การเตรียมตัวอย่างเพื่อตรวจวัดสารกำจัดศัตรูพืชที่ตกค้างในมังคุดคุณภาพส่งออกโดยลิควิดโครมาโทกราฟี-แทนเดมแมสสเปกโทรเมทรี

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SAMPLE PREPARATION FOR THE DETERMINATION OF PESTICIDE RESIDUES IN EXPORT QUALITY MANGOSTEENS BY LC-MS/MS

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A Thesis Submitted in Partial Fulfillment of the Requirements
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วนิสา มีเจริญ : การเตรียมตัวอย่างเพื่อตรวจวัดสารกำจัดศัตรูพืชที่ตกค้างในมังคุดคุณภาพส่งออกโดยลิควิดโครมาโทกราฟี-แทนเดมแมสสเปกโทรเมทรี. (SAMPLE PREPARATION FOR THE DETERMINATION OF PESTICIDE RESIDUES IN EXPORT QUALITY MANGOSTEENS BY LC-MS/MS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร. ณัฐชนัญ ลีพิพัฒน์ไพบูลย์,109 หน้า.

การพัฒนาเทคนิค QuEChERS ในการตรวจวัดสารกำจัดศัตรูพืชที่ตกค้างในมังคุดและวิเคราะห์ด้วยเทคนิคลิควิดโครมาโทกราฟี-แทนเดมแมสสเปกโทรเมทรี ศึกษาตัวแปรที่มีผลต่อประสิทธิภาพของวิธีการเช่นตัวทำละลายอินทรีย์ที่ใช้ในการสกัด, บัฟเฟอร์ และของแข็งที่ใช้ในการดูดซับ วิธีนี้ได้การสกัดด้วยอะซิโตไนไตรล์, แมกนีเซียมซัลเฟตที่ปราศจากน้ำ, โซเดียมคลอไรด์และอะซิเตตบัฟเฟอร์ นำไปเขย่าและปั่นเหวี่ยง และกำจัดสิ่งรบกวนด้วยการกระจายวัฏภาคของแข็งในสารละลายโดยใช้ตัวดูดจับผสมระหว่างอะลูมินาที่เป็นกลางร่วมกับหมู่ฟังก์ชันเอมีนแบบปฐมภูมิและทุติยภูมิในอัตราส่วนหนึ่งต่อหนึ่ง (25 มิลลิกรัมต่อ 25 มิลลิกรัม) ศึกษาสภาวะที่เหมาะสมโดยประเมินจากค่าร้อยละของการคืนกลับ, การทดสอบซ้ำ, ขีดจำกัดต่ำสุดของการตรวจวัด, ขีดจำกัดของการตรวจวัดเชิงปริมาณของสารกำจัดศัตรูพืชกลุ่มคาร์บาเมต 12 ชนิดและกลุ่มออร์กาโนฟอสเฟตที่มีสภาพขั้วสูง 8 ชนิด จากการใช้สารมาตรฐานในเนื้อเมทริกซ์ได้ผลเป็นที่ยอมรับได้คือมีค่าร้อยละการคืนกลับของสารกลุ่มคาร์บาเมตในช่วง 66-116, ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ร้อยละ 9-26 สารกลุ่มออร์กาโนฟอสเฟตที่มีความเป็นขั้วสูงได้ร้อยละการคืนกลับ 75-97, ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ร้อยละ 5-20 ขีดจำกัดต่ำสุดของการตรวจวัด, ขีดจำกัดต่ำสุดของการวิเคราะห์เชิงปริมาณและช่วงความเข้มข้นที่ทำการศึกษาอยู่ที่ 0.005, 0.01 และ 0.01-0.10 มิลลิกรัมต่อกิโลกรัม เทคนิคลิควิดโครมาโทกราฟี-แทนเดมแมสสเปกโทรเมทรีใช้การตรวจวัดค่าการเฝ้าสังเกตปฏิกิริยาหลายชุด ทำการตรวจวัดค่าการแทรนซิชันโดยใช้ค่ามวล 2 ค่า ค่าหนึ่งใช้ในการวิเคราะห์เชิงปริมาณและอีกค่าหนึ่งใช้ในการวิเคราะห์เชิงคุณภาพ วิธีที่พัฒนาขึ้นประสบความสำเร็จในการตรวจสอบความใช้ได้ของวิธีสอดคล้องกับมาตรฐานกลุ่มสหภาพยุโรป EU(EC) 396/2005

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WANISA MEECHAROEN : SAMPLE PREPARATION FOR THE DETERMINATION OF PESTICIDE RESIDUES IN EXPORT QUALITY MANGOSTEENS BY LC-MS/MS. THESIS ADVISOR : ASST. PROF.NATCHANUN LEEPIPATPIBOON,

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QuEChERS technique has been developed for the determination of pesticide residues in mangosteen using liquid chromatography – tandem mass spectrometry (LC-MS/MS) for analysis. QuEChERS parameters affecting efficiency such as extraction solvent, buffering agent and dispersive sorbent were investigated. The extraction method used acetonitrile, anhydrous magnesium sulphate, sodium chloride and acetate buffer. After shaking and centrifugation, cleanup is done by dispersive solid-phase extraction (d-SPE) using primary secondary amine (PSA) and alumina N mixed sorbent in ratio of 1:1 (25:25 mg). The optimized analytical conditions were evaluated in terms of recoveries, repeatabilities, limits of detection, and limits of quantification for 12 carbamates and 8 polar organophosphates. The matrix-matched standards provided acceptable results for all studied pesticides with overall average recoveries between 66-116%, RSDs 9-26% for carbamate and recoveries 75-97%, RSDs 5-20% for polar organophosphate. Limits of detection, limits of quantification and working range of method for all compounds are 0.005 mg/kg, 0.01 mg/kg and 0.01-0.10 mg/Kg, respectively. LC-MS/MS was performed by multiple reaction monitoring (MRM) with two mass transitions for each pesticide; one for quantification and another one for confirmation. This developed method was successfully validated and compliance to the benchmark parameters of Directive EC 396/2005.

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LIST OF ABBREVIATIONS

%	Percentage
ºC	Degree celsius
APCI	Atmospheric pressure chemical ionization
AP-ESI	Atmospheric pressure-electrospray ionization
API	Atmospheric pressure ionization
CID	Collision induced dissociation
CI	Chemical ionization
CEN	Committee for Standardization
d-SPE	dispersive-Solid phase extraction
DC	Direct current
ECD	Electron capture detector
EI	Electron ionization
ESI	Electrospray ionization
FDA	Food and drug administration
FID	Flame ionization detector
FPD	Flame photometric detector
g	Gram
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GCB	Graphitized carbon black
IEC	Ion-exchange chromatography
ISO	International organization for standardization
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LODs	Limit of detections

LOQs	Limit of quantifications
LLE	Liquid-liquid extraction
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
m/z	Mass per charge ratio
mL	Milliter
$MgSO_4$	Magnesium sulfate
M+H	The protonated molecular ion
M-H	The negative ion
MRLs	Maximum residue limits
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MSD	Mass selective detector
MSPD	Matrix solid-phase dispersion
NaCl	Sodium chloride
NPD	Nitrogen phosphorus detector
OCs	Organochlorine
OPs	Organophosphate
ONs	Organonitrogen
PAM	Pesticide analysis manual
PSA	Primary secondary amine
PMT	Photomultiplier tube
R^2	Correlation coefficient
RF	Radio frequency
RSD	Relative standard deviation
S/N	Signal to noise ratio

SPE	Solid phase extraction
SRM	Single reaction monitoring
TOF	Time of flight
TIC	Total ion chromatogram
TCD	Thermal conductivity detector
UV	Ultraviolet
UPLC	Ultra performance liquid chromatography

CHAPTER I

INTRODUCTION

1.1. Problem Definition

Thailand is the world major producer and exporter of mangosteen that earned 1,879 million baths from the trade in 2009. [1] Due to the current health awareness, consumers ever more want their foods to be wholesome, nutritious, and most importantly, safe. The realities are well represented in the recent reinforcement of food safety standards in the European and Japanese markets**;** especially the European Union (EU). Since September 1, 2008 the European Union has implemented regulation EC 396/2005 which 27 member states are mandated to have one unified limit for any pesticide residues that their MRLs (Maximum residue Limit) were not listed as default MRLs at 0.01 mg/Kg. [2] The pesticide residues in mangosteen including pulp and peel are required to prove that the product is safe conforming to Good Agricultural Practice (GAP). However, the trace analysis of pesticide residues in a whole mangosteen is quite complicated due to its intensive colored and plenty of high molecular weight components in the thick peel such as polyphenols and wax, causing serious interferences in pesticide residues analysis. For traditional medicine, the dark purple pericarp (peel, rind, hull or ripe), 6–10 mm in thickness, was used as an antibacterial agent for curing diarrhea as well as treating skin wounds and disorders. Additionally, as a current research of antioxidants, mangosteen can be processed into many types of healthy food products such as jam and beverages.

Whole fruit of exported mangosteen including pulp and peels must be analyzed by homogenization and extraction of wide range of pesticide residues from mangosteen using multi residues method (MRM) and then analyzed by chromatographic technique such as gas chromatography (GC) or high performance liquid chromatography (HPLC). Therefore, additional sample preparation technique with powerful clean up procedure is strongly required to purify the extracts. Purification consists of removing the analyte from interferences matrix and then

concentrate the analyte in a small volume of solvent before further analysis with instrument to obtain the reliable analytical result.

The conventional MRM for wide range of pesticide residues, the sample preparation techniques, liquid-liquid extraction (LLE) and solid–phase extraction (SPE) are still used for clean up steps to remove interferences from matrices. However, LLE is considered as time consuming, multi-stage operation, requires large volume of toxic organic solvent and requires extra evaporation step for pre-concentration of analyte. Another technique, SPE replaces LLE and provides a method that is simpler and requires small volume of organic solvent, but very high cost of SPEs and still as time consuming and difficult for routine analysis at low level residues in mangosteen. Therefore, a simple, quick, low-cost, sensitive and selective method should be developed for pesticide residues determination in mangosteens.

1.2 Regulation for Pesticide Residues in Mangosteen

1.2.1 The European Regulation

The European parliament and the council established Annex I listing the food and feed products to which maximum levels for pesticide residues apply to regulation (EC) No.396/2005. Products of plant to which the MRLs apply were shown in table 1.1 and selected representative matrices vegetables and fruits for validation were shown in table 1.2

Table 1.1 Product of plant to which the MRLs apply [3]

Groups to which the MRLs apply	**Examples of individual products within the groups to which the MRLs apply**	**Examples of related varieties or other products included in the definition to which the MRLs applies**	**Parts of the products to which the MRLs apply**
1. Fruits fresh or frozen; Nuts			
(i) Citrus fruit	- Grapefruit - Orange - Lemons - Limes - Mandarins	-Shaddocks, pomelos, tangelo and other hybrids -Bergamot, bitter orange, chinotto and other hybrids -Citron, lemon -Clementine, tangerine and other hybrids	Whole product
(ii) Tree nuts (shelled or unshelled)	Almonds, cashew nuts, chestnuts, coconuts, macadamia, walnuts		Whole product after removal of shell (except chestnut)
(iii) Pome fruit	-Apple -Pears	-Crab apple -Oriental pear	Whole product after removal of stems

Table 1.1 Product of plant to which the MRLs apply [3] (continued)

Groups to which the MRLs apply	**Examples of individual products within the groups to which the MRLs apply**	**Examples of related varieties or other products included in the definition to which the MRLs applies**	**Parts of the products to which the MRLs apply**
1. Fruits fresh or frozen; Nuts			
(iv) Stone fruit	-Apricots -Cherries -Peaches -Plums	 -Sweet cherries, sour cherries -Nectarines and similar hybrids -Damson, greengage	Whole product after removal of stems
(v) Berries and small fruit (a) Table and wine grapes (b)Strawberries (c) Cane fruit (d) Other small fruit and berries	 -Wine grapes -Blackberries -Dewberries -Raspberries -Blueberries -Rose hips	 -Loganberries, boysenberries and cloudberries	Whole product after removal of caps/crowns and stems except in the case of currants: fruits with stems

Table 1.1 Product of plant to which the MRLs apply [3] (continued)

Groups to which the MRLs apply	**Examples of individual products within the groups to which the MRLs apply**	**Examples of related varieties or other products included in the definition to which the MRLs applies**	**Parts of the products to which the MRLs apply**
1. Fruits fresh or frozen; Nuts			
(vi) Miscellaneous fruit (a) Edible peel (b) Inedible peel, small (c) Inedible peel, large	 Dates, figs, table olives, kumquat Kiwi, lychee (litchi), passion fruit, star apple avocados, bananas, Mangoes, papaya, guava, pineapples, durian -Bread fruit	 -Jackfruit	Whole product after removal of stems or the crown (pine-apples)

Table 1.1 Product of plant to which the MRLs apply [3] (continued)

Groups to which the MRLs apply	**Examples of individual products within the groups to which the MRLs apply**	**Examples of related varieties or other products included in the definition to which the MRLs applies**	**Parts of the products to which the MRLs apply**
2. Vegetables fresh or frozen			
(i) Root and tuber vegetable	potato, sweet potato, carrots, radishes, beetroot		Whole product after removal of tops and adhering soil by rinsing or brushing
(ii) Bulb vegetables	Garlic, onions, shallots, spring onions		Whole product after removal of easily detachable skin and soil (when dry) or roots and soil (when fresh)
(iii) Fruiting vegetables	Tomato, pepper, aubergines, okra, cucumber, sweet corn, pumpkins, melons		Whole product after removal of stems (in case of sweet corn without husks)
(iv) Brassica vegetables	Broccoli, kale, Cauliflower, Chinese cabbage		Whole plant after removal of roots and decayed leaves
(v) Leaf vegetables and fresh herbs	Lettuce, spinach, celery leaves, parsley, basil		Whole product after removal of roots and decayed outer leaves and soil (if any)

Table 1.1 Product of plant to which the MRLs apply [3] (continued)

Groups to which the MRLs apply	**Examples of individual products within the groups to which the MRLs apply**	**Examples of related varieties or other products included in the definition to which the MRLs applies**	**Parts of the products to which the MRLs apply**
2. Vegetables fresh or frozen			
(vi) Legume vegetables (fresh)	-Beans (with pods) -Beans (without pods)	-yard long bean -cowpea	Whole product
(vii) Stem vegetables (fresh)	Asparagus, leek, bamboo shoots		Whole product after removal of decayed tissue, soil and roots
(viii) Fungi	Cultivated	Common mushroom, oyster mushroom	Whole product after removal of soil or growing medium
(ix) Sea weeds			Whole product after removal of decayed leaves

Table 1.2 Annex 1 selection of representative matrices vegetables and fruits for validation. [4]

Commodity groups	**Commodity categories**	**Typical representative commodities included in the category**
High water content	Pome fruit	Apples, pears
	Stone fruit	Apricots, cherries, peaches
	Bulb vegetables	Bulb onion
	Fruiting vegetables/cucurbits	Tomatoes, peppers, cucumber, melon
	Brassica vegetables	Cauliflower, Brussels sprout, cabbage, broccoli
	Leafy vegetables and fresh herbs	Lettuce, spinach, basil
	Stem and stalk vegetables	Leek, celery, asparagus
	Forage/ fodder crops	Fresh alfalfa, fodder vetch, fresh sugar beets
	Fresh legume vegetables	Fresh peas with pods, petit pois, mange tout, broad bean, runner bean, dwarf French bean
	Fresh of root and tuber vegetables	Sugar beet and fodder beet tops
	Fresh Fungi	Champignons, chanterelles
	Root and tuber vegetables or feed	Sugar beet and fodder beet roots, carrot, potato, sweet potato

Table 1.2 Annex 1 selection of representative matrices vegetables and fruits for validation. [4] (continued)

Commodity groups	**Commodity categories**	**Typical representative commodities included in the category**
High acid content and high water content	Citrus fruit	Lemons, mandarins, tangerines, oranges
	Small fruit and berries	Strawberry, blueberry, raspberry, grapes
	Other	Kiwi fruit, pineapple, rhubarb
High sugar and low water content	Dried fruit	Raisins, dried apricots, dried plums, fruit jams
"Difficult or unique commodities"*		Hops, Coffee, Tea, Spices

* Fully validated

From the above data, mangosteen has been classified as miscellaneous fruit. Whole mangosteen after removal of stems is needed in which the MRLs apply. For validation data, mangosteen has not been classified in commodity categories and representative commodities. It is a unique commodity so whole mangosteen needed for fully validation.

