1.64
γ-Chlordane	82	88	88	87	88	81	89	86.14±3.24	3.76
α-Endosulfan	95	91	92	97	97	91	93	93.76±2.69	2.87
α-Chlordane	99	98	94	91	91	95	97	95.00±3.21	3.38
p,p'-DDE	92	92	98	94	91	98	93	94.00±2.89	3.07
Dieldrin	93	93	97	95	98	94	94	94.86±1.95	2.06
Endrin	107	100	105	101	107	103	108	104.40±3.21	3.07
p,p'-DDD	93	92	99	96	93	91	96	94.29±2.81	2.98
o,p'-DDT	99	92	96	91	94	94	97	94.71±2.81	2.97
Endosulfan sulfate	93	97	97	96	96	92	94	95.03±2.22	2.22
p,p'-DDT	98	99	99	95	94	93	97	96.43±2.44	2.53
Bifenthrin	98	92	96	99	94	96	93	95.43±2.57	2.70
Methoxychlor	87	84	82	83	82	89	88	85.02±2.90	3.41
Cyhalothrin	97	91	98	91	99	96	96	95.43±3.21	3.36
Permethrin	84	89	82	80	86	86	85	84.57±2.94	3.47
Cyfluthrin	95	97	94	93	92	96	98	95.00±2.22	2.33
Cypermethrin	98	93	99	98	91	92	97	95.43±3.31	3.47
Fenvalerate	98	95	99	98	98	92	98	96.86±2.48	2.56
Deltamethrin	83	88	85	87	88	89	86	86.51±2.02	2.34

Table 4.26 % Recovery of spiked sample of 26 OCPY at spiking level of 0.10 mg/kg of first day extraction

Pesticide	% Recovery							Mean ±SD	% RSD
	1	**2**	**3**	**4**	**5**	**6**	**7**		
α-BHC	**85**	**88**	**82**	**85**	**86**	**87**	**90**	86.14±2.54	2.95
β-BHC	85	88	81	83	85	88	87	85.29±2.63	3.08
γ-BHC	93	93	96	97	93	93	91	93.71±2.06	2.20
δ-BHC	94	91	92	93	94	96	98	94.00±2.38	2.53
Heptachlor	103	102	101	106	103	104	107	103.71±2.14	2.06
Aldrin	95	95	92	96	95	95	98	95.14±1.77	1.86
Dicofol	85	85	87	89	86	89	86	86.71±1.70	1.97
Hept.Epoxide	102	104	101	107	102	104	105	103.57±2.07	2.00
γ-Chlordane	88	89	89	80	88	89	90	87.57±3.41	3.89
α-Endosulfan	94	95	94	96	94	95	93	94.43±0.98	1.03
α-Chlordane	91	92	92	94	91	92	94	92.29±1.25	1.36
p,p'-DDE	93	94	95	97	93	94	97	94.71±1.70	1.80
Dieldrin	93	94	95	98	93	94	92	94.14±1.95	2.07
Endrin	102	105	102	106	102	100	102	102.71±2.06	2.00
p,p'-DDD	92	95	96	99	92	92	96	94.57±2.70	2.85
o,p'-DDT	93	97	92	91	93	94	95	93.57±1.99	2.12
Endosulfan sulfate	95	96	93	92	95	94	96	94.43±1.51	1.60
p,p'-DDT	96	95	90	93	96	95	97	94.57±2.37	2.51
Bifenthrin	93	98	93	91	96	93	95	94.14±2.34	2.49
Methoxychlor	88	81	84	86	83	89	89	85.71±3.15	3.67
Cyhalothrin	91	97	99	95	91	91	92	93.71±3.30	3.52
Permethrin	88	82	86	90	88	83	87	86.29±2.87	3.33
Cyfluthrin	91	96	98	93	91	90	96	93.57±3.10	3.31
Cypermethrin	94	91	92	94	94	95	92	93.14±1.46	1.57
Fenvalerate	95	92	94	95	95	95	97	94.71±1.50	1.58
Deltamethrin	95	95	96	94	95	95	92	94.57±1.27	1.35

Table 4.27 % Recovery of spiked sample of 26 OCPY at spiking level of 0.10 mg/kg of second day extraction

Pesticide	% Recovery							Mean ±SD	% RSD
	1	2	3	4	5	6	7		
α-BHC	88	85	90	84	89	88	82	86.57±2.94	2.99
β-BHC	81	85	84	87	89	84	89	85.80±2.17	2.53
γ-BHC	95	96	96	97	93	91	96	94.54±2.56	2.71
δ-BHC	97	92	96	92	96	99	91	95.00±3.00	3.16
Heptachlor	101	108	104	109	104	104	108	105.80±2.49	2.35
Aldrin	96	97	97	98	99	92	96	96.59±2.69	2.79
Dicofol	88	87	88	87	84	88	85	86.80±1.64	1.89
Hept.Epoxide	108	106	110	107	107	101	103	106.28±3.15	2.97
γ-Chlordane	88	88	85	89	87	82	87	86.20±2.77	3.22
α-Endosulfan	91	99	97	91	97	92	98	95.20±3.49	3.67
α-Chlordane	93	99	96	91	92	93	98	94.20±3.27	3.47
p,p'-DDE	97	95	95	95	93	99	92	95.40±2.19	2.30
Dieldrin	97	94	93	95	96	97	92	95.00±1.58	1.66
Endrin	103	104	107	104	106	107	108	105.69±1.41	1.33
p,p'-DDD	95	98	97	99	95	91	94	96.0±3.16	3.29
o,p'-DDT	97	93	93	95	94	93	92	93.60±0.89	0.96
Endosulfan sulfate	97	94	97	94	97	93	96	95.00±1.87	1.97
p,p'-DDT	95	99	95	93	90	94	98	94.20±3.27	3.47
Bifenthrin	91	93	94	94	96	97	99	94.80±1.64	1.73
Methoxychlor	87	82	89	81	87	80	83	83.80±3.96	4.73
Cyhalothrin	99	97	92	92	97	95	93	94.60±2.51	2.65
Permethrin	81	84	86	84	81	87	83	84.40±2.30	2.73
Cyfluthrin	91	98	95	97	93	99	99	96.48±2.26	2.35
Cypermethrin	96	90	94	99	99	91	93	94.60±4.28	4.52
Fenvalerate	94	93	98	95	100	91	91	95.40±3.65	3.82
Deltamethrin	88	85	90	84	89	88	82	86.57±2.94	2.99

Table 4.28 % Recovery of spiked sample of 26 OCPY at spiking level of 0.1 mg/kg of third day extraction

Pesticide	% Recovery							Mean ±SD	% RSD
	1	2	3	4	5	6	7		
α-BHC	89	83	88	85	87	88	84	86.22±2.24	2.60
β-BHC	88	89	89	87	88	82	81	86.29±3.35	3.89
γ-BHC	92	97	92	98	97	96	97	95.53±2.48	2.60
δ-BHC	99	98	98	96	95	97	93	96.57±2.07	2.14
Heptachlor	106	102	106	109	101	105	103	104.57±2.76	2.64
Aldrin	96	97	98	96	99	96	98	97.10±1.14	1.18
Dicofol	85	88	89	83	89	85	86	86.46±2.23	2.58
Hept.Epoxide	103	105	104	102	103	105	103	103.59±1.20	1.15
γ-Chlordane	81	84	88	88	87	88	85	85.86±2.67	3.11
α-Endosulfan	91	95	91	92	97	97	95	94.04±2.70	2.88
α-Chlordane	95	99	98	94	91	95	94	95.14±2.67	2.81
p,p'-DDE	98	92	92	98	94	91	91	93.71±3.09	3.30
Dieldrin	94	93	93	97	95	98	99	95.57±2.44	2.55
Endrin	103	107	100	105	105	107	108	104.97±2.84	2.70
p,p'-DDD	91	93	92	99	96	93	91	93.57±2.94	3.14
o,p'-DDT	94	99	92	96	91	94	95	94.43±2.64	2.79
Endosulfan sulfate	92	93	97	97	96	96	91	94.60±2.60	2.75
p,p'-DDT	93	98	99	99	95	94	93	95.86±2.73	2.85
Bifenthrin	96	98	92	96	99	94	91	95.14±2.97	3.12
Methoxychlor	89	87	84	82	83	82	83	84.30±2.65	3.14
Cyhalothrin	96	97	91	98	91	99	94	95.14±3.24	3.40
Permethrin	86	84	89	82	80	86	89	85.14±3.39	3.98
Cyfluthrin	96	95	97	94	93	92	93	94.29±1.87	1.98
Cypermethrin	92	98	93	99	98	91	97	95.43±3.31	3.47
Fenvalerate	92	98	95	99	98	98	93	96.14±2.79	2.91
Deltamethrin	89	83	88	85	87	88	84	86.22±2.24	2.60

Table 4.29 Mean of **%** Recovery in 3 day of 26 OCPY pesticides intermediate precision at spiking level of 0.01 mg/kg in garlic sample

Pesticide	% Recovery					F- value	
	day1	day2	day3	Mean	% RSD	Calculate	Critical (p=0.050)
α-BHC	85.71	86.57	86.37	86.22	2.45		0.21
β-BHC	85.14	85.86	86.86	85.95	2.62		0.74
γ-BHC	93.57	94.81	95.82	94.73	2.49		1.50
δ-BHC	94.00	95.14	97.14	95.43	2.75		2.80
Heptachlor	103.57	105.93	105.29	104.93	2.77		1.42
Aldrin	95.00	95.56	96.67	95.74	2.09		1.17
Dicofol	87.14	86.29	86.38	86.60	1.87		0.42
Hept.Epoxide	103.29	105.88	103.45	104.20	2.33		3.35
γ-Chlordane	86.43	87.14	85.29	86.29	3.10		0.61
α-Endosulfan	95.00	95.29	93.76	94.68	2.70		0.61
α-Chlordane	92.71	93.71	94.71	93.71	2.92		0.81
p,p'-DDE	94.14	95.14	94.57	94.62	2.29		0.31
Dieldrin	93.86	95.00	95.57	94.81	2.25		0.31
Endrin	103.43	105.33	103.68	104.15	2.46		1.06
p,p'-DDD	94.00	95.57	93.71	94.43	2.75	3.55	1.27
o,p'-DDT	94.00	94.00	94.00	94.00	2.26		0.92
Endosulfan sulfate	94.43	94.86	94.39	94.56	2.08		0.10
p,p'-DDT	94.86	94.38	95.86	95.03	2.84		0.10
Bifenthrin	94.57	94.29	95.14	94.67	2.87		0.47
Methoxychlor	84.71	83.95	84.59	84.42	2.82		0.15
Cyhalothrin	94.43	94.86	95.14	94.81	3.06		0.14
Permethrin	86.14	84.00	84.43	84.86	2.85		0.088
Cyfluthrin	93.43	96.20	94.43	94.69	3.37		1.12
Cypermethrin	93.57	94.71	94.86	94.38	2.87		1.25
Fenvalerate	93.71	94.86	96.71	95.10	2.98		0.40
Deltamethrin	85.71	86.57	86.37	95.38	2.92		1.98

Table 4.30 Mean of **%** Recovery in 3 day of 26 OCPY pesticides intermediate precision at spiking level of 0.05 mg/kg in garlic sample

Pesticide	% Recovery					F- value	
	day1	day2	day3	Mean	% RSD	Calculate	Critical (p=0.050)
α-BHC	86.00	86.57	86.51	86.36	2.71		0.11
β-BHC	85.14	85.57	86.57	85.76	3.17		0.48
γ-BHC	94.29	94.81	95.25	94.78	2.15		0.36
δ-BHC	93.71	94.71	96.57	95.00	2.70		2.58
Heptachlor	103.00	105.36	105.29	104.55	2.61		1.83
Aldrin	94.14	96.42	96.24	95.60	2.35		2.58
Dicofol	86.43	86.71	86.18	86.44	2.24		0.12
Hept.Epoxide	103.71	106.04	104.16	104.64	2.35		1.92
γ-Chlordane	86.86	86.57	86.14	86.52	3.32		0.01
α-Endosulfan	94.14	95.00	93.76	94.30	2.79		0.39
α-Chlordane	92.86	94.57	95.00	94.14	3.14		1.03
p,p'-DDE	94.57	95.14	94.00	94.57	2.38		0.43
Dieldrin	94.57	94.86	94.86	94.76	1.88		0.05
Endrin	103.00	105.61	104.40	104.34	2.47		1.98
p,p'-DDD	93.86	95.57	94.29	94.57	2.93	3.55	0.71
o,p'-DDT	94.00	93.86	94.71	94.19	2.46		0.25
Endosulfan sulfate	94.29	95.43	95.03	94.92	1.83		0.76
p,p'-DDT	93.86	94.81	96.43	95.03	2.83		1.75
Bifenthrin	93.43	94.86	95.43	94.57	2.83		1.04
Methoxychlor	85.57	84.14	85.02	84.91	3.57		0.37
Cyhalothrin	93.57	95.00	95.43	94.67	3.26		0.67
Permethrin	86.00	83.71	84.57	84.76	3.25		1.26
Cyfluthrin	92.86	96.06	95.00	94.64	3.48		1.86
Cypermethrin	93.43	94.57	95.43	94.48	3.08		0.82
Fenvalerate	93.86	94.57	96.86	95.10	2.95		2.51
Deltamethrin	94.29	95.57	94.57	94.81	3.07		0.35

Table 4.31 Mean of **%** Recovery in 3 day of 26 OCPY pesticides intermediate precision at spiking level of 0.10 mg/kg in garlic sample

Pesticide	% Recovery					F- value	
	day1	day2	day3	Mean	% RSD	Calculate	Critical (p=0.050)
α-BHC	86.14	86.57	86.22	86.36	2.34		0.11
β-BHC	85.29	85.80	86.29	85.76	2.72		0.48
γ-BHC	93.71	94.54	95.53	94.78	2.04		0.36
δ-BHC	94.00	95.00	96.57	95.00	2.57		2.58
Heptachlor	103.71	105.80	104.57	104.55	2.73		1.83
Aldrin	95.14	96.59	97.10	95.60	2.24		2.58
Dicofol	86.71	86.80	86.46	86.44	1.94		0.12
Hept.Epoxide	103.57	106.26	103.59	104.66	2.46		1.92
γ-Chlordane	87.57	86.20	85.86	86.52	2.87		0.10
α-Endosulfan	94.43	95.20	94.04	94.30	2.63		0.39
α-Chlordane	92.29	94.20	95.14	94.14	2.95		1.03
p,p'-DDE	94.71	95.40	93.71	94.57	2.25		0.43
Dieldrin	94.14	95.00	95.57	94.76	1.79		0.05
Endrin	102.71	105.69	104.97	104.34	2.58		1.98
p,p'-DDD	94.57	96.00	93.57	94.57	2.77	3.55	0.71
o,p'-DDT	93.57	93.60	94.43	94.19	2.32		0.25
Endosulfan sulfate	94.43	95.00	94.60	94.92	1.74		0.76
p,p'-DDT	94.57	94.20	95.86	95.03	2.69		1.75
Bifenthrin	94.14	94.80	95.14	94.57	2.68		1.04
Methoxychlor	85.71	83.80	84.30	84.91	3.04		0.37
Cyhalothrin	93.71	94.60	95.14	94.67	3.09		0.67
Permethrin	86.29	84.40	85.14	84.76	2.76		1.26
Cyfluthrin	93.57	96.48	94.29	94.64	3.30		1.86
Cypermethrin	93.14	94.60	95.43	94.48	2.91		0.82
Fenvalerate	94.71	95.40	96.14	95.10	2.81		2.51
Deltamethrin	94.57	95.60	94.57	94.81	2.91		0.35

4.4.4 The result of Accuracy

From Table 4.32, % recovery of all OCPY pesticides in garlic samples at spiking level of 0.01 mg/kg are in the range of 84.71-103.57 %, at spiking level of 0.050 mg/kg are in the range of 85.14-103.71 % and at spiking level of 100 ng/g are in the range of 84.40-106.28 % . This is within the acceptable range set forth by AOAC Regulations % recovery 0.01-0.10 in mg/kg level. This in turn shows that the developed method can yield high accuracy with samples of low concentration.

4.4.5 The result of determination of organochlorine and pyrethroid pesticide in garlic products at 2 types garlic from 2 Thai markets.

From Table 4.33, all of the four samples above have the concentration of cypermethrin in low concentrations and under the limit of the EU regulations, 0.10 ppm (mg/kg). These likely to be found in cypermethrin substances and their concentration is also found to be low concentrations.

Table 4.32 % Recovery of spiked sample of MSPD method with various of spiking level.

No.	Pesticide	Mean of % recovery ± S.D. (n=7)		
		Spiking level of 0.01 mg/kg	**Spiking level of 0.05 mg/kg**	**Spiking level of 0.10 mg/kg**
1	α-BHC	85.71±2.56	86.00±2.31	86.57±2.94
2	β-BHC	85.14±2.99	85.14±2.54	85.80±2.17
3	γ-BHC	93.57±2.30	94.29±1.81	94.54±2.56
4	δ-BHC	94.00±2.38	93.71±1.89	95.00±3.00
5	Heptachlor	103.57±1.84	103.00±1.63	105.80±2.49
6	Aldrin	95.00±1.64	94.14±1.86	96.59±2.69
7	Dicofol	87.14±1.86	87.43±1.99	86.80±1.64
8	Hept.Epoxide	103.29±1.98	103.71±2.21	106.28±3.15
9	γ-Chlordane	86.43±3.78	86.86±3.34	86.20±2.77
10	α-Endosulfan	95.00±1.15	94.14±1.57	95.20±3.49
11	α-Chlordane	92.71±2.14	92.86±2.48	94.20±3.27
12	p,p'-DDE	94.14±1.46	94.57±1.51	95.40±2.19
13	Dieldrin	93.86±2.41	94.57±1.72	95.00±1.58
14	Endrin	103.43±2.57	103.00±2.08	105.69±1.41
15	p,p'-DDD	94.00±2.95	93.86±2.91	96.0±3.16
16	o,p'-DDT	94.00±2.58	94.00±2.58	93.60±0.89
17	Endosulfan sulfate	94.43±1.51	94.29±1.31	95.00±1.87
18	p,p'-DDT	94.86±2.79	93.86±2.27	94.20±3.27
19	Bifenthrin	94.57±2.76	93.43±2.76	94.80±1.64
20	Methoxychlor	84.71±3.59	85.57±2.99	83.80±3.96
21	Cyhalothrin	94.43±3.41	93.57±3.41	94.60±2.51
22	Permethrin	86.14±2.85	86.00±2.89	84.40±2.30
23	Cyfluthrin	93.43±4.58	92.86±3.98	96.48±2.26
24	Cypermethrin	93.57±1.51	93.43±1.40	94.60±4.28
25	Fenvalerate	93.71±1.98	93.86±1.68	95.40±3.65
26	Deltamethrin	95.30±0.95	86.00±2.31	86.57±2.94

Table 4.33 Result of OCPY pesticides in garlic sample at 2 Thai markets and 2 and 2 typed garlic.(ng/g)

No.	Pesticide	Concentration of OCPY pesticides in Garlic (mg/kg)			
		Market A		Market B	
		Thai garlic	Chinese garlic	Thai garlic	Chinese garlic
1	α-BHC	ND	ND	ND	ND
2	β-BHC	ND	ND	ND	ND
3	γ-BHC	ND	ND	ND	ND
4	δ-BHC	ND	ND	ND	ND
5	Heptachlor	ND	ND	ND	ND
6	Aldrin	ND	ND	ND	ND
7	Dicofol	ND	ND	ND	ND
8	Hept.Epoxide	ND	ND	ND	ND
9	γ-Chlordane	ND	ND	ND	ND
10	α-Endosulfan	ND	ND	ND	ND
11	α-Chlordane	ND	ND	ND	ND
12	p,p'-DDE	ND	ND	ND	ND
13	Dieldrin	ND	ND	ND	ND
14	Endrin	ND	ND	ND	ND
15	p,p'-DDD	ND	ND	ND	ND
16	o,p'-DDT	ND	ND	ND	ND
17	Endosulfan	ND	ND	ND	ND
18	p,p'-DDT	ND	ND	ND	ND
19	Bifenthrin	ND	ND	ND	ND
20	Methoxychlor	ND	ND	ND	ND
21	Cyhalothrin	ND	ND	ND	ND
22	Permethrin	ND	ND	ND	ND
23	Cyfluthrin	ND	ND	ND	ND
24	Cypermethrin	0.02	0.01	0.02	0.02
25	Fenvalerate	ND	ND	ND	ND
26	Deltamethrin	ND	ND	ND	ND

CHAPTER V

CONCLUSIONS AND SUGGESTIONS FOR FURTHER STUDY

A method for the sample preparation and determination of 26 Organochlorine and Pyrethroid (OCPY) pesticides was developed. MSPD was used to extract the 26 analytes before detection with gas chromatography mass spectrometry (GC- MS) with a three way splitter. In this work, the optimization were GC condition and the extraction from garlic sample. The conditions of GC-μECD detection for alpha-Hexachlorocyclohexane (α-HCH), beta- Hexachlorocyclohexane (β-HCH), γ-Hexachlorocyclohexane(γ-HCH), delta-Hexachlorocyclohexane (δ-HCH), Heptachlor, Aldrin, Dicofol, Heptachlor epoxide, γ-Chlordane, alpha-Endosulfan (α-Endosulfan), p,p-DDE, Dieldrin, Endrin, β-Endosulfan (Endosulfan β), p,p-DDD, o,p-DDT, Endosulfan sulfate, p,p-DDT, Methoxychlor , Bifenthrin, lamda Cyhalothrin, Permethrin, Cyfluthrin, Cypermethrin, Fenvalerate and Deltamethrin and described shown in Table 5.1. The confirmation information of organochlorine and pyrethroid pesticide.were previously shown in Table 4.4.