1.2.2 Mangosteen

Mangosteen (*Garcinia mangostana* L.), a tropical fruit originating in Southeast Asia, is also known as "the queen of fruits". The mangosteen as a fresh fruit is in great demand in its native range and is savored by all who find its subtle flavors a refreshing balance of sweet and sour. It should be pointed out that Asians consider many foods to be either 'cooling' such as mangosteen or 'heating' such as durian depending on whether they possess elements that reflect yin and yang.[5] This duality

is commonly used to help describe balance in many aspects of life. Xanthone is one of the most powerful antioxidant to be found in nature. Xanthones are found in the most quantities in mangosteen hull (or pericarp) and can help to stay healthy. Xanthones, the chemically beneficial molecules, are having specific leading properties. These health promoting Xanthones help the body in many ways such as stop pain, reduces swelling and inflammation, and help in the body's healing process. Several literatures reveal about the pharmacology activities as well as nutrient supplements. To illustrate the mangosteen rich in nutrients is shown in table 1.3

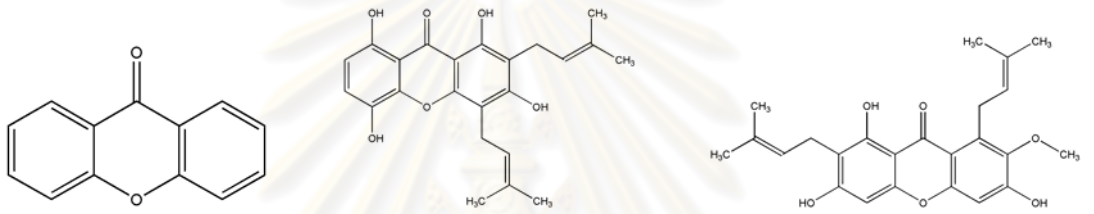
Table 1.3 Nutrition in 100 g of mangosteen peeled [6]

Component	**Quantity**	**Unit**
Water	80.9	gram
Calories	76	Calories
Protein	0.5	gram
Fat	0.1	gram
Carbohydrate	18.4	gram
Fibers	1.7	gram
Calcium	9	gram
Phosphorus	14	gram
Iron	0.5	gram
Copper	0.11	gram
Zinc	0.1	gram
Vitamin B1	0.09	milligram
Vitamin B2	0.06	milligram
Niacin	0.1	milligram
Vitamin C	2	milligram

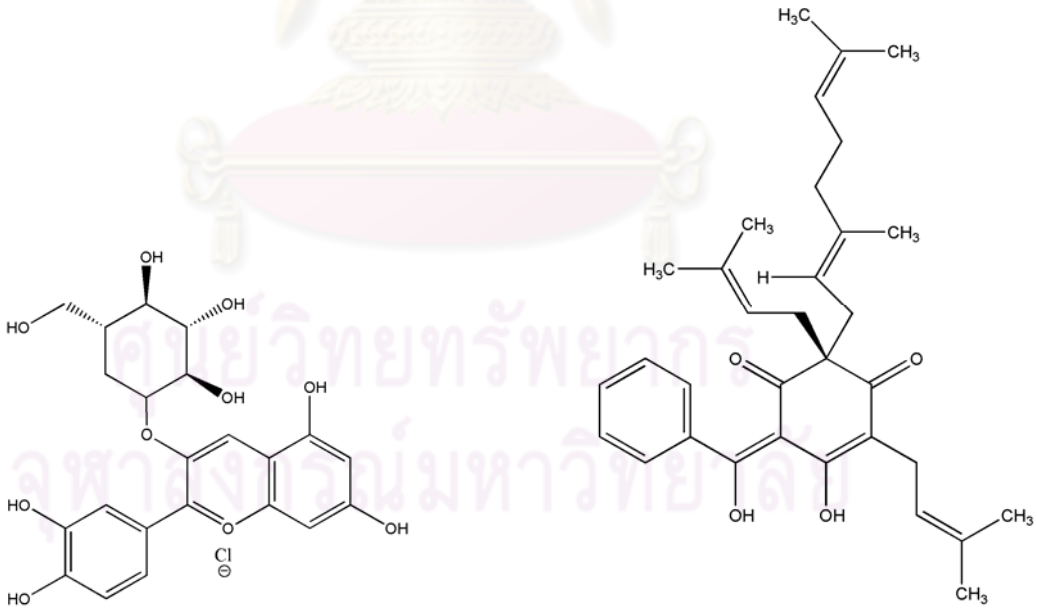
Major components in pericap of mangosteen are resin , taste astringent substances (Tannins group) 7-14% and substances such as Xanthones, Mangostin, Chrysanthemin, Gartanin and Kolanone. Chemical properties and structure were shown in table 1.4 and figure 1.1, respectively.

Table 1.4 Some chemical compositions and properties of mangosteen [7]

Compound	Molecular Formula	Formula Weight	Flash Point (^{O}C)	Boiling Point (^{O}C)
Xanthone	$C_{13}H_8O_2$	196.20	169.8	350.0
Gartanin	$C_{23}H_{24}O_6$	396.43	224.9	644.4
Mangostin	$C_{24}H_{26}O_6$	410.55	220.3	640.1
Chrysanthemin	$C_{21}H_{21}ClO_{11}$	484.84	-	-
Kolanone	$C_{33}H_{42}O_4$	502.68	319.5	581.5



Xanthone Gartanin Mangostin



Chrysanthemin Kolanone

Figure 1.1 Chemical structure of mangosteen matrices

1.2.3 Pesticide Residues

Pesticide is a substance or mixture of substances used to kill pests. A pesticide may be a chemical substance, biological agent such as virus or bacteria. It is used to prevent, destroy and repel pests. Several kinds of pests are destroyed such as insects, mice and other animals including weeds, fungi and microorganisms.

In history, humans had utilized pesticides to protect their crops and first known pesticide was elemental sulfur dusting used in Sumeria about 4500 year ago. In 1939, Paul Muller discovered that DDT and claimed to be a very effective insecticide.

Currently**,** more than 1100 official pesticide names have been recorded by the international organization for standardization (ISO) [8]. There are lists of pesticides classified in term of application or uses such as;

- Algicides or Algaecides for the control of algae
- Avicides for the control of birds
- Bactericides for the control of bacteria
- Miticides or Acaricides for the control of mites
- Molluscicides for the control of slugs
- Nematicides for the control of nematodes
- Rodenticides for the control of rodents
- Vilucides for the control of viruses
- Fungicides for the control of fungi
- Herbicides for the control of weeds
- Insecticides for the control of insects, such as;
    - Organophosphate
    - Organochlorine
    - Carbamate
    - Pyrethroid

Insecticides are mostly used to control insects in food plants and become more serious conditions in term of health certification for export. Currently Thai fruits and

vegetables for export have been controlled by official authority for monitoring 34 pesticide residues comprising organophosphates (23), organochlorines (1), pyrethroids (6), and carbamates (4) [9].

1.3 Literature Review

The multi-residue methods of pesticides are used most often for monitoring of food, risk assessment studies, and routine pesticide residues analysis.

In 1963, U.S Food and Drug Administration (FDA) by chemist P.A.Mills [10] had developed the first multiresidue method (MRM). The Mill method could analyze only nonpolar pesticides in nonfatty foods, especially, organochlorine insecticides (OCs), which was the main focus for analysis at that time. OCs were extracted from nonfatty foods with acetonitrile, then diluted with water, and partitioned into petroleum ether. The water was removed in a liquid-liquid extraction (LLE) step. Later, new methods were developed to extend the analytical polarity range to cover more polar pesticides such as organophosphate (OPs) and organonitrogen insecticides (ONs) in a single method. Sodium chloride (NaCl) was added to increasing the polarity range.

In 1973 Luke et al. in U.S Food and Drug Administration (FDA) [11] had developed the new MRM method for analysis of OCs, OPs and ONs . The Luke method used acetone for the initial extraction and partition with methylene chloride-petroleum ether to remove water. In this LLE step sodium chloride was added to saturate water phase, which forced more acetone into organic layer, thus increased its polarity and lead to higher recoveries of the polar analytes. In 1975 Steinwandter [12] had combined the extraction and partition steps into one step by saturating the extraction solvents with NaCl and simultaneously driving away the water by dichloromethane or hexane, dried with sodium sulfate (Na_2SO_4) and evaporated for instrument analysis. The sample was extracted with acetone added NaCl and dichloromethane, followed by blending at high speed. Water was removed from the sample extract, and organic phase was measured for volume of acetone and dichloromethane. This method was tested with different matrix samples e.g. fruits and vegetables are extracted directly whereas cereals, tea, coffee, dried fruits are presoaked with water before extract with solvent.

Luke method was replaced by Mills method in the FDA and became the official U.S. FDA Pesticide Analysis Manual (PAM) method [13], and later became AOAC Official Method 985.22 [14]. This MRM method is still widely used by pesticide residue monitoring laboratories worldwide.

Due to the environmental and public health concern, many new methods have been developed to reduce solvent usage especially, chlorinated solvents. Casanova [15] used Solid Phase Extraction (SPE) for isolation and cleans up replaced LLE in pesticide residues analysis method. Fernandez et al. [16] developed method determination of carbamate insecticides in fruits and vegetables by matrix solid-phase dispersion (MSPD) for clean up technique.

In 2003, Anastassiades and Lehotay et al. who worked for the U.S. Department of Agriculture (Original version) [17] had developed a simple, rapid, and inexpensive multiclass MRM that provided high quality results and minimized the number of analytical steps as well as required less glassware. This new method has been a sample preparation technique applied to pesticide multiresidue method, known as QuEChERS (quick, easy, effective, rugged and safe) method. It has been demonstrated to be a very effective sample clean-up procedure for simultaneous analysis of pesticides in a variety of fruit and vegetable matrices. This method was applied for Lettuce and strawberry based on unbuffered in extraction/partitioning step. The method had been collaboratively studied on a large number of commodity/pesticide combinations. This version added citrate buffering agents to induce liquid separation and stabilize acidic and basic labile pesticides. It became the European Committee for Standardization (CEN) standard method EN 15662:2007 [18] (EN version). The method scope is for the analysis of pesticide residues in foods of plant origin, such as fruits (including dried fruits), vegetables, cereals and processed products. In 2005, Lehotay et al. [19] had developed technique to improve results of problematic pesticides by added 1 % acetic acid in extraction solvents and acetate buffer in extraction/partitioning step. The method has been collaboratively studied for fortified pesticides in grapes, lettuces, and oranges. [20] The method is recognized by AOAC to become AOAC official method 2007.01 first action 2007 [21] (AOAC version).

Generally, the QuEChERS procedure employs acetonitrile in initial extraction and then partitions the extracts with additional salts, followed by clean-up with dispersive SPE technique (d-SPE). The final extracts are injected to gas chromatography and/or liquid chromatography coupled to mass spectrometry systems. This method becomes an analysis tool to fulfill the versatility of QuEChERS applications in case of rapid, simultaneous determination, sensitivity and selectivity.

The QuEChERS method has been well-known as challenging sample preparation technique for multiresidue method with wide range of pesticide residues in fruit and vegetable matrices. Based on original version, there were applications on. tomato/carrot/apple/cabbage [22], apple/strawberries/tomato/spinach (no clean up and direct inject to UPLC-TOF-MS) [23], EN version having citrate buffering agents was applied on grape/lemon/onion/tomato [24], lemon/ cucumber/orange/red grape [25], Banana [26], AOAC version having acetate buffering agents was applied on cucumber/peach/green pepper/plum/orange/lettuce [19], peach/orange/pineapple/ apple/multifruit [27], fruit-based baby food [28], Leeks (sulphur compounds matrix) [29]. Moreover, several studies have been developed to combine between unbuffering original version with acidic solvent as in AOAC version on cabbage/radish [30], Korean herb [31], tomato/pear/orange (original solvent and acetate buffer) [32].

In 2008, QuEChERS had been compared in term of matrix effect with other sample preparation methods (i.e. Luke method (AOAC 985.22), and matrix solid-phase dispersion (MSPD)) in different fruits and vegetables [33]. The observation indicated both QuEChERS and Luke method gave good and satisfactory result but QuEChERS could overcome Luke method by means of simplicity, high sample throughput, time and cost saving as well as high recovery yield while MSPD was influenced by matrix effect offering unacceptable low recoveries for some compounds. Matrix effect and recovery were both dependent on the matrix.

QuEChERS method is developed to multiclass, multi residue method for determination of pesticide residues in matrices containing high contents of water, sugar and low fat such as fruits and vegetables. Furthermore, several studies have been proved for the strong potential of QuEChERS in extraction and clean-up even trace analysis in complex food matrices such as sugarcane juice [34], honeybees [35],

milk [36-37], rice & grain [38], tobacco [39], for fat containing matrices such as cereal [40], olive & olive oil [41], soybean oil [42] including very complicate matrices as soil [43] and whole blood [44].