For the sample preparation of organochlorine and pyrethroid pesticides, MSPD was employed as illustrated in Figure 5.1. MSPD was investigated in this work to improve the 26 OCPY pesticides with optimization of related parameters. Solid support type and composition, elution solvent type and volume of elution solvent were considered. The MSPD procedure with optimized parameters is summarized in Figure 5.2

Table 5.1 GC/MS condition for the analysis of 26 OCPY pesticides

	Parameters	**Condition**
GC	Analytical Column Restrictor Column	HP-5 MS(25 m 0.25mm I.D.2.5 mm film thickness)
		µECD: I.D.2.5 mm 2.018 M,MSD : I.D.2.5 mm 5.483 M
	Temperature Program	50 ºC , rate A 35 ºC /min to 200 ºC (5 min)
		200ºC rate B of 5 ºC /min to 250 ºC (5 min)
		250ºC rate C of 35 ºC /min to 290 ºC (10 min)
	Injection Mode	PTV solvent vent mode
	Injection Volume	4µL
	Injection Temperature	50 ºC (1.1 min) rate700 ºC /min to 300 ºC
	Flow Rate of carrier Gas (He)	1.44 mL /min
	Flow Rate of Nitrogen gas	60 mL /min
	PCM makeup supply	4.5 mL/min
	Detector	micro-Electron Capture Detector (µ-ECD)
	Detector Temperature	300 ºC
	Solvent Delay Time	3 min
MS	Transfer line Temperature	280ºC
	Ionization source temperature	300 ºC
	Quardapole temperature	150 ºC
	Analysis	SIM mode
	Ionization Type	EI 70 eV

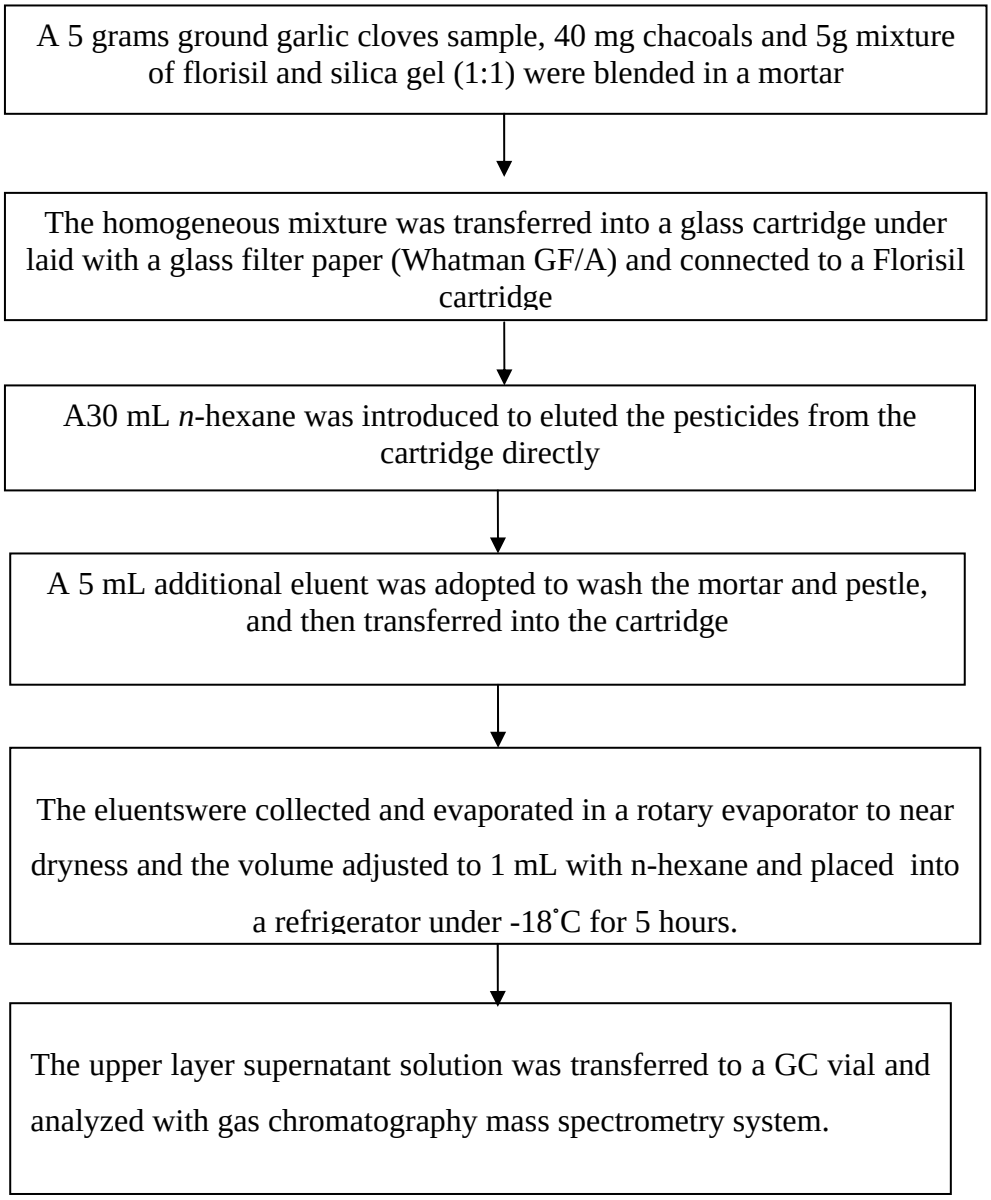

Figure 5.1 Schematic diagram of MSPD procedure with optimized condition

Table 5.2 The optimum conditions of MSPD

MSPD parameter	Condition
Type of solid support	florisil : silica gel
Composition of solid support	Ratio 1:1
Type of elution solvent	n-Hexane
Volume of elution solvent	30 mL
ratio of sample to solid support	1:1

All optimized parameters in MSPD were summarized in Table 5.2. After, this optimized condition of MSPD method was validated to observe the performance of the method before studying the application with a real sample. The summary of MSPD method validation is reported in Table 5.3

The linearity from standard calibration curve of 26 OCPY pesticides revealed correlation coefficient value (R^2) of over 0.995 representing good linear dynamic range of the method. The method recovery representing accuracy ranged from 84.71to 103.57 % at 10 μg/L spiking level. The intra-assay precision was reported as relative standard deviation (%R.S.D.) and the value of %R.S.D. for within-day precision ranged from 1.55 to 4.90%. The %R.S.D. values obtained from the experiments were lower than %R.S.D calculated from Horwitz equation, which indicates the satisfactory of method capability. For intermediate precision, the %R.S.D. was calculated from the results on three analytical days and single factor Anova were used to evaluate the significance of different %R.S.D. three days. The values of %R.S.D did not significantly differ on three working days because the calculated F values were lower than the critical F values (P=0.05). Both intra-assay and intermediate precisions were in acceptable ranges.

In real sample analysis, Thai and Chinese garlic samples are chosen to study with MSPD method because OCPY pesticides were found to create residual problem in both. types of sample. For garlic sample analysis, was optimized MSPD condition and detected by GC-μECD. All of the four samples above have the concentration of cypermethrin in low concentrations and under the limit of the EU regulations, 0.01-0.10 ppm (mg/kg). This shows that the method is very effective and can detect slight traces of contaminants, the developed sample preparation technique is most suitable for the standard method for Thailand.

Table 5.3 Method performance of MSPD with GC-μECD detector for 26 OCPY pesticides

Pesticide	Linear range (mg/L)	Correlation coefficient (R^2)	LODs (mg/L)	LOQs (mg/L)	% Recovery	Intra-assay precision (% SD)	Intermediate precision (% RSD)
α-BHC	0.005-0.50	0.9981	0.003	0.01	85.71-86.57	2.71	3.39
β-BHC	0.005-0.50	0.9976	0.003	0.01	85.14-85.80	3.17	3.89
γ-BHC	0.005-0.50	0.9977	0.003	0.01	93.57-94.54	2.49	2.90
δ-BHC	0.005-0.50	0.9976	0.003	0.01	94.00-95.00	2.75	3.61
Heptachlor	0.005-0.50	0.9975	0.003	0.01	103.57-105.80	2.77	2.96
Aldrin	0.005-0.50	0.9972	0.003	0.01	95.00-96.59	2.58	3.12
Dicofol	0.005-0.50	0.9953	0.003	0.01	87.14-86.80	1.94	2.81
Hept.Epoxide	0.005-0.50	0.9955	0.003	0.01	103.29-106.28	2.45	2.80
γ-Chlordane	0.005-0.50	0.9973	0.003	0.01	86.43-86.20	3.10	3.89
α-Endosulfan	0.005-0.50	0.9969	0.003	0.01	95.00-95.20	3.32	3.96
α-Chlordane	0.005-0.50	0.9951	0.003	0.01	92.71-94.20	3.14	3.68
p,p'-DDE	0.005-0.50	0.9988	0.003	0.01	94.14-95.40	2.38	3.22
Dieldrin	0.005-0.50	0.9961	0.003	0.01	93.86-95.00	2.25	2.57
Endrin	0.005-0.50	0.9950	0.003	0.01	103.43-105.69	2.58	3.07
p,p'-DDD	0.005-0.50	0.9950	0.003	0.01	94.00-96.01	2.93	3.14
o,p'-DDT	0.005-0.50	0.9964	0.003	0.01	94.00-93.60	2.46	2.90
Endosulfan sulfate	0.005-0.50	0.9950	0.003	0.01	94.43-95.00	2.08	3.16
p,p'-DDT	0.005-0.50	0.9991	0.003	0.01	94.86-94.20	2.84	3.47
Bifenthrin	0.005-0.50	0.9972	0.003	0.01	94.57-94.80	2.87	3.45
Methoxychlor	0.005-0.50	0.9984	0.003	0.01	84.71-83.80	3.57	3.81
Cyhalothrin	0.005-0.50	0.9976	0.003	0.01	94.43-94.60	3.26	3.61
Permethrin	0.005-0.50	0.9957	0.004	0.01	86.14-84.40	3.25	3.98
Cyfluthrin	0.005-0.50	0.9972	0.003	0.01	93.43-96.48	3.48	4.90
Cypermethrin	0.005-0.50	0.9972	0.003	0.01	93.57-94.60	3.08	4.52
Fenvalerate.II	0.005-0.50	0.9962	0.003	0.01	93.71-95.40	2.98	4.01
Deltamethrin	0.005-0.50	0.9953	0.004	0.01	95.30-86.57	3.07	4.01

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APPENDICES

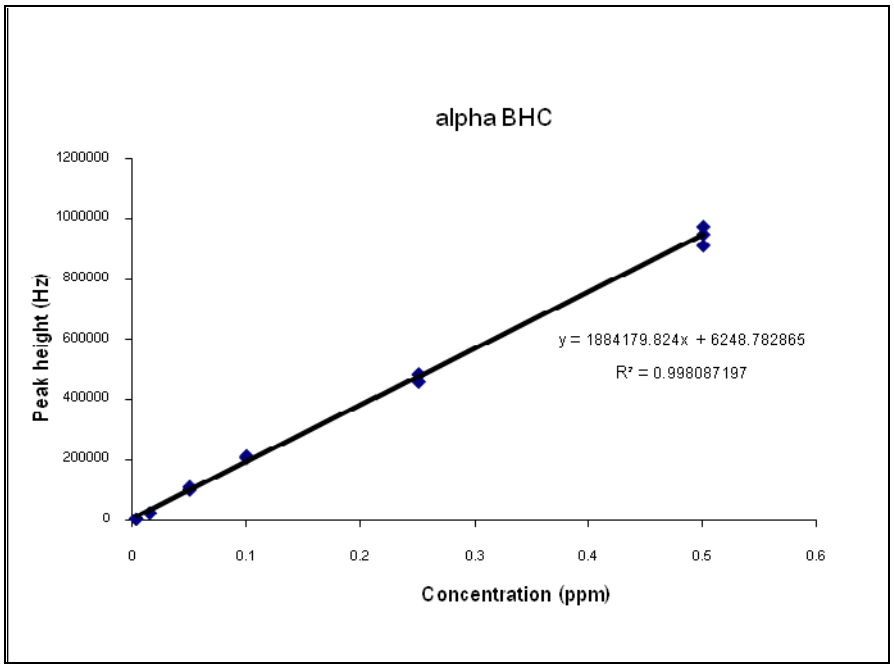

Figure A-1 The calibration curves of mixed standard of alpha-BHC by GC condition in Table 4.2

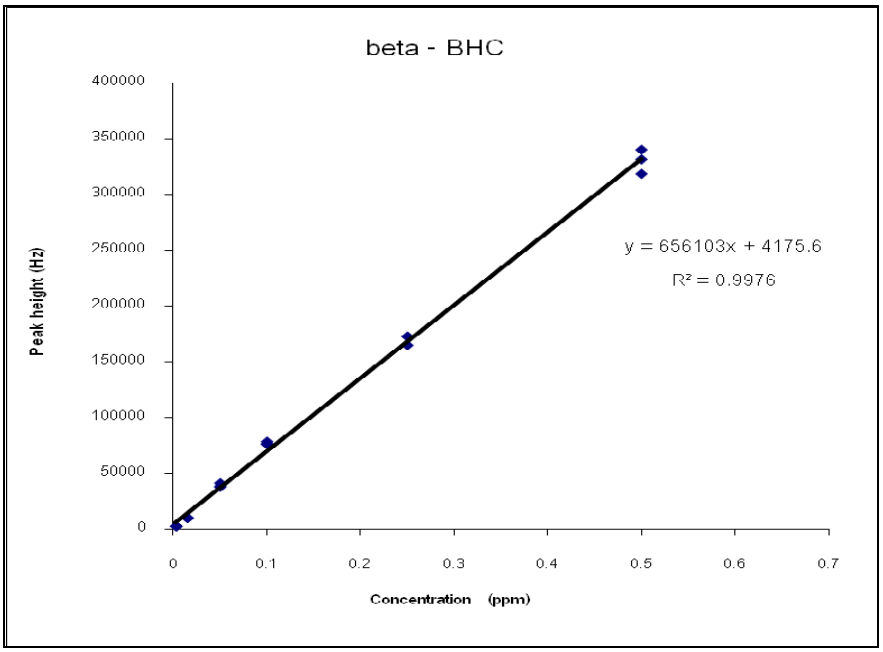

Figure A-2 The calibration curves of mixed standard of beta-BHC by GC condition in Table 4.2

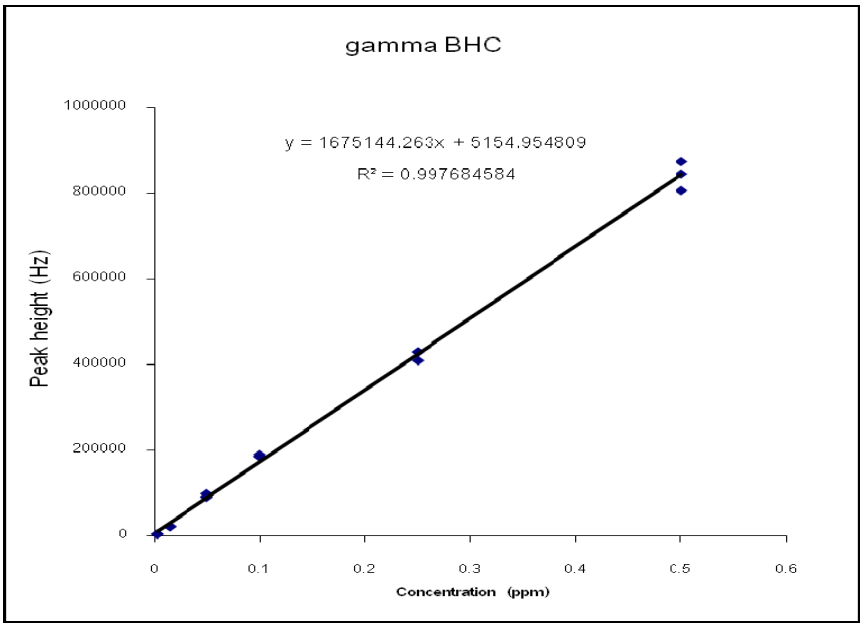

Figure A-3 The calibration curves of mixed standard of gamma-BHC by GC condition in Table 4.2

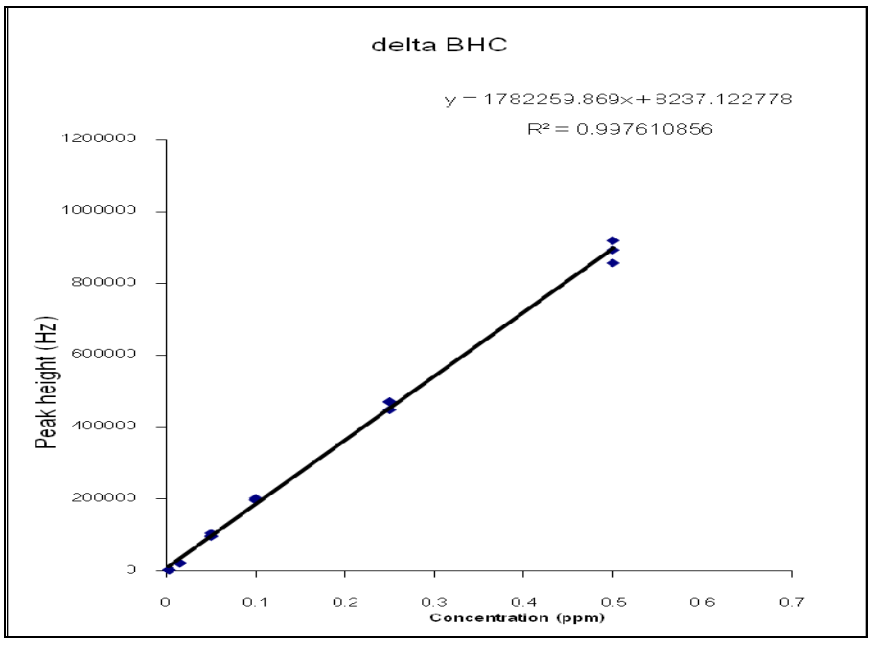

Figure A-4 The calibration curves of mixed standard of delta-BHC by GC condition in Table 4.2

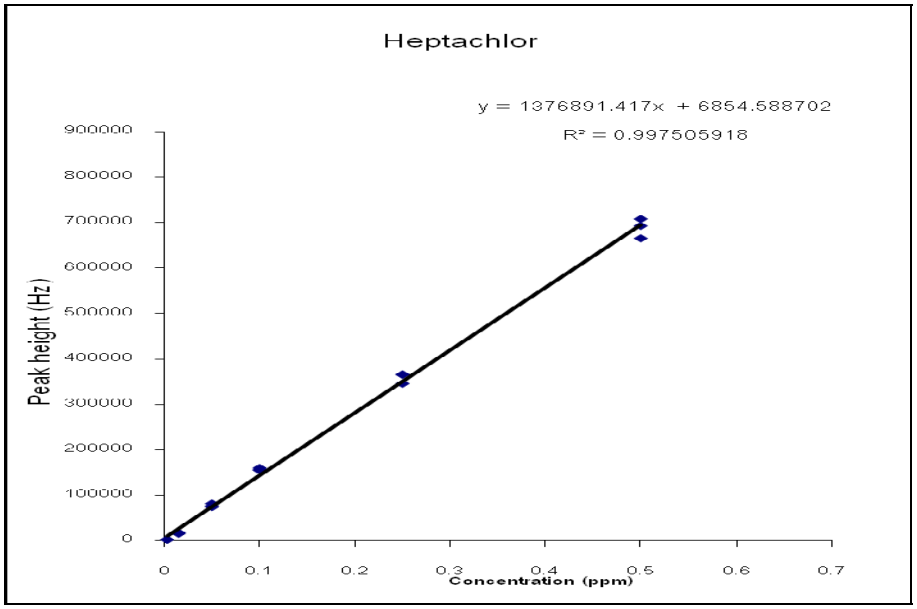

Figure A-5 The calibration curves of mixed standard of Heptachlor by GC condition in Table 4.2