Multi residue method based on QuEChERS technique provides high throughput results, hence chromatography instruments are required for analysis both gas and liquid chromatography techniques. As the international trade and regulation established maximum residue limits for pesticide residues in fruits and vegetables at low level for food safety, hyphenated technique between chromatography with mass spectrometry is used to provide good selectivity, high sensitivity and confirmation. At first, QuEChERS method used GC-MS (Quadrupole) for analysis the 22 pesticide residues. In 2005 Lehotay et al. [19] developed method for 229 pesticide residues using gas and liquid chromatography equipped with mass spectrometric detector (GC-MS ion trap with PTV large volume injection and LC-MS/MS). GC and LC Quadrupole tandem mass spectrometric detector were used to analyze 80 pesticides by Paya and Anastassiades et. al. [25] For the lowest 111 MRLs in fruit based baby food the PTV-LP-GC-HR-TOF-MS was used by Cajka and Lehotay et al [28]. Presently, UPLC-TOF-MS is used to enable rapid and comprehensive analysis of 212 pesticides in food plants within 24 min. [23]. The method did not use d-SPE for clean up but the extracts were centrifuged at 11,000 RPM for 5 min. and supernatant was filtered through 0.2 μm filter.

In 2010, 3 versions of QuEChERS were compared to answer the question on which version is better for sample preparation for analysis of pesticide residues in fruits and vegetables. [45] For the 3 versions, 3 matrices were compared; original unbuffered method published in 2003, EN 15662:2007 used citrate buffering and AOAC 2007.01 used acetate buffering, both of which analysed by GC-MS and LC-MS/MS. The QuEChERS method is also flexible and rugged and matrix dependent. Only a few pesticides needed buffering to improve the results for pH-dependent pesticides.

1.4 Purpose of the Study

Since September 1, 2008 the European Union has implemented regulation EC 396/2005 which 27 member states are mandated to have one unified limit for any pesticide residues that their MRLs (Maximum Residue Limits) are not listed as default MRLs at 0.01 mg/Kg. The pesticide residues in mangosteen including pulp and peels are required to prove that the product is safe conforming to Good Agricultural practice (GAP). However, the trace analysis of pesticide residues in a whole mangosteen is quite complicated due to its intensive colored and plenty of high molecular weight components such as polyphenols and wax in the peel. Therefore, additional cleanup is demanded for obtaining the reliable analysis result.

As mentioned, the QuEChERS method offers a great opportunity to determine multiresidue of pesticide with various physico-chemical properties in a wide variety of samples. The method can reduce matrix interferences as well as its simplicity, high sample throughput, time and cost saving and high recovery yield. It can be modified to proper with analytes and matrices. The analytical technique could develop by varying organic solvent, buffer, salt, sorbent type. However, even though QuEChERS has been modified and utilized in many types of fruits to reduce effect from matrices but not for mangosteen.

In this study, the modified QuEChERS method was developed for multiresidue determination of pesticides in exported mangosteen regarding EU guideline by which the matrix effect from mangosteen composition in whole fruit could be reduced using dispersive-SPE technique combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS). The critical QuEChERS parameters were optimized to increase the effectiveness of removal interferences from mangosteen samples and pesticides residues were determined. The proposed method offered efficient extraction and clean up process for the extremely complex matrices with good selectivity and high sensitivity.

CHAPTER II

THEORY

Pesticide residues analysis in food and environment has been determined by various methods. Some laboratories still used methods developed long time ago when solvent usage was not a problem as well as time consuming, labor and technology was considered. When modern residue monitoring programs are expected to be initiated the new, more rapid, and effective analytical approaches are essential for laboratories to improve overall analytical quality and laboratory efficiency. The multi-residue methods of pesticides are needed for monitoring of food, risk assessment studies. Multi residues method based on QuEChERS technique provided high throughput results and required for analysis using gas and liquid chromatography techniques. As the international trade and regulation established maximum residue limits for pesticide residues in fruits and vegetables at low level for food safety, hyphenated technique between chromatography with mass spectrometry is used to provided good selectivity, high sensitivity the results and confirmation.

2.1 QuEChERS Technique [17, 21, 46, 47]

QuEChERS was a new technique for multiresidue analysis of pesticides in foods and agricultural samples. Steven J. Lehotay, a chemist at the Microbial Biophysics and Residue Chemistry Research Unit, Eastern Regional Research Center, Wyndmoor, Pennsylvania, and a visiting scientist, Michelangelo Anastassiades, from a government laboratory in Stuttgart, Germany, had developed the new extraction technique called QuEChERS method(pronounced "catchers") which stood for quick, easy, cheap, effective, rugged, and safe. The streamlined approach makes it easier and less expensive for analytical chemists to examine fruits and vegetables for pesticide residues. The technique used simple glassware, a minimal amount of organic solvent and various salt/buffer additives to partition analytes into an organic phase for clean up by dispersive solid-phase extraction (d-SPE). QuEChERS was developed between 2000 and 2002 and first reported in "QuEChERS Method Catches Pesticide Residues" in the July 2003 issue of Agricultural Research magazine. It was published in Journal of AOAC 2003 [17].The method has already been widely accepted by the

international community of pesticide residues analysis, it was appeared as the AOAC official method in 2007[21].

The method reduced many procedural steps thus, lessened the chance of mistaking. A single, easy-to-clean Teflon tube is the only item to be washed and reused, eliminating all the glassware used in conventional methods. Furthermore, less than 10 mL of solvent waste is generated but much less than the 75-450 mL generated by older methods.

The new key approach is the development of a rapid procedure, a dispersive solid-phase extraction. This technique quickly removed water and non-target compounds with magnesium sulfate and a primary-secondary amine sorbent. The method was primarily designed for low-fat commodities.

QuEChERS was 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 d-SPE. Since its inception, there have been several modifications of techniques depending on analytes, matrices, instrumentation and analyst preferences. 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 or acetate) to induce liquid separation and stabilize acidic and basic labile pesticides. 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 concentrated before solvent exchanging step depend on type of instrument. QuEChERS has 5 core step processes for sample preparation and additional 2 steps for quality control.

Step 1: Sample Comminution

The sample mass (10–15 g) used in the QuEChERS technique is reduced compared with more traditional extraction approaches, it is important to ensure that

the original sample, typically kilograms, is homogeneous. Thus, a powerful chopping device is recommended to homogenize the sample to maximize surface area for better extraction efficiencies. Such a homogenization procedure will ensure that the 10–15 g subsample is representative of the original. Fruit and vegetable samples contain about 80–95% water, therefore, the following steps will emphasize phase separation between this water and an organic solvent so that the pesticides of interest will be in organic phase.

Step 2: Extraction–Partitioning

The other nonhalogenated solvents such as acetone and ethyl acetate may be used in the extraction, acetonitrile is the recommended solvent for QuEChERS because after addition of salts, it will separate more easily from water than acetone. Ethyl acetate has an advantage of partial miscibility with water but it co-extracts lipids and waxes resulting lower recoveries for acid–base pesticides and provides less clean up in d-SPE. Acetonitrile extracts contain less lipophilic materials, compared with acetone. The use of acetonitrile allows better removal of residual water with magnesium sulphate. It is compatible with HPLC mobile phases, and is less volatile than the other common organic solvents, thus, making evaporative concentration steps more time consuming.

Step 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. The concentration of salt could influence the percentage of water in the organic phase and could adjust its "polarity". In QuEChERS, acetonitrile alone was sufficient to perform good extraction efficiency. Anastassiades and colleagues 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 sulphate as a drying salt to reduce the water phase helped to improve recoveries by promoting partitioning of the pesticides into organic layer. The supplemental use of sodium chloride helps to control the polarity of the extraction solvents and thus influences the degree of matrix clean up of the

QuEChERS method but too much salt will reduce the organic layer's ability to partition polar pesticides.

In some instances, the pH of the extraction must be controlled due to most 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. Of course, the pH at which the extraction is performed can also influence the coextraction of matrix compounds and pesticide stability.

Step 4: Dispersive - Solid Phase Extraction (d- SPE)

In general, SPE clean up used plastic cartridges containing various amount 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 clean up process. The sorbent is then separated by centrifugation and an aliquot of the supernatant is subjected to further analysis. The sorbent is chosen to retain matrix components and not the analytes of interest. In some instances, 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, 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.

Step 5: Instrumental Analysis

The sample aliquot from Step 4 can be injected directly into a HPLC or GC system without further work-up. 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 is needed.

Additional Step 1: Internal Standard Addition

To minimize error generating 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 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, who recommended the use of more than one internal standard as quality control measures to enable recognition of errors as a result of mispipetting or discrimination during partitioning or clean up. In most instances, the internal standard is employed at an early stage of the analytical procedure. However, in cases of samples with high fat content, the 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).

Additional Step 2: Addition of 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, nonvolatile compounds from previous injection, on the inlet liner or on the precolumn (guard column). In this instance, 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 flowsteam. Thorough studies were devoted to selecting the appropriate analyte protectants, 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 were also reduced dramatically with the help of analyte protectants.

2.2 Clean up Technique

2.2.1 Solid Phase Extraction (SPE) [48]

Solid phase extraction (SPE) is a method of sample preparation that concentration and purified analytes from solution by sorption onto a disposable solid phase cartridge, followed by elution of the analyte with an appropriate solvent for instrumental analysis. The mechanisms of retention include reversed phase, normal phase, and ion exchange. Traditionally, sample preparation consisted of sample dissolution, purification, and extraction that was carried out with liquid-liquid extraction including the use of large volumes of organic solvent, cumbersome glassware, and cost. Furthermore, liquid-liquid extraction often creates emulsion with aqueous samples that are difficult to extract, and liquid-liquid extraction is not easily automated. These difficulties are overcome by solid phase extraction. The solid phase extraction is an analogous term to liquid-liquid extraction, and in fact, solid phase extraction might also be called liquid-solid extraction.

2.2.2 Matrix Solid Phase Dispersive (MSPD) [49]

Matrix solid-phase dispersion is a sample preparation technique widely applied to solid, semisolid or viscous samples, including animal tissues and foods with a high lipid content. The process consists of blending the matrix onto a solid support, allowing the matrix cell disruption and the subsequent extraction of target analytes by means of suitable elution solvent.

2.2.3 Dispersive - Solid Phase Extraction (d-SPE)

Dispersive-SPE (d-SPE), often referred to as the “QuEChERS” (Quick, Easy, Cheap, Effective, Rugged, and Safe) method is an emerging sample preparation technique that is becoming increasingly popular in the area of multi-residue pesticide analysis in food and agricultural products. Dispersive solid-phase extraction is similar in some respects to matrix solid-phase dispersion but in this instance, 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 before an aliquot of the sample cleaning up.

. In dSPE, food/agricultural samples are first extracted with an aqueous miscible solvent in the presence of high amounts of salts and/or buffering agents to induce liquid phase separation and stabilize acid and base labile pesticides. Upon shaking and centrifugation, an aliquot of the organic phase is taken to further clean up using SPE. Unlike traditional methods using SPE tubes, in dispersive SPE, clean up is done by mixing bulk amounts of SPE with the extract. After sample clean up, the mixture is centrifuged and the resulting supernatant can either be analyzed directly or can have further treatment before analysis. Typically, dispersive SPE replaces SPE and LLE as a pesticide residues sample preparation tool and provides a method that is simple and safe to use. The benefits of dispersive SPE are having high recoveries of analytes, purified extract, reduction of the volume of organic solvent used and no use of vacuum manifold and glasswares.

Compared with SPE, dispersive solid-phase extraction takes less time and uses less labour and lower volume of solvent. In addition, no concern over channeling, analyte or matrix breakthrough, or preconditioning of SPE cartridges. Magnesium sulphate a drying agent is sometimes added to the top of an SPE cartridge with the SPE sorbent to remove much of the excess water and improve analyte partitioning to provide better clean up.

2.3 SPE Sorbent

Solid state extraction is an extremely efficient method for isolating and concentrating solutes from relatively large volumes of liquid. Materials extracted in this way can be used for subsequent chromatographic separation. The apparatus consists of a simple tube, usually packed with an appropriate bonded phase. The choice of sorbent is shown in table 2.1

Table 2.1 Type of sorbents, description and application [50]

Sorbent/Surface	Description	Properties
-Reversed phase Oasis® HLB (N-Vinylpyrolidone-DVB copolymer)	Waters patented, strongly hydrophobic, yet water-wettable, polymer with unique hydrophilic-lipophilic balance retains high retention and capacity even if it runs dry after conditioning, enabling high-throughput applications. Highly cross-linked polymer is stable in organic solvents.	Particle sizes: 30 and 60 µm Pore size: 80 Å Surface area: 830 m^2/g pH range: 0-14
C_{18} $-Si(CH_3)_2C_{18}H_{37}$	Hydrophobic silica-based bonded phase used to adsorb analytes from aqueous solutions. Most widely referenced SPE product for applications such as: drugs and metabolites in biofluids; desalting and isolation of peptides, oligonucleotides; trace organics in environmental water samples; synthetic radiolabeled compound isolation	Particle sizes: 55-105 µm Pore size: 125 Å Surface area: 325 m^2/g Carbon load: 12% pH range: 2-8
-Reversed or nornal phase NH_2 (Aminoprropyl**)** $(-Si(CH_2)_3NH_2)$	Moderately polar, silica-based-bonded phase with weakly basic surface used as a polar sorbent, like silica, with different selectivity for acidic/basic analytes, or as a weak anion exchanger in aqueous medium below pH8.	Particle sizes: 55-105 µm Pore size: 125 Å Surface area: 325 m^2/g Carbon load: 3.5% pH range: 2-8

Table 2.1 Type of sorbents, description and application [50] (continued)

Sorbent/Surface	Description	Properties
-Normal phase Silica SiO_2	Polar sorbent binds analytes in non-aqueous solvents by H-bonding or dipole interaction; also used as an intermediate-strength cation exchanger in aqueous media, a support for liquid-liquid partition separations., or a solid-phase reagent when suitable coated [*e.g.*, see DNPH below].	Particle sizes: 55-105 µm Pore size: 125 Å Surface area: 325 m^2/g Activity: High [≤3.2% water]
Florisil® MgO SiO$_2$	Polar, highly active, weakly basic sorbent [a co-precipitate of magnesia and silicar] for the adsorption of low to moderately polar species from nonaqueous solutions.	Particle sizes: 50-200 µm Pore size: 60 Å Activity: High [≤2.5% water] pH of 10% aqueous slurry: 8.5
Alumina (A,N,B) Al_2O_3	Highly surface-active, polar, acidic [A] neutral [N], and basic [B] sorbents. Unlike silica, alumina exhibits specific π-electron interactions with aromatic hydrocarbons. Acidic and basic alumina are also low-capaciy ion exchangers in aqueous media, unaffected by high-energy radioactivity [unlike polymers].	Particle sizes: 50-300 µm Pore size: 125 Å pH of 10% aqueous slurry: A:4 N:7.5 B:10