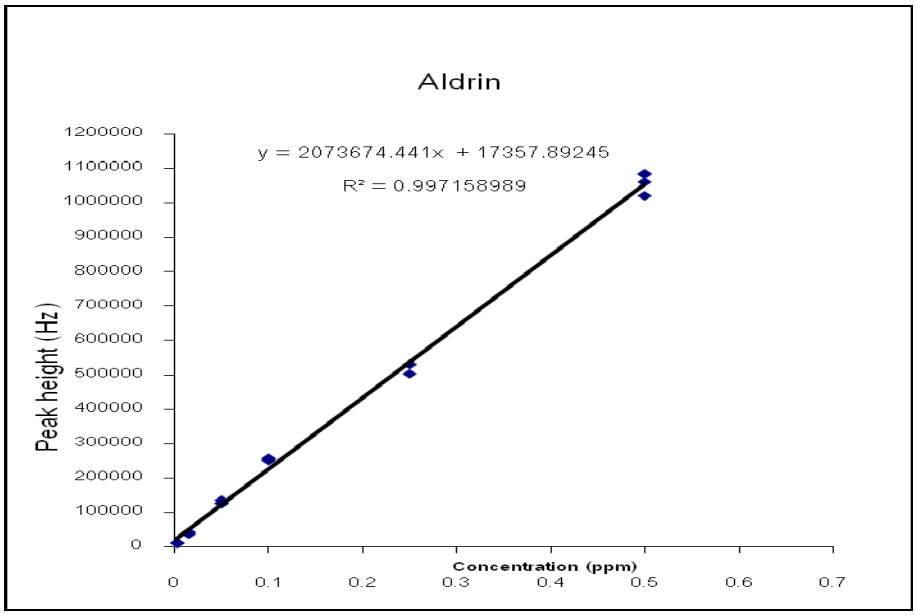

Figure A-6 The calibration curves of mixed standard of Aldrin by GC condition in Table 4.2

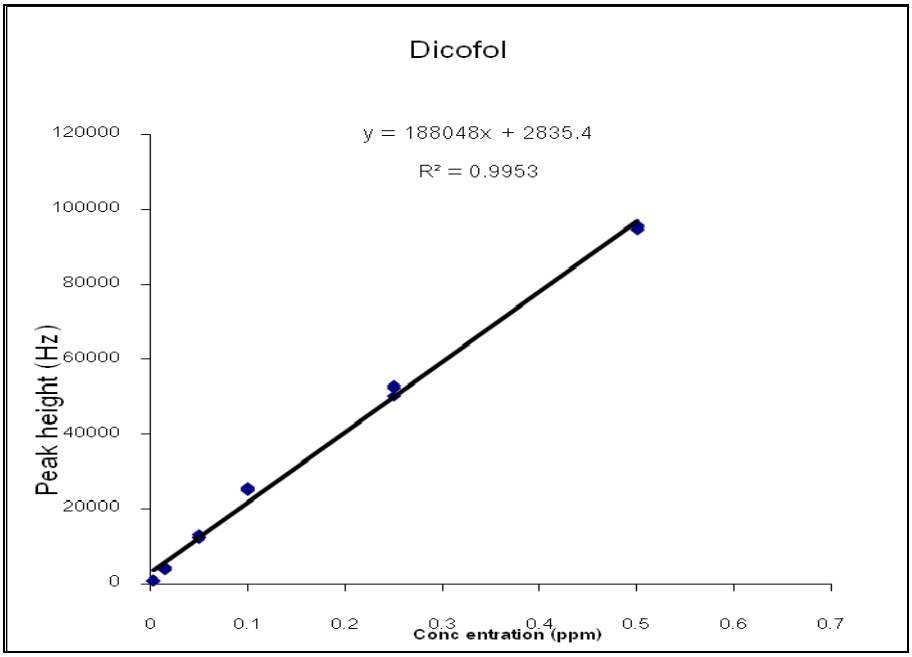

Figure A-7 The calibration curves of mixed standard of Dicofol by GC condition in Table 4.2

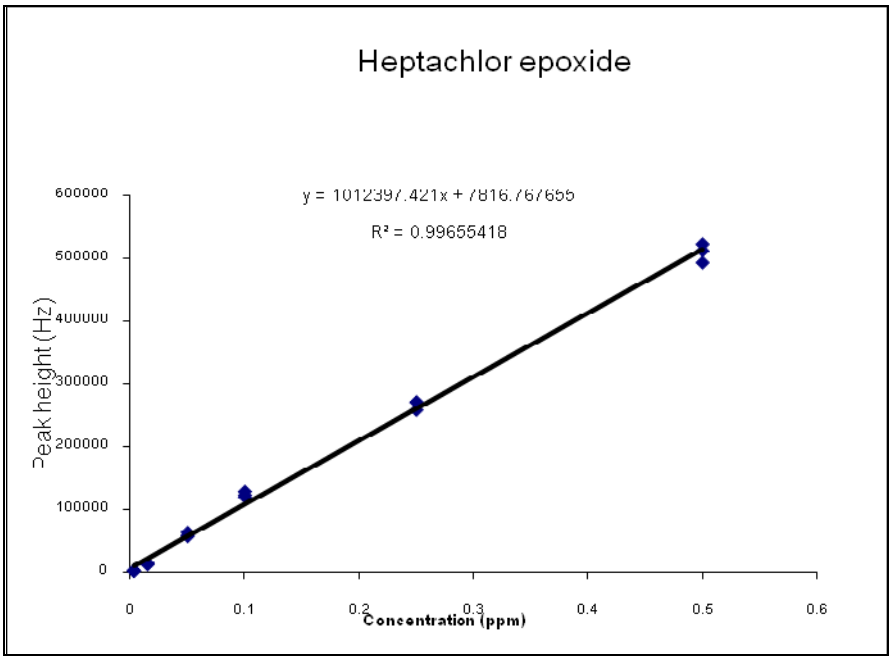

Figure A-8 The calibration curves of mixed standard of Heptachlor epoxide by GC condition in Table 4.2

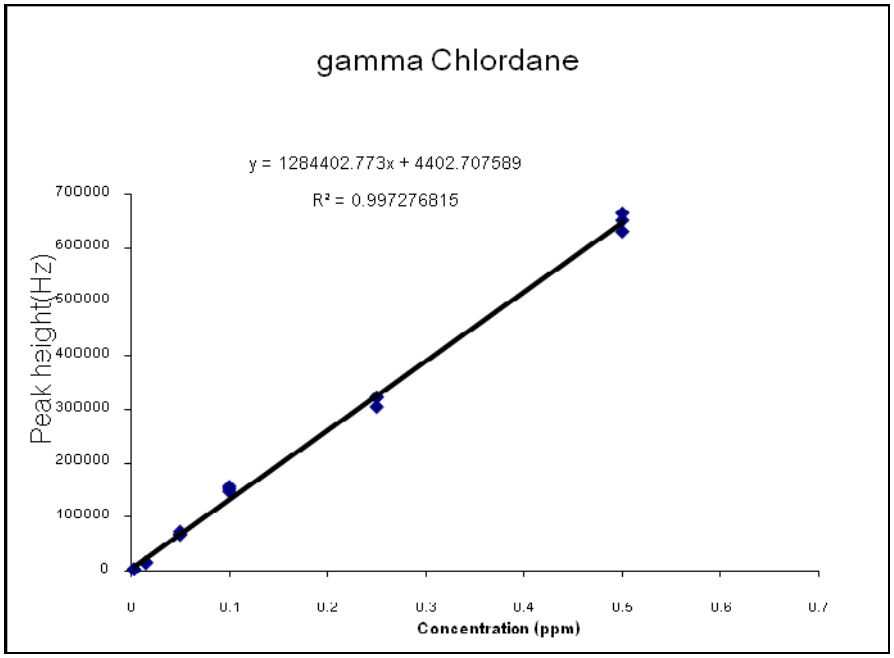

Figure A-9 The calibration curves of mixed standard of gamma Chlordane by GC condition in Table 4.2

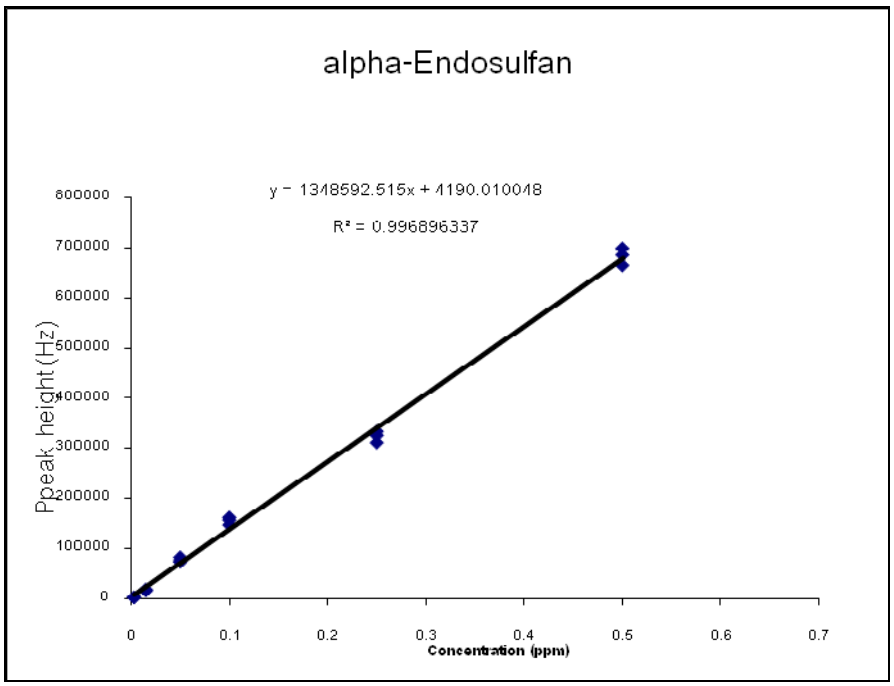

Figure A-10 The calibration curves of mixed standard of alpha-Endosulfan by GC condition in Table 4.2

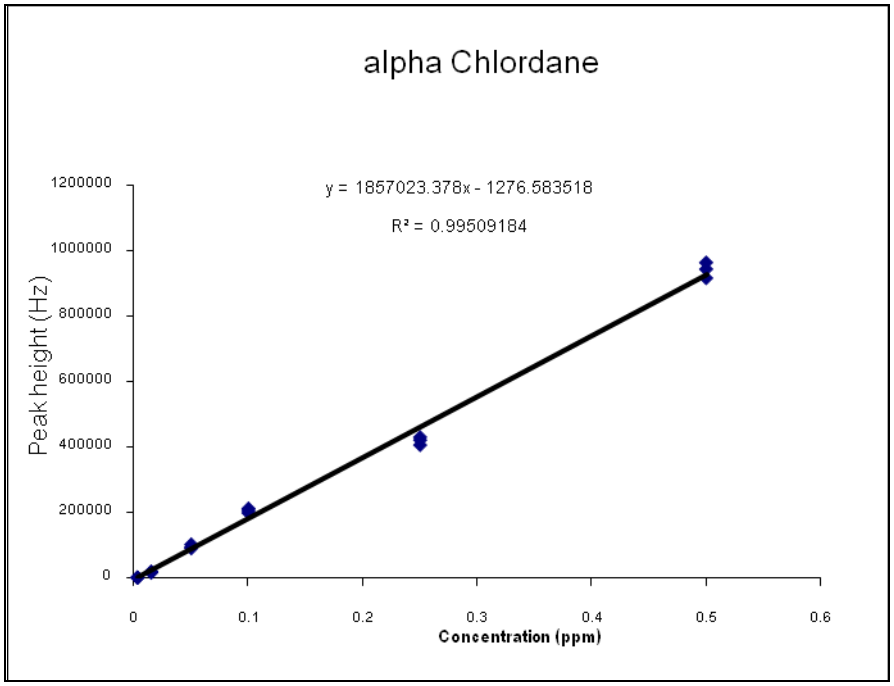

Figure A-11 The calibration curves of mixed standard of alpha- Chlordane by GC condition in Table 4.2

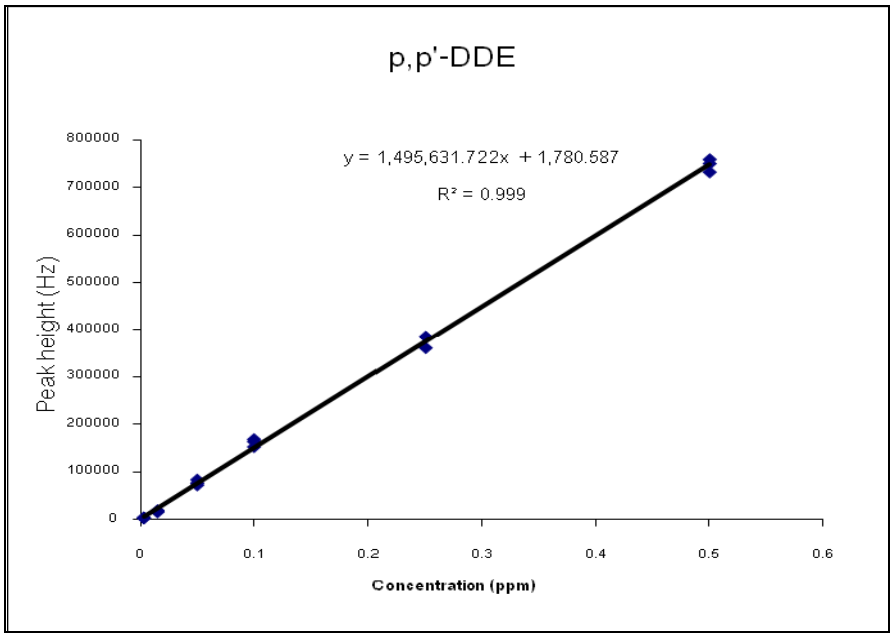

Figure A-12 The calibration curves of mixed standard of p,p'-DDE by GC condition in Table 4.2

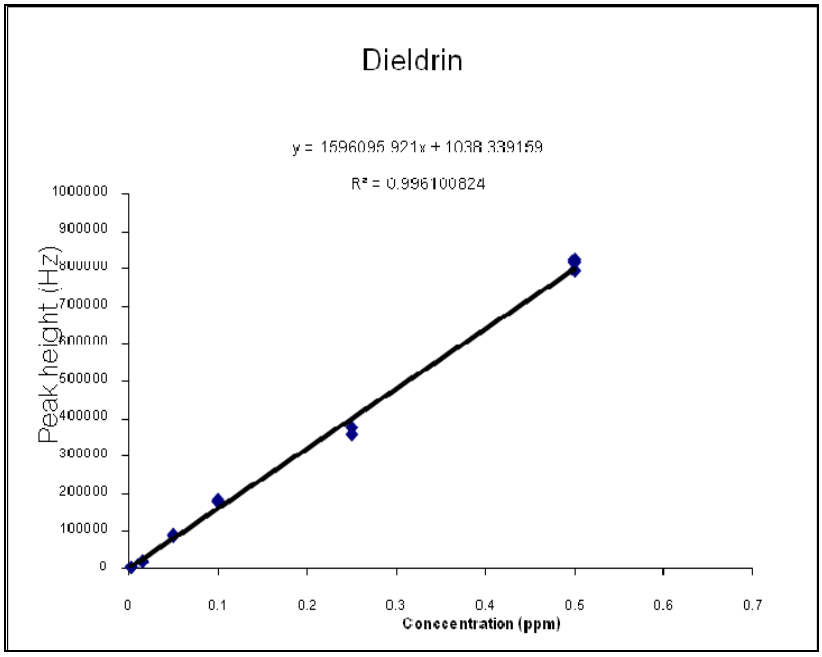

Figure A-13 The calibration curves of mixed standard of Dieldrin by GC condition in Table 4.2

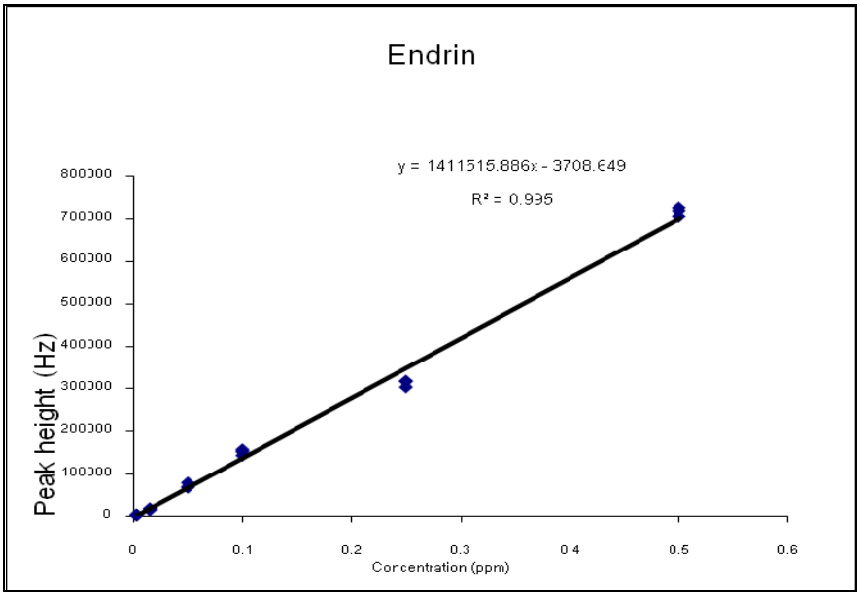

Figure A-14 The calibration curves of mixed standard of Endrin by GC condition in Table 4.2

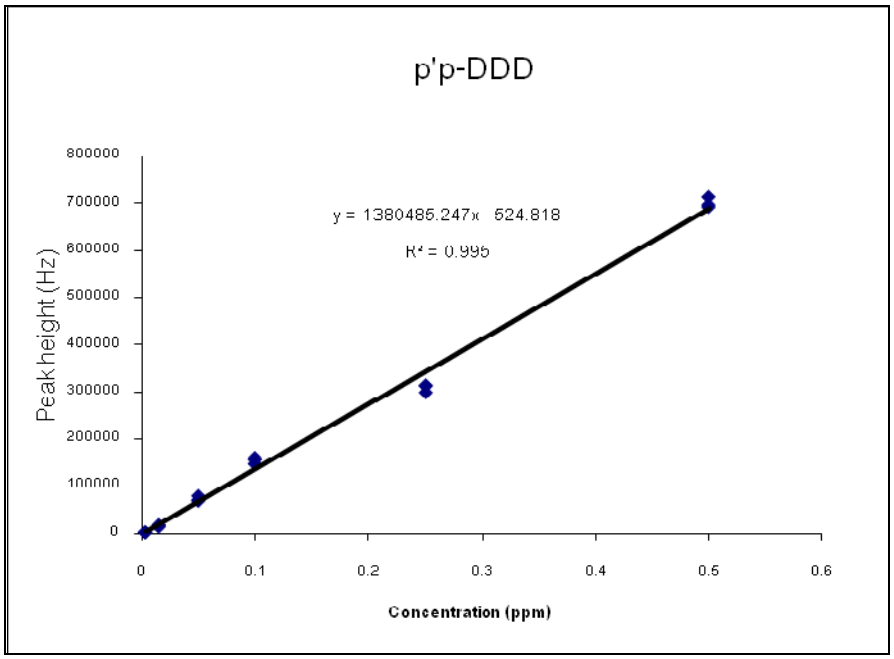

Figure A-15 The calibration curves of mixed standard of p,p-DDD by GC condition in Table 4.2

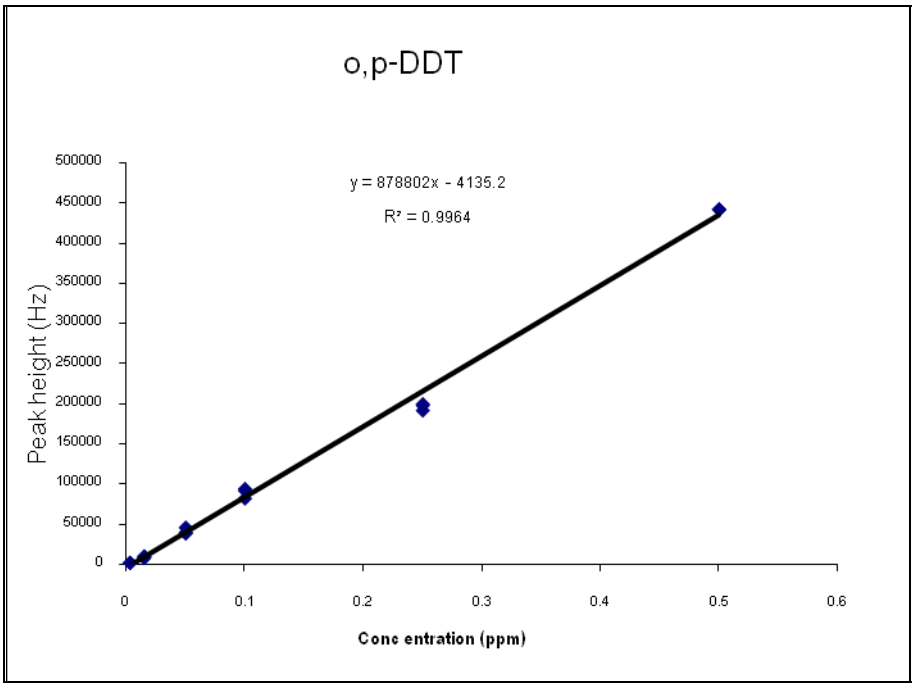

Figure A-16 The calibration curves of mixed standard of o,p-DDT by GC condition in Table 4.2