Table 2.1 Type of sorbents, description and application [50] (continued)

Sorbent/Surface	Description	Properties
-Ion exchanged mode Oasis® MCX (N-Vinylpyrrolidone-DVB Copolymer) $-SO_3H$	Waters patented mixed-mode, reversed-phase/strong cation-exchange, water-wettable polymer, highly selective for bases, used to isolate basic, neutral and acidic compounds with high recoveries. Highly cross-linked polymer is stable in organic solvents.	Particle sizes: 30 and 60 µm Pore size: 80 Å Surface area: 830 m^2/g pH range: 0-14 [pK_a:<1] IEX capacity: 1 meq/g
Accell™ Plus CM (Acrylic acid/acrylamide copolymer on diol-silica) $-COO^- Na^+$	Silica-based, hydrophilic, weak cation-exchanger with large pore size used to extract cationic analytes in aqueous and non-aqueous solutions.	Particle sizes: 37 and 55 µm Pore size: 300 Å pH range: 2-9
Oasis® MAX (N-Vinylpyrolidone-DVB copolymer) $-CH_2N(CH_3)_2C_4H_9^+$	Waters patented mixed-mode, reversed-phase/strong anion-exchange, water-wettable polymer, highly selective for acids, used to isolate basic, neutral and basic compounds with high recoveries. Highly cross-linked polymer is stable in organic solvents.	Particle sizes: 30 and 60 µm Pore size: 80 Å Surface area: 830 m^2/g pH range: 0-14 [pK_a:<18]

Table 2.1 Type of sorbents, description and application (continued)

Sorbent/Surface	Description	Properties
PSA (Primary Secondary Amine) | — Si — $CH_2CH_2NHCH_2CH_2NH_2$ |	PSA is a week anion exchanger. It can be useful for the extraction of the analytes with a permanent negative charge such as strong acids. PSA used for analytes containing sulphate or phosphate groups.	Particle sizes: 40-70 µm Pore size: 40 Å pKa : 10.1-10.9
SAX (Silica based trimethylaminopropyl) | — Si — $CH_2CH_2CH_2NCH_3CH_3CH_3$ |	SAX is a strong anion exchanger is manufactured with chloride as the counter ion. It maintains a permanent positive change over the whole pH range	Particle sizes: 40-70 µm Pore size: 40 Å pH range: 1-14
GCB (Graphitized carbon black)	GCB has a strong affinity towards planar molecules and can isolate/remove pigments (such as chlorophyll and carotinoids) and sterols commonly present in foods and natural products	

2.4 Chromatography [51]

Chromatography is a separation process, distributing the components of a mixture between two phases, a stationary phase and a mobile phase. Those components held preferentially in the stationary phase are retained longer in the system than those that are distributed selectively in the mobile phase. As a consequence, solutes are eluted from the system as local concentrations in the mobile phase in the order of their increasing distribution coefficients with respect to the stationary phase. In practice, the distribution system can take the form of a column such as a tube packed with particulate matter on which the stationary phase is bonded or coated. The mobile phase (which may be a gas or a liquid) passed under pressure through the column to elute the sample. The column form may also be a long, small-diameter open tube that has the stationary phase coated or bonded to the internal surface. The sample is injected into the mobile phase stream before the front of the columns. The column is designed to allow two processes to take place to produce the separation. Firstly, as a result of different forces between each molecular type and the stationary phase, each solute is retained to a different extent and, thus, the more weakly held will elute first and the more strongly held elute last.

Chromatography is probably the most powerful and versatile technique available to the modern analyst. In a single step process it can separate a mixture into its individual component and simultaneously provided a qualitative and quantitative estimate of each component. Moreover, the analysis can be carried out, at one extreme, on a very costly and complex instrument, and at the other, on a simple, inexpensive thin layer plate.

2.4.1 Gas Chromatography

The modern gas chromatograph is described and included gas supplies, pressure controllers, flow controllers and flow programmers, together with injection devices for both packed and capillary columns. Sample is volatized at high temperature (temperature higher than boiling point of analyte) at the injector port then flow to separate in column (in column oven) by carrier gas. Analyte can been separated with oven temperature program and detected by detector. The GC diagram is shown in figure 2.1. Selection of the GC detector depended on chemical property.

The design and function of the common GC detectors such as the flame ionization detector, the nitrogen phosphorus detector and the electron capture, etc.

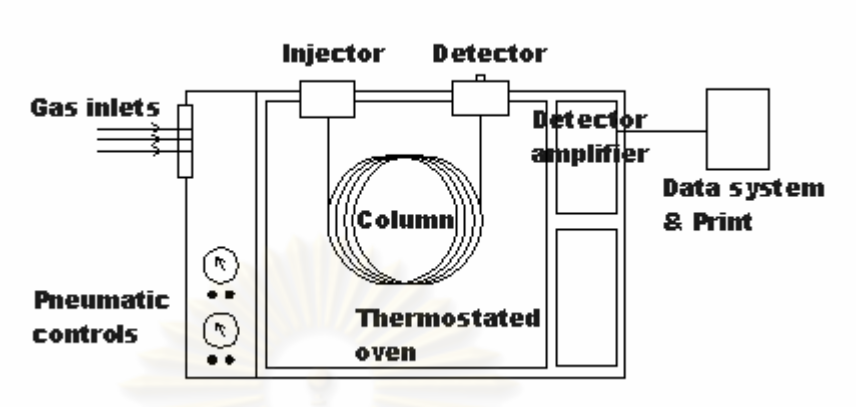


Figure 2.1 Gas Chromatography (GC) diagram [52]

2.4.1.1 Gas Chromatography (GC) Detectors

After the components of a mixture are separated using gas chromatography, they must be detected as they exit the GC column. The links listed below provide the details of some general GC detectors. The thermal-conductivity (TCD) and flame-ionization (FID) detectors are the two most common detectors on commercial gas chromatographs. The requirements of a GC detector depended on the separation application. As capillary column based gas chromatography takes its place as the major, highest resolution separation technique available for volatile, thermally stable compounds, the requirements for the sensitive and selective detection of these compounds increases. Thus, specific detector was used to differentiate between the sample components using the GC detector as a means of compounds discriminating is more and more common. In addition, each detector has its own characteristics (selectivity, sensitivity and linear range) such as electron-capture detector (ECD) specific for halogens compound and flame-photometric detector (FPD) specific for phosphorus and sulfur compounds.

2.4.1.1.1 Flame Ionization Detector (FID)

An FID consists of a hydrogen/air flame and a collector plate. The effluent from the GC column passes through the flame, which breaks down organic molecules and produces ions. The ions are collected on a biased electrode and produce an electrical signal. The FID is general detector, large dynamic range, its only disadvantage is that it destroys the sample.

2.4.1.1.2 Electron Capture Detector (ECD)

The ECD is sensitive detector but has a limited dynamic range and finds its greatest application in analysis organic molecules that contain electronegative functional groups, such as halogens. The ECD uses a radioactive^{63}Ni source to produce Beta emitter (electrons) to ionize some of the carrier gas and produce a small standing current between a biased pair of electrodes. When organic molecules that contain electronegative functional groups, such as halogens pass by the detector, they capture some of the electrons and reduce the current measured between the electrodes. The mobility of the captured electrons are much reduced compared with the free electrons and, furthermore, are more likely to be neutralized by collision with any positive ions that are also generated. As a consequence, the electrode current falls dramatically. The ECD is greatest application in analysis of halogenated compounds.

2.4.1.1.3 Flame Photometric Detector (FPD)

The Flame photometric detector is to achieve selective and/or highly sensitive detection of sulfur or phosphorus containing compounds. The carrier gas and burnt hydrogen from the chromatography column at a small jet similar to the flame ionization detector. The light from the flame was focused on a photoelectric cell, the output from which was electronically modified and fed to a recorder. Any aromatic burning in the flame rendered it strongly luminous and, thus, the aromatic compounds could be selectively identified. 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.

2.4.1.1.4 Nitrogen Phosphorus Detector (NPD)

The nitrogen phosphorus detector (NPD) is a very sensitive, specific detector the design of which is based on the FID. Physically the sensor appears to be very similar to the FID but operates on an entirely different principle. NPD differs from the FID by a rubidium or cesium chloride bead contained inside a heater coil situated close to the hydrogen jet. The bead is situated above a jet and heated by a coil over the nitrogen carrier gas mixed with hydrogen passes. If the detector responds to both nitrogen and phosphorus, the hydrogen flow should be minimal so that the gas does not ignite at the jet. If the detector responds to phosphorus, only a large flow of hydrogen can be used and the mixture burnt at the jet. The heated alkali bead emits electrons by thermionic emission which is collected at the anode and provides background current through the electrode system. When a solute that contains nitrogen or phosphorus is eluted, the partially combusted nitrogen and phosphorus materials are adsorbed on the surface of the bead.

2.4.1.1.5 Mass Spectrometry Detector (MS)

Mass spectrometry detector is GC combined with MS. The Gas Chromatography/Mass Spectrometry (GC-MS) instrument separates chemical mixtures (the GC technique) and identifies the components at a molecular level (the MS technique). GC/MS is a technique that can be used to separate volatile organic compounds and pesticides. The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. A “library” of known mass spectra is stored on a computer. Mass spectrometry is considered the only definitive analytical detector.

2.4.2 Liquid Chromatography [53, 54, 55]

The HPLC, coined by the late Prof. Csaba Horváth for his 1970 Pittcon paper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi (35 bar). This was called high pressure liquid chromatography, or HPLC. These new HPLC instruments could develop up to 6,000 psi (400 bar) of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time (smaller particles, even higher pressure), the HPLC remained the same, but the name was changed to high performance liquid chromatography.

High performance liquid chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, qualitative and quantitative the compounds that are present in any sample that can be dissolved in a liquid. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, cosmetics, environmental matrices, forensic samples, and industrial chemicals.

The basic liquid chromatograph is described; including mobile phases supply systems, high pressure and low pressure gradient programmers, pump, valves (sample and switching), column compartment and detector. A reservoir holds the solvent (called the mobile phase, because it moves). A high-pressure pump (solvent delivery system) is used to generate and meter a specified flow rate of mobile phase. An injector (auto sampler) is able to inject the sample into the continuously flowing mobile phase stream that carries the sample into the HPLC column to separate and detect signal by detector. HPLC system is shown in figure 2.2. The column contains the chromatographic packing material needed for the separation. The column (stationary phases) used in LC are considered and the description of the different types of bonded phase. The properties of the mobile phase are outlined and their interaction with silica gel and the different types of bonded stationary phases. A detector is needed to see the separated compound bands as they elute from the HPLC column. HPLC detectors are described, including the UV detector (fixed and variable wavelength), the fluorescence detector and the refractive index detector. The mobile phase exits from detector will become waste.

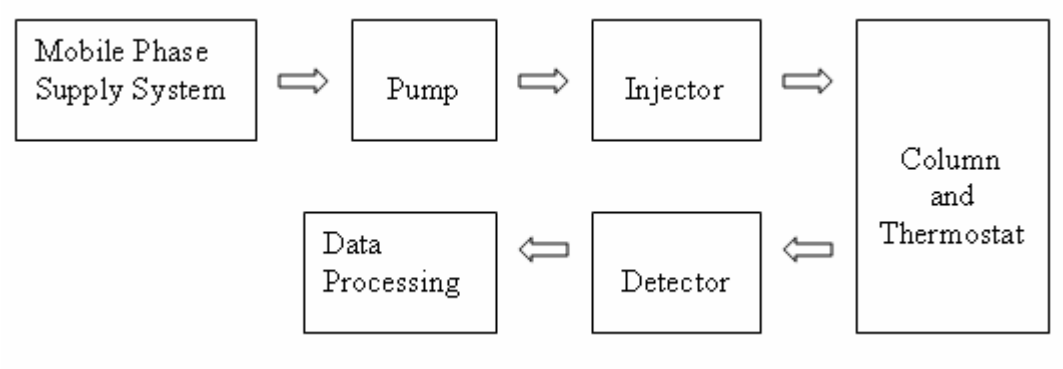


Figure 2.2 High Performance Liquid Chromatography (HPLC) System

2.4.2.1 HPLC Separation Modes

Chromatographic separations based on polarity depend upon the stronger attraction between likes and the weaker attraction between opposites. “Like attracts like” in polarity-based chromatography. The chromatographer will choose the best combination of a mobile phase and particle stationary phase with appropriately opposite polarities. Then, as the sample analytes move through the column, the rule like attracts like will determine which analytes slow down and which proceed at a faster speed.

2.4.2.1.1 Normal Phase

Normal phase is a classical mode of chromatography separation. The stationary phase is polar and retains the polar compound most strongly. The relatively non-polar is won in the retention competition by the mobile phase, a non-polar solvent, and elutes quickly. Since the non polar is most like the mobile phase (both are non-polar), it moves faster. It is typical for normal-phase chromatography on silica that the mobile phase is 100% organic; no water is used.

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2.4.2.1.2 Reversed Phase

The silica column is modified to make it non polar by attaching long hydrocarbon chains to its surface typically with either 8 or 18 carbon atoms in them. A polar solvent is used for example, a mixture of water or buffer and polar organic solvent, such as acetonitrile or methanol. In this case, there will be a strong attraction between the polar solvent and polar molecules in the mixture being passed through the column. Polar molecules in the mixture will therefore spend most of their time moving with the solvent. Non-polar compounds in the mixture will tend to form attractions with the hydrocarbon groups because of van der wals dispersion forces. They therefore spend less time in solution in the solvent and this will slow them down on their way through the column. That means it is the polar molecules that will travel through the column more quickly. Reversed phase HPLC is the most commonly used form of HPLC.

2.4.2.1.3 Ion Exchange Chromatography (IEC)

In ion exchange chromatography, the separation is based on electrical charge. Stationary phases for ion-exchange separations are characterized by the nature and strength of the acidic or basic functions on their surfaces and the types of ions that they attract and retain. Cation exchange is used to retain and separate positively charged ions on a negative surface. Conversely, anion exchange is used to retain and separate negatively charged ions on a positive surface.

2.4.2.2 Part of HPLC System

2.4.2.2.1 Pump

There are a number of different types of pumps that can provide the necessary pressures and flow-rates required by the modern liquid chromatograph. There were two types of pump in common use; they were the pneumatic pump, where the necessary high pressures were achieved by pneumatic amplification and the syringe pump, which was simply a large, strongly constructed syringe with a plunger that was driven by a motor. The pneumatic pump has a much larger flow capacity. The pneumatic pump can provide extremely high pressures and is relatively inexpensive,

but the high pressure models are a little cumbersome and, at high flow rates, can consume considerable quantities of compressed air. The HPLC, modern LC pumps need to operate at these pressures and remain sensibly inert to the wide variety of solvents used HPLC pumps usually have sapphire pistons, stainless steel cylinders and return valves fitted with sapphire balls and stainless steel seats.