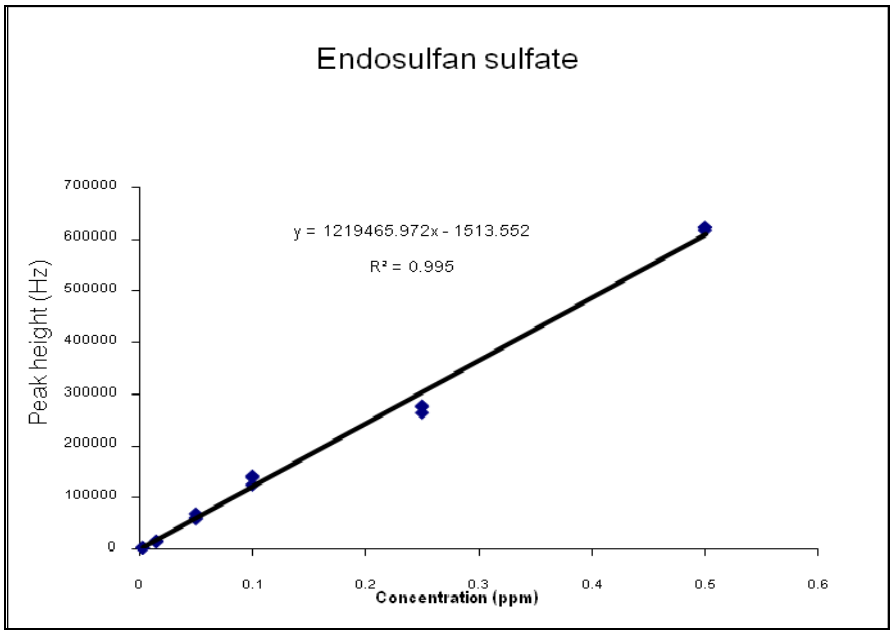

Figure A-17 The calibration curves of mixed standard of Endosulfan sulfate by GC condition in Table 4.2

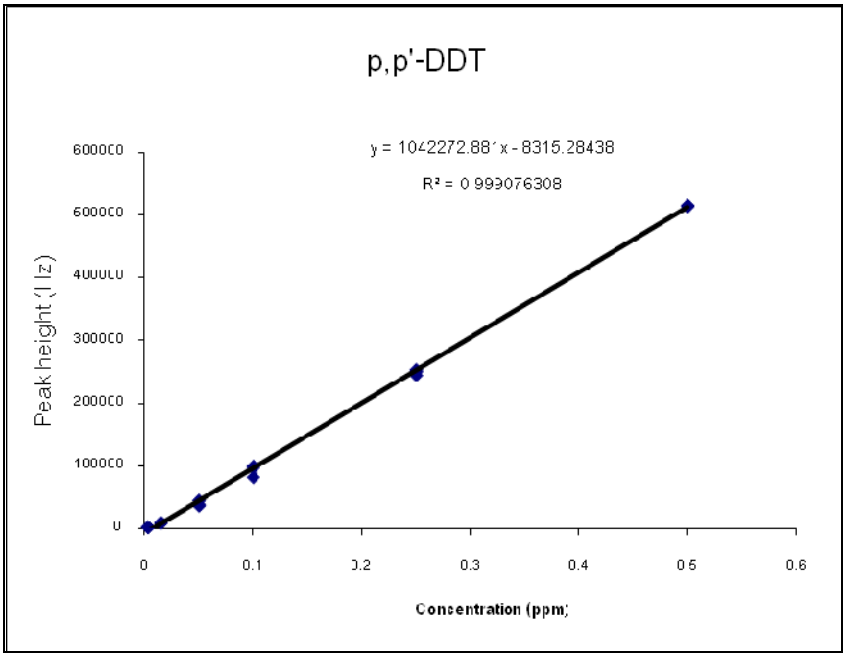

Figure A-18 The calibration curves of mixed standard of p,p'-DDT by GC condition in Table 4.2

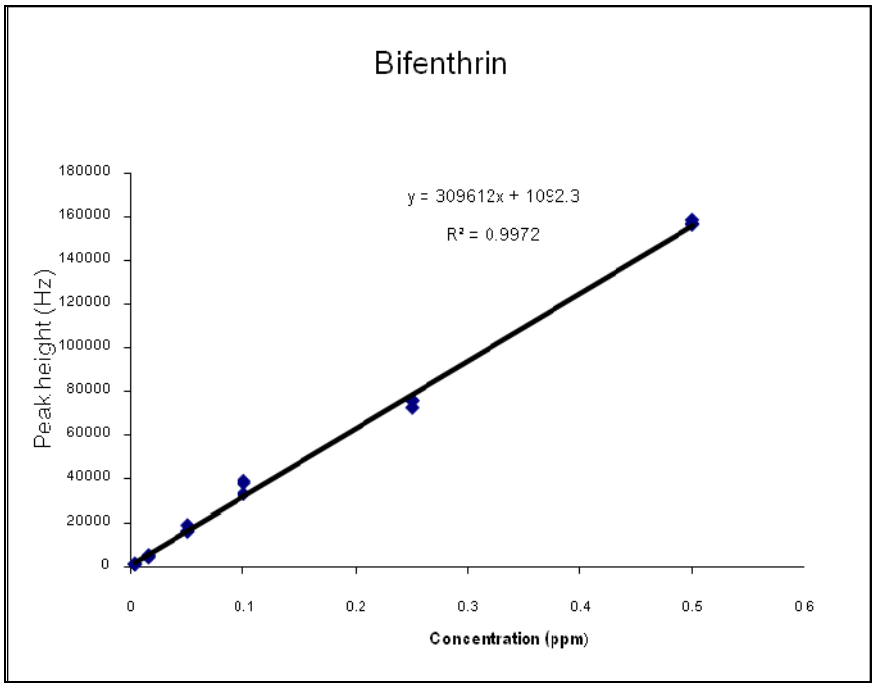

Figure A-19 The calibration curves of mixed standard of Bifenthrin by GC condition in Table 4.2

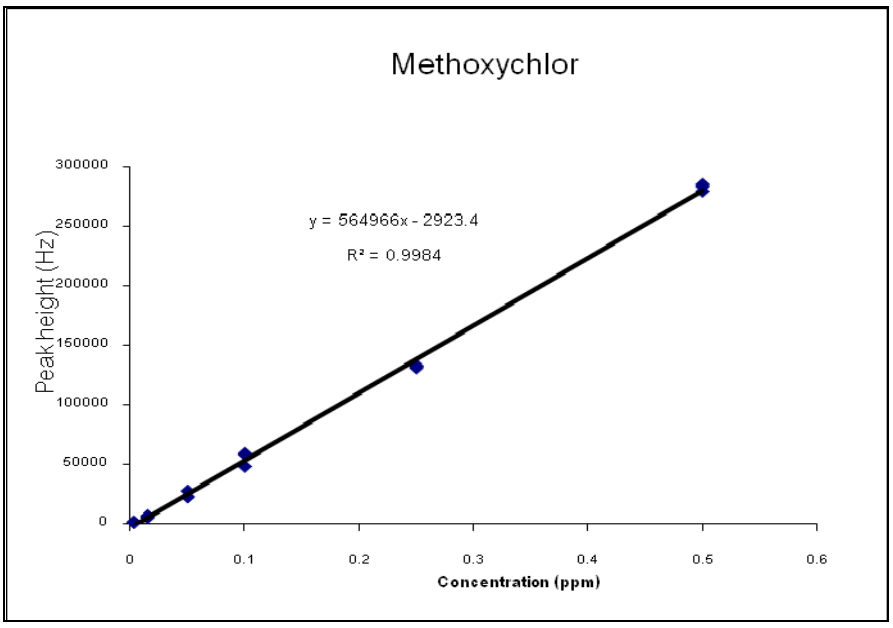

Figure A-20 The calibration curves of mixed standard of p,p'-DDT by GC condition in Table 4.2

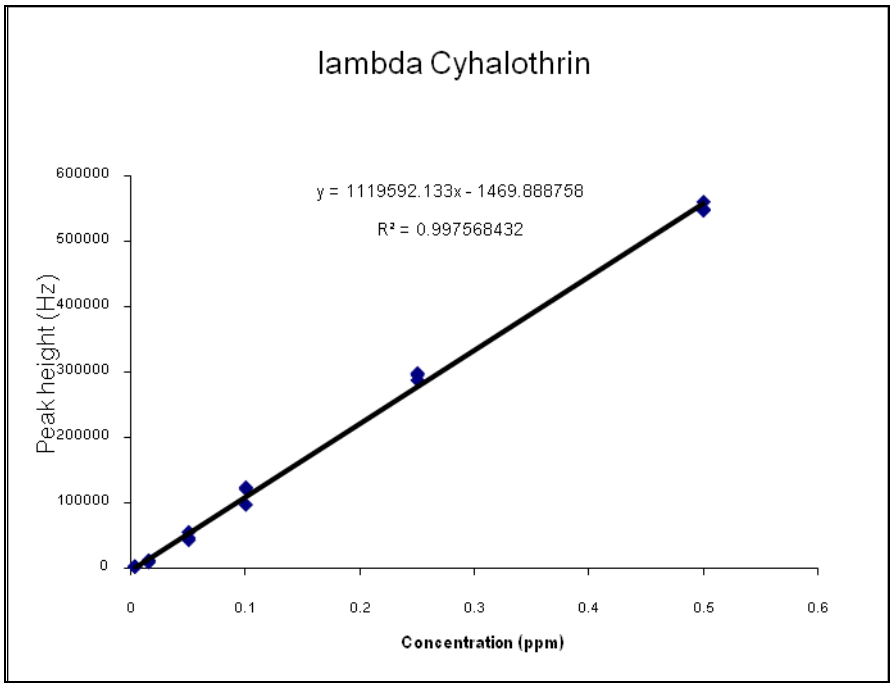

Figure A-21 The calibration curves of mixed standard of lambda Cyhalothrin by GC condition in Table 4.2

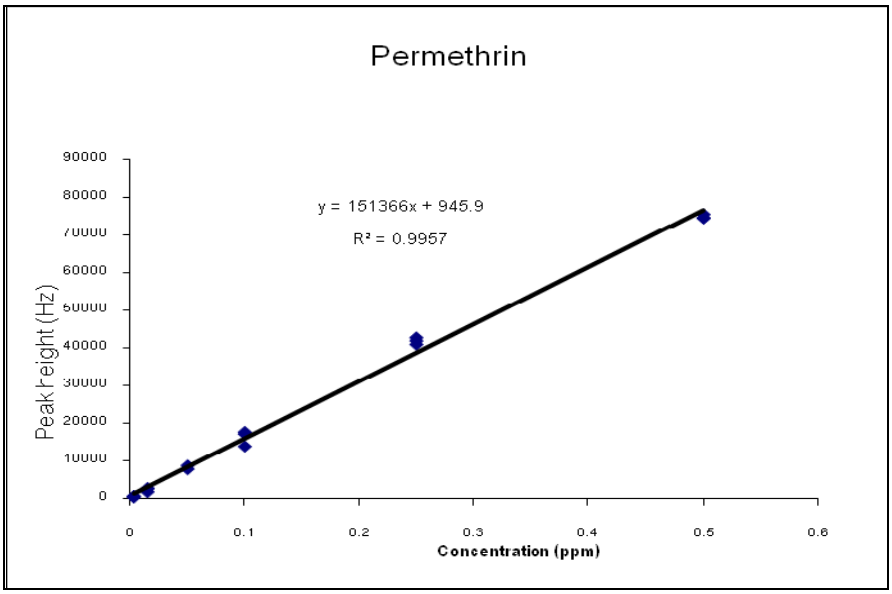

Figure A-22 The calibration curves of mixed standard of Permethrin GC condition in Table 4.2

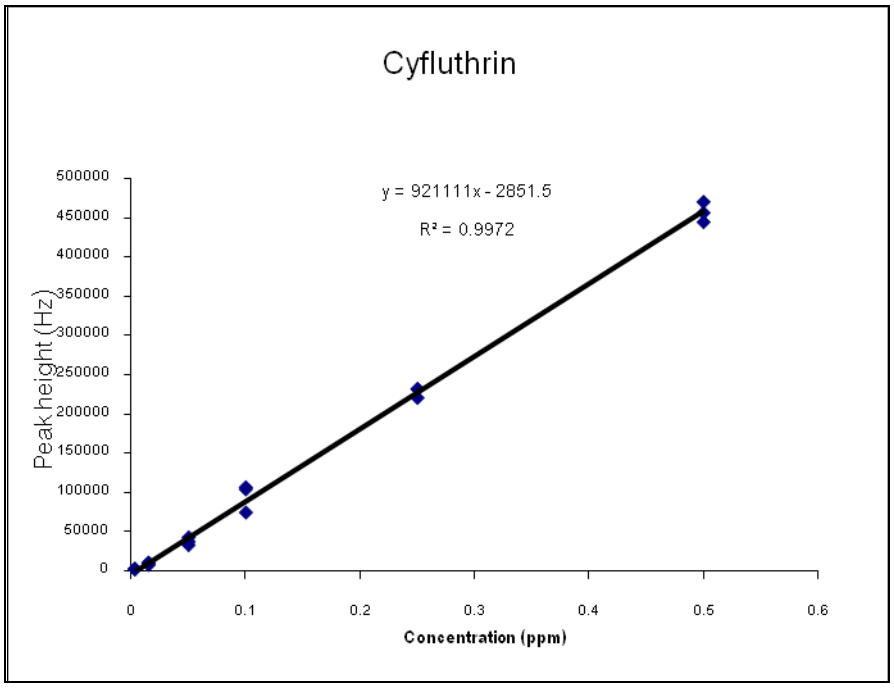

Figure A-23The calibration curves of mixed standard of Cyfluthrin GC condition in Table 4.2

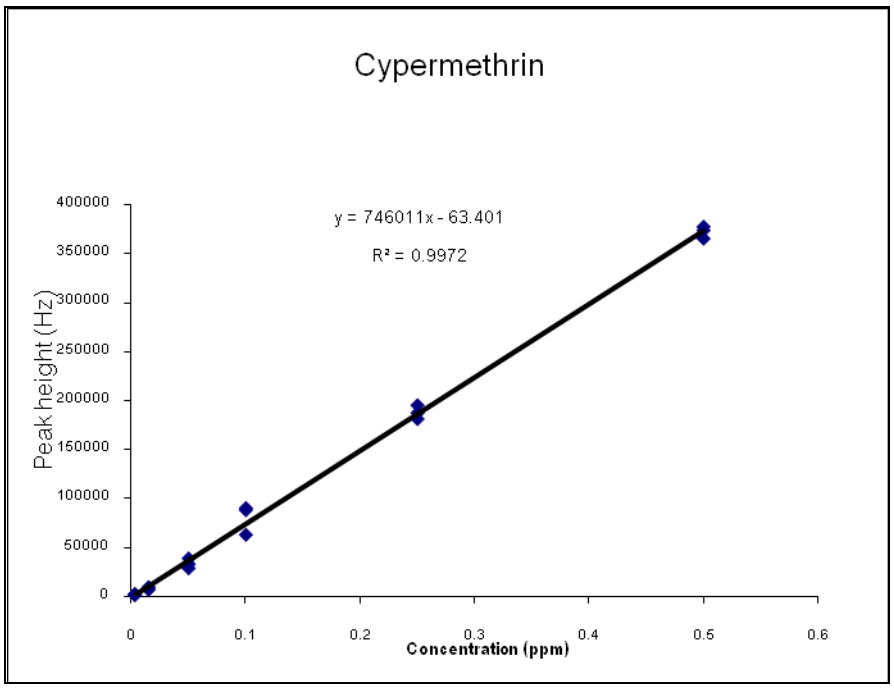

Figure A-24 The calibration curves of mixed standard of Cypermethrin GC condition in Table 4.2

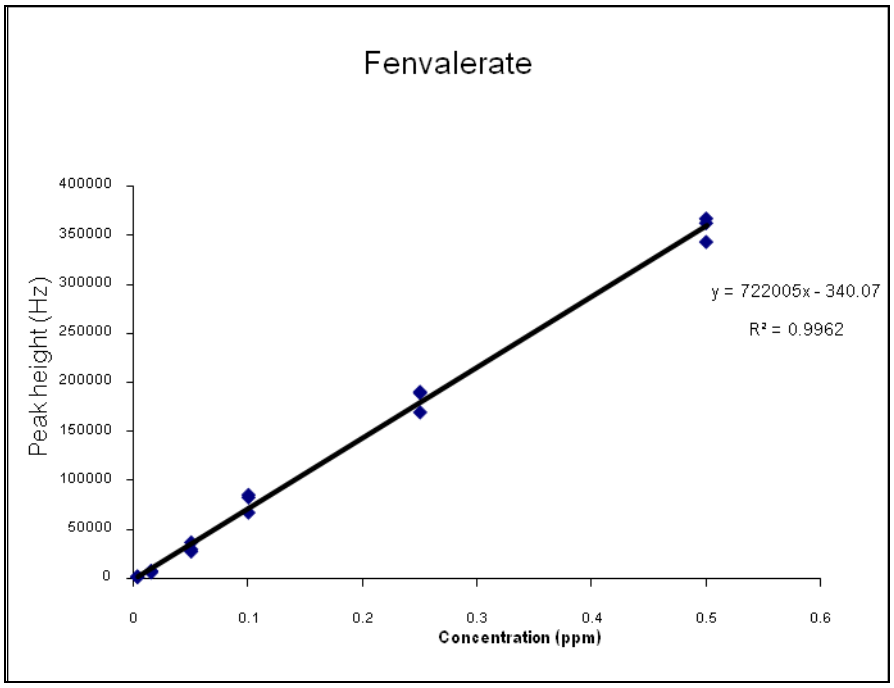

Figure A-25 The calibration curves of mixed standard of Fenvalerate GC condition in Table 4.2

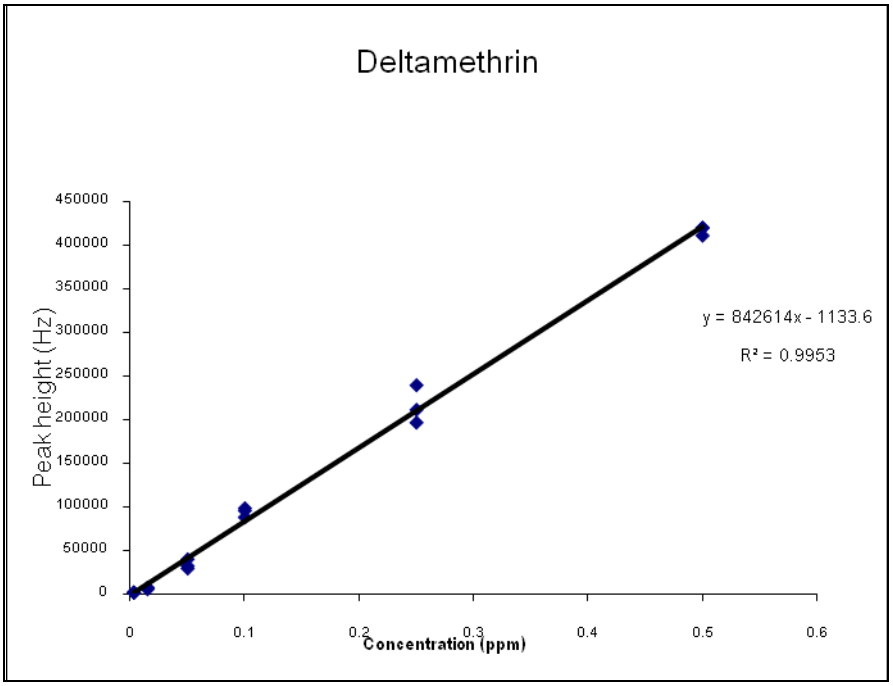

Figure A-26 The calibration curves of mixed standard of Deltamethrin GC condition in Table 4.2

VITA

Mrs. Supaporn Sayaroon was born on March 13, 1974, in Bangkok, Thailand. She had graduated with a Bachelor's degree of Science in Chemistry from Chandrakasam University. She had completed the program in May 1999 .