2.4.2.2.2 Injector

Injection of the sample is entirely automated. An injector (auto sampler) is able to applied the sample extract into the into the HPLC column.

2.4.2.2.3 HPLC Column

A column tube and fittings must contain the chromatographic packing material (stationary phase) that is used to effect a separation. It must withstand backpressure created both during manufacture and in use. Also, it must provide a well-controlled (leak-free, minimum-volume, and zero-dead-volume) flow path for the sample at its inlet, and analyte bands at its outlet, and be chemically inert relative to the separation system such as sample, mobile, and stationary phases. A column is uniformly packed; its mechanical separation power is determined by the column length and the particle size. Mechanical separation efficiency is often measured and compared by a plate number. Smaller-particle size has higher efficiency and higher backpressure. For a given particle size, more mechanical separation power is gained by increasing column length. However, it is longer chromatographic run times, greater solvent consumption, and higher backpressure. Shorter column lengths minimize all these variables but also reduce mechanical separation power.

2.4.2.2.4 Liquid Chromatography Detectors

A detector is needed to see the separated compound bands as they elute from the HPLC column, when a substance has passed through the column, they pass immediately into the detector. An appropriate detector has the ability to sense the presence of a compound and send its corresponding electrical signal to a computer data station. A wide range of different detectors are described including refractive index measurement, UV absorption and fluorescence detection. Many different types

of detectors are selected depending on the characteristics and concentrations of the compounds that need to be separated and analyzed. For example, UV detector selected to be used for a compound can absorb ultraviolet light. If the compound fluoresces, a fluorescence detector is used. The most powerful approach is the use multiple detectors in series. For example, a UV may be used in combination with a mass spectrometer (MS) to analyze the results of the chromatographic separation. This provides, from a single injection, more comprehensive information about an analyte. The practice of coupling a mass spectrometer to an HPLC system and tandem mass are called LC-MS and LC-MS/MS, respectively.

2.5 Mass Spectrometry (MS)

Mass spectrometry is one of the most important analytical techniques to provide information about chemical composition and abundance of isotopes. A mass spectrometer can measure the mass of a molecule only in form of a gas phase ion**.** The ions are separated, detected and measured according to their mass-to-charge ratios (m/z). Relative ion current (signal) is plotted versus m/z producing total ion chromatogram. The three major components of MS instrument are ion source, mass analyzer and detector. Sample molecules are ionized into gas phase ion at ion source and ions are accelerated into mass analyzer for mass separation. The separated ions are determined with a detector and signals are delivered to data system analysis. All MS instrument required high vacuum system to increase the mean free path of ions and minimize the collision of ion to prevent the loss of ions. Figure 2.4 shows a schematic diagram of the mass spectrometer.

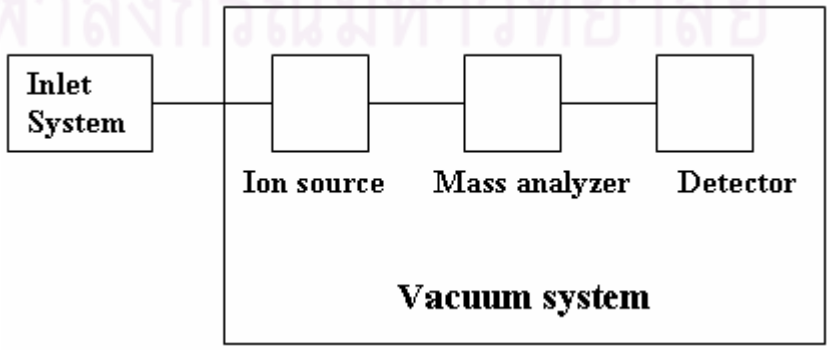


Figure2.3 Diagram of the mass spectrometry system

Mass spectrometers were proved themselves as both qualitative and quantitative instruments. MS, replacing the less-certain results of immunoassays for drug testing and screening food safety and environmental researchers.

2.5.1 Ion Source

2.5.1.1. Electron Ionization (EI)

EI (electron ionization) was a hard ionization technique since sufficient energy is imparted to disrupt internal chemical bonds requiring high kcal/mol. Ionizing voltage (typically 70eV) refers to the difference in voltage causing acceleration of the electrons used to induce electron ionization. EI, samples must be thermally stable since heating in the source causes vaporization. The analyzer operates at even higher vacuum (10^{-4} to 10^{-6} torr). The energy of the electrons interacting with the molecule of interest is generally much greater than that contained in its bonds, so ionization occurs. The excess energy breaks bonds in a well-characterized way. The result is predictable, identifiable fragments from which we can deduce the molecule's identity. The EI technique is fairly independent of the source design. A spectrum produced by one EI instrument looks much like a spectrum of the same compound from another EI instrument, a fact that lends itself to creating spectral libraries to match unknowns to reference spectra.

2.5.1.2. Chemical Ionization (CI)

Molecules that fragment excessively call for "soft" techniques. Chemical ionization (CI) produces ions by a gentler proton transfer process that preserves and promotes the appearance of the molecular ion itself. The ionization mechanism of CI relies on EI for the initial ionization step but within the source is a chemical reagent gas, such as methane, isobutane or ammonia, at high pressure forms the protonated molecular ion ($M+H$). The reverse process can produce negative ions. Transferring the proton to the gas molecule can, in some cases, produce the negative ion ($M-H$).

2.5.1.3 Atmospheric Pressure Electrospray Ionization (AP-ESI)

Electrospray ionization (ESI) was soft ionization technique. The most widely employed of the atmospheric pressure ionization (API) techniques. ESI, the less polar and more volatile ones introduced into a mass spectrometer from a condensed phase, or liquid stream. The liquid from the liquid chromatograph enters the ESI probe and pumped through a stainless steel capillary which energy (voltages in the 3-5kV range) applied to a conductive tube (stainless steel capillary). The liquid aerosolizes as it exits the capillary at atmospheric pressure, the desolvating droplets shedding ions that flow into the mass spectrometer, induced by the combined effects of electrostatic attraction and vacuum. The mechanism by which potential transfers from the liquid to the analyte, creating ions, remains a topic of controversy. Firstly, the charge residue mechanism in which hypothesized that as a droplet evaporates, its charge remains unchanged. The droplet's surface tension, ultimately unable to oppose the repulsive forces from the imposed charge, explodes into many smaller droplets. These Coulombic fissions occur until droplets containing a single analyte ion remain. When the solvent evaporated from the last droplet; a gas phase ion forms. The ESI process is shown in figure 2.5. The ions are typically protonated and detected in the form M+H+ in positive ionization mode or M-H- in negative ion mode.

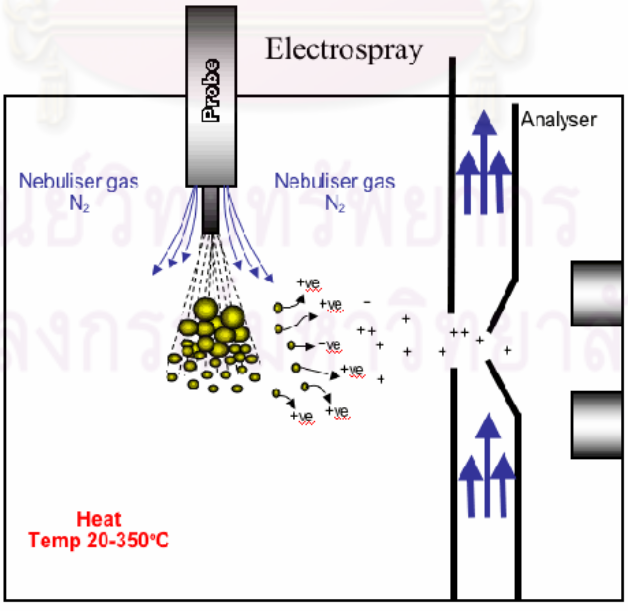


Figure2.4 Atmospheric pressure electrospray ionization process [56]

2.5.1.4. Atmospheric Pressure Chemical Ionization (APCI)

Atmospheric Pressure Chemical Ionization (APCI**)** was more often successfully applied to neutral molecules that do not ionize easily directly out of solution. The ionizing potential is applied, at the tip of a needle as a plasma, or corona, through which the droplets pass, create plasma of metastable ions from the solvent itself and transfer the charge from these ions to the analyte as it passes through the plasma. Heating a probe through which the LC or solvent stream passes creates the aerosol. Hence the early name given APCI: "solvent-mediated electrospray".

2.5.2 Mass Analyzer

Mass analyzer is the heart of a mass spectrometer. The analyzer is an instrument's means of separating or differentiating introduced ions. Both positive and negative ions (as well as uncharged, neutral species) form in the ion source. However, only one polarity is recorded at a given moment. Modern instruments can switch polarities in milliseconds, yielding high fidelity records even of fast, transient events like those typical of ultra performance liquid chromatography (UPLC) or GC separations in which peaks are only about one second wide.

2.5.2.1 Quadrupole

Quadrupole mass spectrometer is superimposed radio frequency (RF) and constant direct current (DC) potentials between four parallel rods were shown in figure 2.6 to act as a mass separator, or filter, where only ions within a particular mass range, exhibiting oscillations of constant amplitude, could collect at the analyzer. The instruments target them for specific applications. Single quadrupole mass spectrometers require a clean matrix to avoid the interference of unwanted ions, and they exhibit very good sensitivity.

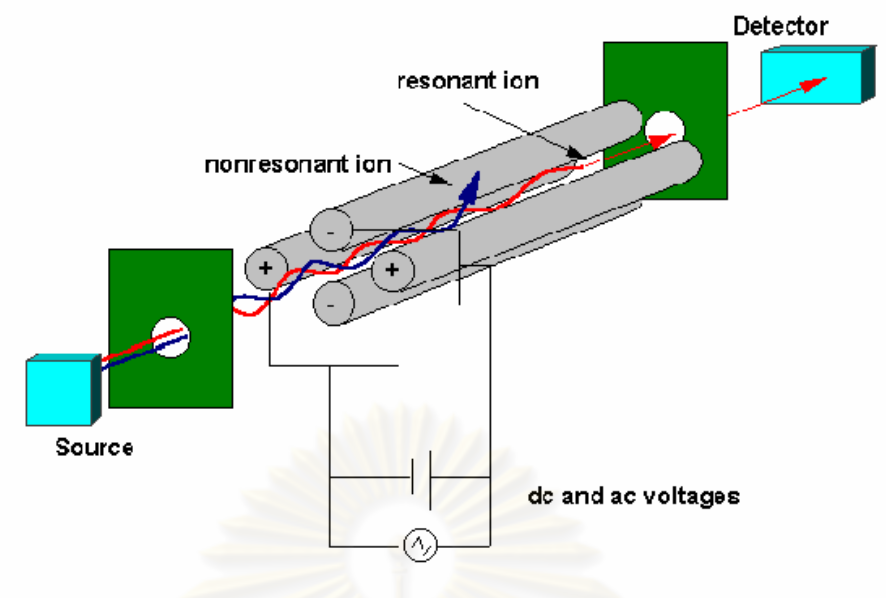


Figure 2.5 Schematic diagram of Quadrupole mass analyzer [57]

2.5.2.2 Triple Quadrupoles

Triple quadrupoles, or tandem**,** mass spectrometers (MS/MS) add to a single quadrupole instrument an additional quadrupole, which can act in various ways. One way is simply to separate and detect the ions of interest in a complex mixture by the ions' unique mass-to-charge (*m/z*) ratio. Another way that an additional quadrupole proves useful is when used in conjunction with controlled fragmentation experiments. Such experiments involved colliding ions of interest with another molecule (typically a gas like argon). In such an application, a precursor ion fragments into product ions, and the MS/MS instrument identifies the compound of interest by its unique constituent parts.

MS/MS is described a variety of experiments-multiple-reaction monitoring (MRM) and single-reaction monitoring (SRM). That is monitoring the transition of precursor ions, or fragmentations, to product ion(s), which in general tend to improve the selectivity, specificity, and/or sensitivity of detection over a single-stage-instrument experiment. Two mass analysers in series or two stages of mass analysis, in a single instrument are used.

In a triple quadrupole mass spectrometer, there are three sets of quadrupole filters, although only the first and third function as mass analyzers. More recent

designs have sufficiently differentiated the middle device (replacing the quadrupole of earlier designs) adding increased function so the term or tandem quadrupole is often used instead. The first quadrupole (Q1), acting as a mass filter, transmits and accelerates a selected ion towards Q2, which is called a collision cell. Although in some designs Q2 is similar to the other two quadrupoles, RF is imposed on it only for transmission, not mass selection. The pressure in Q2 is higher, and the ions collide with neutral gas (argon) in the collision cell. The result is fragmentation by collision-induced dissociation (CID). The fragments are then accelerated into Q3, another scanning mass filter, which sorts them before they enter a detector, is shown in figure 2.7.

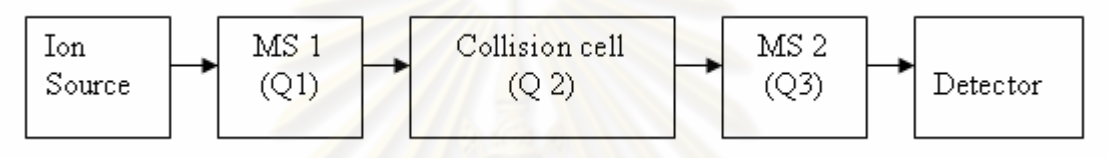


Figure 2.6 Schematic diagram of tandem mass spectrometer (MS/MS)

CHAPTER III

EXPERIMENTAL

3.1 Instruments and Apparatus

3.1.1 Liquid chromatography-tandem mass spectrometer (LC-MS/MS): Waters alliance® HPLC system with an autosampler, a binary pump and water 2695 separation module coupled to a Micromass Quattro Ultima™ FS benchtop tandem quadrupole mass spectrometer using an atmospheric pressure electrospray (AP-ESI) interface and Masslynx 4.0 software processing, Water Corporation, MA, USA.

3.1.2 Gas chromatography equipped with Flame Ionization Detector (GC/FID): Agilent model 6890N and Chemstation software processing from Agilent technology, Wilmington, USA.

3.1.3 Spectrophotometer: model 8453, Agilent technology, Wilmington, USA.

3.1.4 HPLC column: Luna C_{18} (150mm x 2.0mm I.D., 3 μm) connection with guard column. Phenomenex.

3.1.5 GC column: HP-5 (Agilent, Folsom, CA) capillary column 30m x 0.25 mm id 0.25 μm film thickness.

3.1.6 Milli-Q, Ultrapure W-Q, water systems with Simpak® 40 Filter unit 0.22 μm, model ZFMQ050RG, Millipore, Billerica, MA, U.S.A.

3.1.7 Ultrasonicate: model crest 575d, Crest Ultrasonic Corporation, NY, USA.

3.1.8 Analytical balance (5 digits), model AB 204-S, Mettler-Toledo, Inc., OH, USA.

3.1.9 Analytical balance (2 digits), model PB 3002-S, Mettler-Toledo, Inc., OH, USA.

3.1.10 Centrifuge: Heraeus centrifuge, model Megafuge 1.0R, Thermo Fisher Scientific, MA, USA.

3.1.11 Micro centrifuge, model BR 4 from Jouan.

3.1.12 pH meter, Mettler-Toledo, Inc., OH, USA.

3.1.13 Blender, MARA

3.1.14 Vacuum pump with pressure regulator, Model SUE 300E, Heto-Holten A/S 17-19 DK-3450 Allerod, Denmark.

3.1.15 Vortex mixer, Model KMS1, IKA-works Industries, Willmington, U.S.A.

3.1.16 Liquid dispenser: An adjustable volume solvent dispenser provided 10 mL.

3.1.17 Micro-pipettes 10-100 µL, 20-200 µL, 100-1000 µL and tips, Eppendorf, Hamburg, Germany.

3.1.18 Refrigerator, SANTO Medical Freeze Coperation, Scientific, Co., Ltd., Tokyo, Japan.

3.1.19 HPLC amber vials, size 2 mL with PTFE cap

3.1.20 Insert flat vial, size 300 µL

3.1.21 Micro centrifuge tube, size 1.5 mL

3.1.22 Volumetric flasks 5.00mL, 10.00 mL, 25.00 mL, 50.00 mL.

3.1.23 Oak Ridge Centrifuge Tubes, polypropylene copolymer; polypropylene screw closure, NALGENE®

3.1.24 Graduate centrifuge tube, size 15 mL

3.1.25 Spatular

All experimental glasswares were cleaned with detergents and rinsed with deionized water and followed by acetone before used.

3.2 Chemicals

3.2.1 Standard Compounds

Organophosphate group such as omethoate, methamidophos, mevinphos, dimethoate, monocrotophos, dicrotophos, diazinon and DDVP (dichlovos), carbamate group such as oxamyl, methomyl, carbaryl, carbofuran, carbofuran-3-hydroxy, isoprocarb, fenobucarb, methiocarb, bendiocarb, propham, propoxur, carbosulfan, alanycarb and benfuracarb were purchased from Dr. Ehrenstorfer (Augsburg,Germany) with more than 95.00 % purity.

3.2.2 Organic Solvents

All solvents such as acetonitrile, methanol, acetone, and ethyl acetate were purchased from RCI Labscan (Bangkok Thailand). Acetonitrile, methanol in HPLC

grage, ethyl acetate in pesticide grade and acetone for rinse grassware were sufficient quality for pesticide residues analysis.

3.2.3 Reagents

Sodium chloride, anhydrous sodium acetate and trisodium citrate dihydrate in analytical reagent grade were purchased from Merck**.** Di sodium hydrogen citrate sesquihydrate was obtained form Fluka (Buchs, Switzerland). Anhydrous magnesium sulfate ($MgSO_4$) in powder and glanular form were purchased from UCT (Bristol, USA) and Fluka (Buchs, Switzerland), respectively.

3.2.4 Sorbents

Florisil, Silica, Alumina N, Alumina B, C-18, NH_2, MAX, MCX, SAX, HLB and CM were purchased from waters. Graphitized carbon black (GCB) was obtained form Supelco, INC (Bellfonte, Pennsylvania). Primary secondary amine sorbent (PSA) was supplied by UCT (Bristol, USA).

3.3 Preparation of Standard Solutions

3.3.1 Stock Standard Solutions

Reference standards of the pecticides were prepared as stock solution at concentration 1000 mg/L in ethyl acetate for organophospate and methanol for carbamate pesticide, respectively. Individual standard solution was prepared by weighing 2.5 mg (by weight corrected to 100 % purity) of each standard and dissolved in appropriate solvents in 25.00 mL volumetric flasks. These stock standard solutions were kept in amber glass bottle with screw cap and stored at -20 ºC in the freezer.

3.3.2 Intermediate Standard Solutions

The intermediate standard mixture solutions of organophosphate group and carbamate group were prepared at concentration 10 mg/L in acetonitrile. Mixed

intermediate standards of each group were prepared by pipette of each stock standard solution into a 50.00 mL volumetric flask and diluted to volume with acetonitrile. Both mixed solutions were kept in amber glass bottle with screw cap and stored at -20 ºC in the freezer.

3.3.3 Working Standard Solutions

The standard calibration curves were prepared by matrix-matched calibration standard to compensate for matrix effects. The organophosphate and carbamate standards were added to blank mangosteen extracts to concentration at 5, 25, 50, 100, 125 μg/L, respectively. Quantitative determination was analyzed by bracketing calibration in external standard.

3.4 GC/FID System

Gas chromatography (agilent) model 6890 was equiped with Flam Ionization detector. The GC condition was HP-5 (Agilent, Folsom, CA) capillary column of 30m, 0.25mm id, 0.25 μm film thickness, Helium at constant flow 2 mL/min, inlet temperature 250 ^{o}C, injection volume 2 μL (splitless), temperature program was from 95 ^{o}C for 1.5 min, then 20 ^{o}C/min ramp to 190 ^{o}C followed by 5 ^{o}C/min ramp to 230 ^{o}C and 25 ^{o}C ramp to 290 ^{o}C and held for 20 min. Total run time was 36.67 min. The chemstation solfware was used for instrument control and data analysis.

3.5 GC-MS System

Gas chromatography (Agilent) model 6890 was coupled with 5973 mass-selective detector (MSD). The GC condition was a HP-5ms (Agilent, Folsom, CA) capillary column of 30 m, 0.25 mm id, 0.25 μm film thickness, Helium at constant flow 1 mL/min, inlet temperature 250 ^{o}C, injection volume 1 μL (splitless), MS transfer line temperature 290 ^{o}C, temperature program was from 95 ^{o}C for 1.5 min, then 20 ^{o}C/min ramp to 190 ^{o}C followed by 5 ^{o}C/min ramp to 230 ^{o}C and 25 ^{o}C ramp to 290 ^{o}C and held for 20 min. Total run time was 36.67 min. Full-scan analysis (50-

500 m/z) was used to determin interference effect from cleanup. The chemstation solfware was used for instrument control and data analysis.

3.6 LC-MS/MS System

A water performance liquid chromatography was connected to a Micromass Quattro Premier™ XE benchtop tandem quadrupole mass spectrometer (Milford, MA, USA). Electrospray ionization (ESI) was used as ionizing source in positive mode. The LC system was performed by injecting 10 µL via autosampler on a Luna C-18 (2.0 mm x 150 mm x µm) column (Phenomenex, USA) connected with guard column at 40 ^{o}C, 0.2 ml/min flow rate. The mobile phase, solution A (0.01 M ammonium acetate) and solution B (methanol) was set at linear gradient from 0 % B to 95 % B in 14 min and held for 6 min. The chromatographic separations of the 20 compounds were achieved within 15 min.

The tandem mass spectrometer parameters were ion spray voltage at 4000 V, cone gas flow at 0-55 L/hr., desolvation gas flow at 600-650 L/hr., desolvation temp. at 350 ^{o}C and the ion source temperature at 120 ^{o}C. Estimation of the residues was performed by multiple reaction monitoring (MRM), with two mass transitions for each pesticide; one for quantification and the other for confirmation. The detail of MRM transitions of all analytes were shown in table 3.1. Instrument control and data acquisition and evaluation were performed by MassLynx 4.0 solfware package provided by Micromass™.

Table 3.1 Multiple Reaction Monitoring (MRM) setting for 22 pesticides in the MS/MS analysis

Pesticide	The MRM transition			
	Quantitation MRM 1	Confirmation MRM 2	Collision energy1 (V)	Collision energy 2 (V)
Oxamyl	237.01>71.99	237.01>89.94	10	8
Methomyl	162.98>88.01	162.98>105.96	8	8
Carbofuran-3-OH	238.05>162.97	238.05>181.03	18	10
Carbaryl	202.04>117.04	202.04>145.04	20	13
Carbofuran	222.07>122.99	222.07>164.96	20	12
Isoprocarb	194.07>95.04	194.07>137.04	13	8
Fenobucarb	208.12>95.08	208.12>152.07	13	8
Methiocarb	226.04>121.07	226.04>169.02	18	10
Bendiocarb	224.08>109.04	224.08>167.01	15	8
Propoxur	210.26>111.09	210.26>168.08	13	8
Propham	180.05>120.04	180.05>138.03	15	8
Carbosulfan	381.16>118.08	381.16>160.13	18	13
Alanycarb	400.32>238.27	400.32>138.08	8	23
Benfuracarb	411.06>195.01	411.06>252.10	23	15
Omethoate	214.03>142.97	214.03>182.98	18	12
Methamidophos	142.03>112.01	142.03>124.97	10	12
Mevinphos	225.04>126.96	225.04>193.03	15	8
Dimethoate	229.99>170.98	229.99>198.97	15	8
Monocrotophos	224.09>127.03	224.09>192.98	15	8
Dicrotophos	238.03>112.05	238.03>127.01	13	15
Diazinon	305.06>153.08	305.06>169.06	20	20
DDVP	221.08>108.96	221.08>127.02	15	15

3.7 Sample Preparation of Mangosteen

3.7.1 Study of QuEChERS Method for Mangosteen Matrix.

The experimental procedures were studied with the 3 versions of QuEChERS method for analysis of pesticide residues in mangosteen. The different 3 versions QuEChERS were applied with the extraction with acetonitrile and 1% acetic acid in acetonitrile and furthered partition with salt (unbuffer), citrate buffer and acetate buffer. This study can be designed into 6 methods as shown in table3.2. The organophosphate and carbamate standard were spiked into sample to obtain recovery at concentration level of 0.10 mg/Kg in 3 replicates. The sample were extracted with 6 methods and analysis by LC-MS/MS (for preliminary study in term of recovery).

Table 3.2 Six methods for determination of pesticide residues in mangosteen based on QuEChERS method

Method / **Process**	**Method I Original Version**	**Method II EN15662 Version**	**Method III**	**Method IV**	**Method V**	**Method VI AOAC Version**
Weighed	10 g					
Add extracting solution	10 mL Acetonitrile			10 mL 1 % Acetic acid In Acetronitrile		
Extraction/partition	$MgSO_4$ 4g NaCl 1 g	$MgSO_4$ 4 g NaCl 1 g *Tri 1g **Di 0.5g	$MgSO_4$ 4g NaCl 1 g NaOAc 1g	$MgSO_4$ 4g NaCl 1 g	$MgSO_4$ 4g NaCl 1 g *Tri 1g **Di 0.5g	$MgSO_4$ 4g NaCl 1 g NaOAc 1g
Centrifuged	Shake & Centrifuge					
Aliquat taken	1 mL					
Cleaned up	PSA 50 mg $MgSO_4$ 150 mg					
	Vortex & Centrifuge					
Final volume adjusted	1 mL					

* Trisodium citrate dihydrate

** Di sodium hydrogen citrate sesquihydrate

Extraction Method I (analysis in 3 replicates)

1. Weighed: 10 g of homogenized mangosteen was weighed into 50 mL centrifuge tube.
2. Spiked: 100 µL of 10 mg/L mixed standards were spiked at 0.10 mg/Kg into sample. The tubes containing spiked samples were vortexed for 30 sec and left standing for 10 min to allow pesticides residues interacted with matrix.
3. Added extracting solution: 10 mL acetonitrile were added into spiked samples.
4. Extraction/Partition: 4 g anhydrous $MgSO_4$ and 1 g NaCl were added into centrifuge tube, cap tightly and mix on vortex mixer immediately for 1 min then centrifuged the extract for 10 min at 3500 rpm.
5. Cleaned up: 1 mL of the upper extract was pipetted into 1.5 mL microcentrifuge tube containing 150 mg anhydrous $MgSO_4$ and 50 mg PSA. After that, cap tightly, shaked and vortex 30 sec then centrifuge the extract for 5 min at 10000 rpm.
6. The clear extract was transfered into 2 mL amber vial for inject LC-MS/MS.

Extraction Method II (analysis in 3 replicates)

1 Weighed: 10 g of homogenized mangosteen was weighed into 50 mL centrifuge tube.

2 Spiked: 100 µL of 10 mg/L mix standard were spiked at 0.10 mg/Kg into sample. The tubes containing spiked samples were vortexed for 30 sec and left standing for 10 min to allow timing for pesticides interact with matrix.

3 Add extracting solution: 10 mL of acetonitrile were added into spiked sample.

4 Extraction/Partition : 4 g of anhydrous $MgSO_4$, 1 g of NaCl, 1 g of trisodium citrate dehydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added into centrifuge tube, capped tightly and mixed on vortex mixer immediately for 1 min. then centrifuged the extract for 10 min at 3500 rpm.

5 Cleaned up: 1 mL of the upper layer was pipetted into 1.5 mL microcentrifugal tube containing 150 mg anhydrous $MgSO_4$ and 50 mg PSA, capped tightly, shaked and vortexed for 30 sec then centrifuged the extract for 5 min at 10,000 rpm.

6 The clear extract was transfered into 2 mL amber vial for inject LC-MS/MS.

Extraction Method III (analysis in 3 replicates)

1 Weighed: 10 g of homogenized mangosteen was weighed into 50 mL centrifuge tube.
2 Spiked: 100 µL of 10 mg/L mix standard were spiked at 0.10 mg/Kg into sample. The tubes containing spiked samples were vortexed for 30 sec and left standing for 10 min to allow pesticide residues interacted with matrix.
3 Add extracting solution: 10 mL of acetonitrile were added into spiked sample.
4 Extraction/Partition: 4 g of anhydrous $MgSO_4$, 1 g of NaCl and 1 g of anhydrous sodium acetate were added into centrifugal tube, capped tightly and mixed on vortex mixer immediately for 1 min. then centrifuged the extract for 10 min at 3500 rpm.
5 Cleaned up: 1 mL of the upper extract was pipetted into 1.5 mL microcentrifugeal tube containing 150 mg anhydrous $MgSO_4$ and 50 mg PSA, capped tightly, shaked and vortexed for 30 sec then centrifuged for 5 min at 10,000 rpm.
6 The clear extracts were transfered into 2 mL amber vial for inject LC-MS/MS.

Extraction method IV (analysis in 3 replicates)

1 Weighed: 10 g of homogenized mangosteen was weighed into 50 mL centrifuge tube.
2 Spiked: 100 µL of 10 mg/L mix standard were spiked at 0.10 mg/Kg into sample. The tubes containing spiked samples were vortexed for 30 sec and left standing for 10 min to allow timing for pesticides interact with matrix.
3 Add extracting solution: 10 mL 1% acetic acid in acetonitrile were added into spiked sample.
4 Extraction/Partition: 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added into centrifugal tube, capped tightly and mixed on vortex mixer immediately for 1 min. then centrifuged the extract for 10 min at 3,500 rpm.
5 Cleaned up: 1 mL of the upper extract was pipetted into 1.5 mL microcentrifugal tube containing 150 mg anhydrous $MgSO_4$ and 50 mg PSA,

capped tightly, shaked and vortexed for 30 sec then centrifuged for 5 min at 10,000 rpm.

6 The clear extracts were transfered into 2 mL amber vial for inject LC-MS/MS.

Extraction method V (analysis in 3 replicates)

1 Weighed: 10 g of homogenized mangosteen was weighed into 50 mL centrifuge tube.
2 Spiked: 100 µLof 10 mg/L mix standard were spiked at 0.10 mg/Kg into sample. The tubes containing spiked samples were vortexed for 30 sec and left standing for 10 min to allow timing for pesticides interact with matrix.
3 Added extracting solution: 10 mL of 1% acetic acid in cetonitrile were added into spiked sample.
4 Extraction/Partition: 4 g of anhydrous $MgSO_4$, 1 g of NaCl, 1 g of trisodium citrate dehydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added into centrifugal tube, capped tightly and mixed on vortex mixer immediately for 1 min. then centrifuged for 10 min at 3,500 rpm.
5 Cleaned up: 1 mL of the upper extract was pipetted into 1.5 mL microcentrifuge tube containing 150 mg anhydrous $MgSO_4$ and 50 mg PSA. After that, cap tightly, shaked and vortex 30 sec then centrifuge the extract for 5 min at 10000 rpm.
6 The clear extracts were transfered into 2 mL amber vial for inject LC-MS/MS.

Extraction method VI (in 3 replicates)

1. Weighed: 10 g of homogenized mangosteen was weighed into 50 mL centrifuge tube.
2. Spiked: 100 µL of 10 mg/L mix standard were spiked at 0.10 mg/Kg into sample. The tubes containing spiked samples were vortexed for 30 sec and left standing for 10 min to allow pesticide residues interacted with matrix.
3. Added extracting solution: 10 mL of 1% acetic acid in acetonitrile were added into spiked sample.
4. Extraction/Partition: 4 g of anhydrous $MgSO_4$, 1 g of NaCl and 1 g of anhydrous sodium acetate were added into centrifuge tube, capped tightly and

mixed on vortex mixer immediately for 1 min. then centrifuged for 10 min at 3,500 rpm.

5. Cleaned up: 1 mL of the upper extract was pipetted into 1.5 mL microcentrifugal tube containing 150 mg anhydrous $MgSO_4$ and 50 mg PSA. After that, cap tightly, shaked and vortexed for 30 sec then centrifuged for 5 min at 10,000 rpm.
6. The clear extracts were transfered into 2 mL amber vial for inject LC-MS/MS.

3.7.2 Study on the Effect of Extraction Solvent

3.7.2.1 Weighed: 10 g of homogenized mangosteen were weighed into 50 mL centrifugal tube (6 tubes).

3.7.2.2 Spiked: 100 μL of 10 mg/L mix standard were spiked at 0.10 mg/Kg into sample. The tubes containing spiked samples were vortexed for 30 sec and left standing for 10 min to allow pesticide residues interacted with matrix.

3.7.2.3 Added extracting solution: Extraction solvent was studied in 2 compositions. Both compositions were conducted in tree replicates.

Composition I: 10 mL of acetonitrile were added into spiked sample.

Composition II: 10 mL of 1% acetic acid in acetonitrile were added into spiked sample.

3.7.2.4 Extraction/Partition: 4 g of anhydrous $MgSO_4$, 1 g of NaCl and 1 g of anhydrous sodium acetate were added into centrifugal tube, capped tightly and mixed on vortex mixer immediately for 1 min. then centrifuged for 10 min at 3,500 rpm.

3.7.2.5 Cleaned up: 1 mL of the upper extract was pipetted into 1.5 mL microcentrifugal tube containing 150 mg anhydrous $MgSO_4$ and 50 mg PSA, capped tightly, shaked and vortexed for 30 sec then centrifuge for 5 min at 10,000 rpm.

3.7.2.6 The clear solutions were transfered into 2 mL amber vial for injecting LC-MS/MS

3.7.3 Study of Effect of Buffering Agent

3.7.3.1 Weighed: 10 g of homogenized mangosteen were weighed into 50 mL centrifuge tube. (6 tubes)

3.7.3.2 Spiked: 100 µL of 10 mg/L mix standard were spiked at 0.10 mg/Kg into sample. The tubes containing spiked samples were vortexed for 30 sec and left standing for 10 min to allow timing for pesticides interact with matrix.

3.7.3.3 Addded extracting solution: 10 mL of acetonitrile were added into spiked sample.

3.7.3.4 Extraction/Partition: Anhydrous magnesium sulfate, sodium chloride and buffering agent were studied in 3 compositions. Each composition was studied with tree replicates.

Composition I: 4 g of anhydrous $MgSO_4$ and 1 g of NaCl were added into centrifugal tube.

Composition II: 4 g of anhydrous $MgSO_4$, 1 g of NaCl, 1 g of trisodium citrate dehydrate and 0.5g of disodium hydrogen citrate sesquihydrate was added into centrifugal tube.

Composition III: 4 g of anhydrous $MgSO_4$, 1 g of NaCl and 1 g of anhydrous sodium acetate were added into centrifugal tube.

Samples were capped tightly and mixed on vortex mixer immediately for 1 min. then centrifuged for 10 min at 3,500 rpm.

3.7.3.5 Cleaned up: 1 mL of the upper layer was pipetted into 1.5 mL microcentrifugal tube containing 150 mg anhydrous $MgSO_4$ and 50 mg PSA, capped tightly, shaked and vortexed for 30 sec then centrifuged the extract for 5 min at 10,000 rpm.

3.7.3.6 The clear extract were transfered into 2 mL amber vial for injecting LC-MS/MS

3.7.4 Study of Sorbent Type for Dispersive-SPE Clean up

3.7.4.1 Study of Suitable Sorbents for Mangosteen Matrix

Mangosteen extracts were cleaned up with 13 sorbents such as GCB, PSA, Florisil, Silica, Alumina N, Alumina B, C-18, NH_2, MAX, MCX, SAX, HLB and CM as follows:

3.7.4.1.1 **Study of wight of residues after cleaned up with different sorbents**

3.7.4.1.1.1 The stems of fresh mangosteen fruits were removed and the whole fruit including pulp and peel were homogenized in a high speed blender for 3-5 min.

3.7.4.1.1.2 Weighed 50 g homogenized mangosteen into 250 mL centrifuge tube.

3.7.4.1.1.3 The sample was extracted with 50 mL acetonitrile and shaked by a shaker for 10 min.

3.7.4.1.1.4 Added 20 g $MgSO_4$ and 5 g NaCl and mix on vortex mixer immediately for 1 min and centrifuge the extract for 10 min at 3500 rpm. (The mangosteen extract)

3.7.4.1.1.5 Transfered 2 mL aliquot of upper acetonitrile layer into 15 mL centrifuge tube with screw cap containing 300 mg anhydrous $MgSO_4$ and 100 mg of each sorbent (dispersive-SPE), capped tightly, shaked and vortexed 30 sec then centrifuged for 5 min at 10000 rpm.

3.7.4.1.1.6 Transfered 1 mL aliquot into 1.5 mL weighed centrifuge tube then evaporated to dryness with nitrogen evaporater.

3.7.4.1.1.7 Weighed residue from 1 mL after dryness.

3.7.4.1.2 Study in term colour of extract after clean up with different sorbents by study ability to absorb UV-VIS light.

3.7.4.1.2.1 The mangosteen extract was d-SPE cleaned up with 13 sorbents.

3.7.4.1.2.2 The clear solution was determined their absorbance by uv-vis spectrometer.

3.7.4.1.3 Study cleanup effect with chromatography instrument

3.7.4.1.3.1 The mangosteen extract was d-SPE cleaned up with 13 sorbents.

3.7.4.1.3.2 The clear solution was analytzed by GC/FID and GC-MS.

3.7.4.1.4 Study of effect of temperature for clean up

The experimental procedures study the effect of temperature to removed matrix interference by freezing out and centrifuging at low temperature, as following procedures:

3.7.4.1.4.1 2 mL of the mangosteen extract was d-SPE cleaned up with 13 sorbents.

3.7.4.1.4.2 The extracts were centrifuged and compared temperature control at 25^{o}C and - 4 ^{o}C for 5 min at 10,000 rpm.

3.7.4.1.4.3 Transfered 1 mL aliquot into 15 mL centrifugal tube then kept overnight at -20 ºC in the freezer.

3.7.4.1.4.4 Transfered clear aliquot into 2 mL GC vial and injected to GC/FID.

3.7.4.2 Study of Effect of Sorbent Type

3.7.4.2.1 Weighed: 10 g of homogenized mangosteens were weight into 50 mL centrifugal tube. (6 tubes)

3.7.4.2.2 Spiked: 100 µL of 10 mg/L mix standards was spiked at 0.10 mg/Kg into sample. The tubes containing spiked samples were vortexed for 30 sec and left standing for 10 min to allow timing for pesticides interact with matrix.

3.7.4.2.3 Added extracting solution: 10 mL of acetonitrile was added into spiked sample.

3.7.4.2.4 Extraction/Partition: 4 g of anhydrous $MgSO_4$, 1 g of NaCl and 1 g of anhydrous sodium acetate were added into centrifuge tube.After that, cap tightly and mix on vortex mixer immediately for 1 min. then centrifuged for 10 min at 3500 rpm.

3.7.4.2.5 Cleaned up: 1 mL of the upper extract was cleaned up by varying 5 sorbent types. Each sorbent type was conducted with three replicates.

Sorbent I: dispersive –SPE with 150 mg anhydrous $MgSO_4$ and 50 mg PSA.

Sorbent II: dispersive –SPE with 150 mg anhydrous $MgSO_4$ and 50 mg alumina N.

Sorbent III: dispersive – SPE with 150 mg anhydrous $MgSO_4$ and 50 mg florisil.

Sorbent IV: dispersive –SPE with 150 mg anhydrous $MgSO_4$ and 50 mg MCX.

Sorbent V: dispersive –SPE with 150 mg anhydrous $MgSO_4$ and 50 mg SAX.

All samples were capped tightly, shaked and vortexed for 30 sec then centrifuged for 5 min at 10,000 rpm.

3.7.4.2.6 The clear extracts were transfered into 2 mL amber vial for injecting LC- MS/MS

3.7.5 Study of the Effect of Mixed and Weight of Sorbent

3.7.5.1 Weighed: 10 g of homogenized mangosteen were weighed into 50 mL centrifuge tube.

3.7.5.2 Spiked: 100 µL of 10 mg/L mix standards were spiked at 0.10 mg/Kg into samples. The tubes containing spiked samples were vortexed for 30 sec and left standing for 10 min to allow pesticide residues interacted with the matrix.

3.7.5.3 Added extracting solution: 10 mL of acetonitrile was added into spiked samples.

3.7.5.4 Extraction/Partition: 4 g of anhydrous $MgSO_4$, 1 g of NaCl and 1 g of anhydrous sodium acetate were added into centrifugal tubes, capped tightly and mixed on vortex mixer immediately for 1 min. then centrifuged the extract for 10 min at 3,500 rpm.

3.7.5.5 Cleaned up: 1 mL of the upper extract was cleaned up by varying weight of mixed sorbents. Each composition was conducted in tree replicates as follows:

Composition I: dispersive –SPE with 150 mg anhydrous $MgSO_4$ and 25 mg PSA: 25 mg alumina N

Composition II: dispersive –SPE with 150 mg anhydrous $MgSO_4$ and 50 mg PSA: 50 mg alumina N

Composition III: dispersive –SPE with 150 mg anhydrous $MgSO_4$ and 75 mg PSA: 75 mg alumina N

.**Composition IV:** dispersive –SPE with 150 mg anhydrous $MgSO_4$ and 25 mg PSA: 25 mg florisil

Composition V: dispersive –SPE with 150 mg anhydrous $MgSO_4$ and 50 mg PSA: 50 mg florisil

Composition VI: dispersive –SPE with 150 mg anhydrous $MgSO_4$ and 75 mg PSA: 75 mg florisil

All samples were capped tightly, shaked and vortexed for 30 sec then centrifuged the extract for 5 min at 10,000 rpm.

3.7.5.6 The clear extracts were transfered into 2 mL amber vial for injecting LC-MS/MS.

3.7.6 Study of Effect of Mixed Sorbents in Term of Precision

3.7.6.1 Weighed: 10 g of homogenized mangosteen was weighed into 50 mL centrifugal tube. (6 tubes)

3.7.6.2 Spiked: 100 µL of 10 mg/L mixed standards were spiked at 0.10 mg/Kg into samples. The tubes containing spiked samples were vortexed for 30 sec and left standing for 10 min to allow pesticide residues interacted with matrix.

3.7.6.3 Added extracting solution: 10 mL of acetonitrile was added into spiked samples.

3.7.6.4 Extraction/Partition: 4 g of anhydrous $MgSO_4$, 1 g of NaCl and 1 g of anhydrous sodium acetate were added into centrifuge tube, capped tightly and mixed on vortex mixer immediately for 1 min. then centrifuged for 10 min at 3,500 rpm.

3.7.6.5 Cleaned up: 1 mL of the upper extract was cleaned up with mixed sorbents between PSA: alumina N and PSA: florisil. Both compositions were studied in ten replicates as follow:

Composition I: dispersive –SPE with 150 mg anhydrous $MgSO_4$ and 25 mg PSA: 25 mg alumina N

Composition II: dispersive –SPE with 150 mg anhydrous $MgSO_4$ and 25 mg PSA: 25 mg florisil

All samples were capped tightly, shaked and vortexed for 30 sec then centrifuged the extract for 5 min at 10,000 rpm.

3.7.6.6 The clear extracts were transfered into 2 mL amber vial for injecting LC-MS/MS.

3.8 Method Validation [4]

Method validate was performed to provide the evidence that a method was fit for the purpose to be used. The method tested to assess for sensitivity covered mean recovery (as a measure of trueness or bias), precision, limit of detections (LODs) and limit of quantifications (LOQs). The method was optimized condition as follow:

1. Weighed: 10g of homogenized mangosteen was weighed into 50 mL centrifugal tube.
2. Spiked: Mix standards were spiked into sample. The tubes containing spiked samples were vortexed for 30 sec and left standing for 10 min to allow pesticide residues interacted with matrix.
3. Added extracting solution: 10 mL of acetonitrile was added into spiked sample.
4. Extraction/Partition: 4 g of anhydrous $MgSO_4$, 1 g of NaCl and 1 g of anhydrous sodium acetate were added into centrifugal tube, capped tightly and mixed on vortex mixer immediately for 1 min. then centrifuged for 10 min at 3,500 rpm.

5. Cleaned up: 1 mL of the upper extract was cleaned up with mixed sorbents between PSA:Alumina N (25:25mg), capped tightly, shaked and vortexed 30 sec then centrifuged for 5 min at 10,000 rpm.
6. The clear extracts were transfered into 2 mL amber vial for injecting LC-MS/MS.

3.8.1 Standard Calibration Curve

The standard calibration curves were prepared by matrix-matched calibration standard to compensate for matrix effects. The 22 organophosphate and carbamate standards were added to blank mangosteen extracts. The performances of the methods were conducted at concentration range from 5-125 μg/L. The process began at the lowest calibrated level (LCL) 5 μg/L where represented the practical LOQ and 25 μg/L increments was added to 125 μg/L. Six concentrations were conducted in three replicates. The calibration curves obtained were plotted between concentration and peak area of each analytes and then evaluated.

3.8.2 Linearity

Linearity of method was obtained from standard calibration curve of range 5-125 μg/L with three replicates. The calibration curves exhibited their intercepts, slopes and coefficient of determination (R^2) where the coefficient of determination (R^2) represents the linearity of the proposed method. The slope represents the sensitivity of method. The results obtained such as slope, y-intercept, and coefficient of determination (R^2) of all compounds were shown in table 4.11.

3.8.3 Limit of Detections (LODs)

In trace analysis, it is important to know the lowest concentration of analyte or property value that can be confidently detected by the method .The limit of detection is the lowest concentration of analytes applied to the complete analytical method The method employed was determined by analyzing the lowest spiked sample of all analytes at concentration 0.005 mg/L under the optimized condition with ten

replicates. The results and chromatograms of limit of detections LODs) were shown in figure 4.15 and table 4.11, respectively.

3.8.4 Limit of Quantifications (LOQs)

The limit of quantification is the minimum concentration of analyte that can be quantified with acceptable accuracy and precision. It should be applied to the complete analytical method. LOQs are referred to the MRL at 0.01 mg/Kg.The LOQs must not be lower than the corresponding lowest calibrationed level (LCL). This method, LOQs were determined by analyzing the spiked samples at concentration 0.01 mg/Kg under the optimized condition in ten replicates. The concentration level obtained was evaluated in term of accuracy and precision by calculating the percentage recovery and relative standard deviation (RSD). The limit of quantifications (LOQs) obtained were shown in table 4.11.

3.8.5 Accuracy and Precision

The performance of method in term of accuracy and precision was obtained by analyzing the spiked recovery samples to determine theirs accuracy at different concentrations and 5 replicates. The LOQs is defined as the lowest spiked level that meet the method performance acceptability criteria. Ten replicates for accuracy and precision data at spiked levels of 0.01, 0.02, 0.05 and 0.10 mg/Kg were analyzed under the optimized condition and calculated the percentage of recovery to determine accuracy and %RSD for precision according to the acceptable criteria. The accuracy and precision data were shown on table 4.11.

CHAPTER IV

RESULTS AND DISCUSSION

The components in the thick peel such as polyphenols and wax were extracted with QuEChERS technique and analyzed by liquid chromatography coupled tandem mass spectrometry. The pesticide residues of polar organophosphate and carbamate group were analyzed with the QuEChERS method. QuEChERS parameters affecting efficiency such as extraction solvent, buffering agent and dispersive sorbent were studied.

4.1 Study of QuEChERS Method for Mangosteen Matrix

The 3 versions of QuEChERS method to determine pesticide residues in mangosteen were compared for preliminary study. The different of 3 versions were designed to 6 methods as shown in table 3.2. The organophosphate and carbamate standards were spiked to samples for recovery study at 0.10 mg/Kg concentration with 3 replicates and extracted with 6 methods analyzed by LC-MS/MS analysis. The mean recovery of polar organophosphate and carbamate were shown in table 4.1. All residues were founded when extracted with Method III and Method VI. However, the recoveries of all compounds extracted with 6 methods obtained are out of recovery range 60-120 %.

Table 4.1 Mean of percentage recovery of carbamate & polar organophosphate at spiked level 0.10 mg/Kg (n=3).

No	Compound	Method					
		I	II	III	IV	V	VI
1	Oxamyl	53	69	96	162	125	113
2	Methomyl	107	140	108	149	184	168
3	Carbofuran-3-OH	122	117	103	384	204	145
4	Carbaryl	175	136	154	322	128	104
5	Carbofuran	129	172	186	288	186	178
6	Isoprocarb	113	156	184	264	135	120
7	Fenobucarb	65	76	85	269	191	167
8	Methiocarb	73	124	158	383	139	126
9	Bendiocarb	116	104	159	317	140	120
10	Propoxur	193	115	179	207	136	120
11	Propham	80	52	0	128	119	146
12	Carbosulfan	0	33	196	0	0	138
13	Alanycarb	0	0	45	0	0	337
14	Benfuracarb	1	1	110	0	1	150
15	Omethoate	161	60	98	96	67	86
16	Methamidophos	87	88	99	122	105	84
17	Mevinphos	90	107	86	157	100	93
18	Dimethoate	91	99	88	78	82	109
19	Monocrotophos	117	116	136	168	196	193
20	Dicrotophos	95	103	111	170	129	128
21	Diazinon	62	85	54	229	113	133
22	DDVP	142	124	126	136	97	91

4.2 Study on the Effect of Extraction Solvent

The extraction solvents were evaluated for their extraction efficiency. The comparison between using of pure acetonitrile and 1% acetic acid in acetonitrile as extractant were shown in figure 4.1 where carbamate and polar organophosphate residues spiked at 0.10 mg/kg in 3 replicates extracted with acetonitrile and 1% acetic acid in acetonitrile. The extraction with acetonitrile gave better recovery than using 1% acetic acid in acetonitrile.

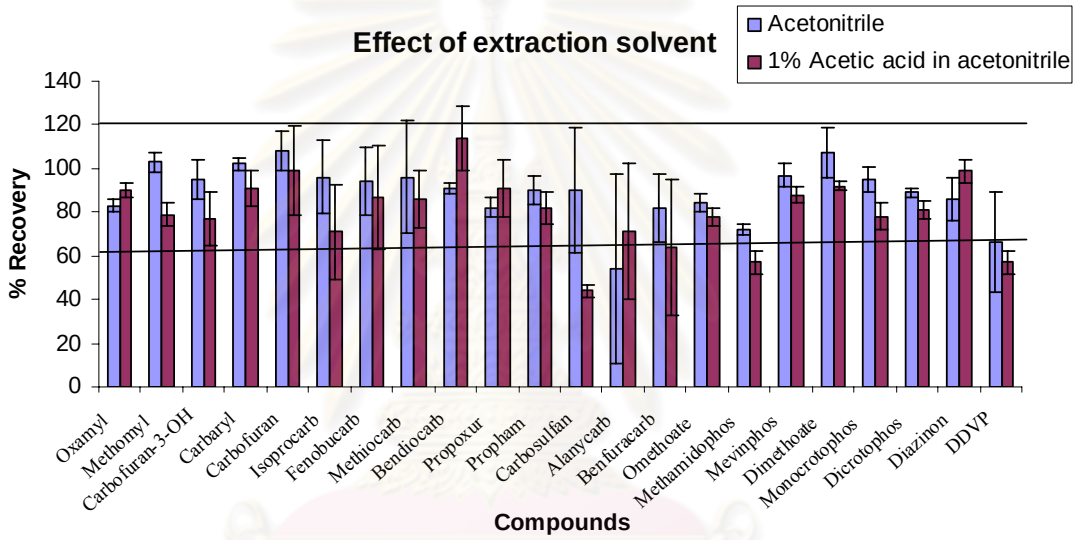


Figure 4.1 Effect of extraction solvent on mean recovery data (n=3).

4.3 Study of Effect of Buffering Agent

Salt and buffering agent induced liquid phase separation as well as stabilizes acid and base labile pesticides. The type of salts and amounts were evaluated for their extraction efficiency. The different of % recovery when using 4 g of $MgSO_4$+ 1 g of NaCl (original version unbuffering), 4 g of $MgSO_4$ + 1 g of NaCl + 1 g of trisodium citrate dehydrate + 0.5 g disodium hydrogen citrate sesquihydrate (EN version which citrate buffering) and 4 g of $MgSO_4$ + 1 g of NaCl + 1 g of anhydrous sodium acetate (AOAC version acetate buffering) were also shown in figure 4.2. Carbamate and polar organophosphate residues spiked at 0.10 mg/kg in 3 replicates then extracted with

acetonitrile and added mixture of 4g magnesium sulfate, 1 g sodium chloride and 1g anhydrous sodium acetate in the extraction solution gave the best recovery.

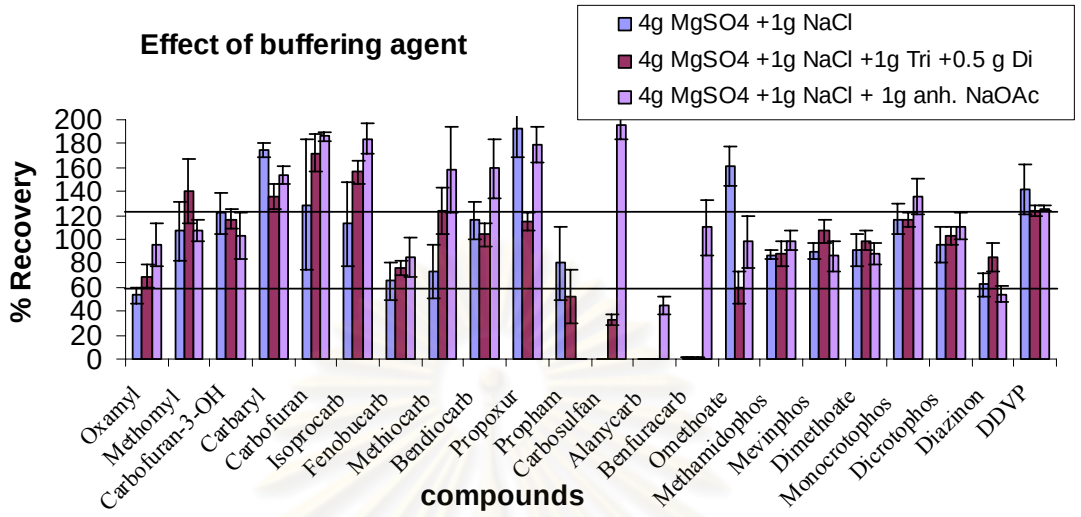


Figure 4.2 Effect of buffers added on mean percentage recovery (where n=3)

4.4 Study of Sorbent Type for Dispersive-SPE Clean up

4.4.1 Study of Suitable Sorbents for Mangosteen Matrix

Mangosteen extracts were cleaned up with 13 sorbents such as GCB, PSA, Florisil, Silica, Alumina N, Alumina B, C-18, NH_2, MAX, MCX, SAX, HLB and CM**.** The extraction after clean up with 13 sorbents were evaluated for their removing interference efficiency.

1 mL of the mangosteen extract was cleaned up its interferences by dispersive-SPE comparing with 13 sorbents such as GCB, PSA, Florisil, Silica, Alumina N, Alumina B, C-18, NH_2, MAX, MCX, SAX, HLB and CM and determined the residuals after clean up of each sorbent**.** MAX, MCX, HLB, NH_2, PSA and Alumina N sorbent showed to remove some interference more than 30 %, the results were shown in table 4.2 and figure 4.3. The mangosteen extract after d-SPE cleaned up with MCX sorbent provided most clear solution; the results were shown in figure 4.4. The extracts after cleaned up with each sorbent was determined their absorbance by spectrophotometer.The UV-VIS spectrum of mangosteen extract was scaned to find

out the max wavelength at 3 wavelengths such as 623, 655, and 664 nm. The results of UV-VIS spectrums were shown in figure 4.5 and absorbance of the extract after d-SPE various sorbent compared with sample blank mangosteen extract were shown in table 4.3. The extract affer clean up with MCX, CM, HLB, PSA, alumina N and SAX sorbents gave lower absorbance than MAX, florisil, GCB, NH_2, alumina B, C-18 and silica.

Table 4.2 Wieght of the residues from 1 mL mangosteen extract after clean up with different sorbents

No.	Sorbent Types	Weight of residues	% Residues	
			Retained	Removed
1	GCB	0.0269	76.64	23.36
2	PSA	0.0226	64.39	35.61
3	Florisil	0.0305	86.89	13.11
4	Silica	0.0295	84.05	15.95
5	Alumina N	0.0242	68.95	31.05
6	C-18	0.0296	84.33	15.67
7	NH_2	0.0212	60.40	39.60
8	Alumina B	0.0258	73.50	26.50
9	MAX	0.0182	51.85	48.15
10	MCX	0.0183	52.14	47.86
11	SAX	0.0269	76.64	23.36
12	HLB	0.0207	58.97	41.03
13	CM	0.0247	70.37	29.63
14	Sample Blank	0.0351	100.00	0.00

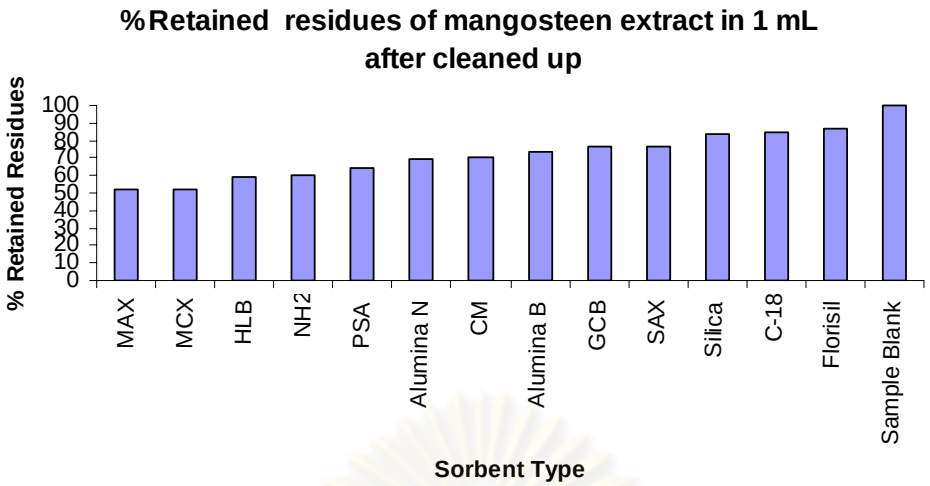
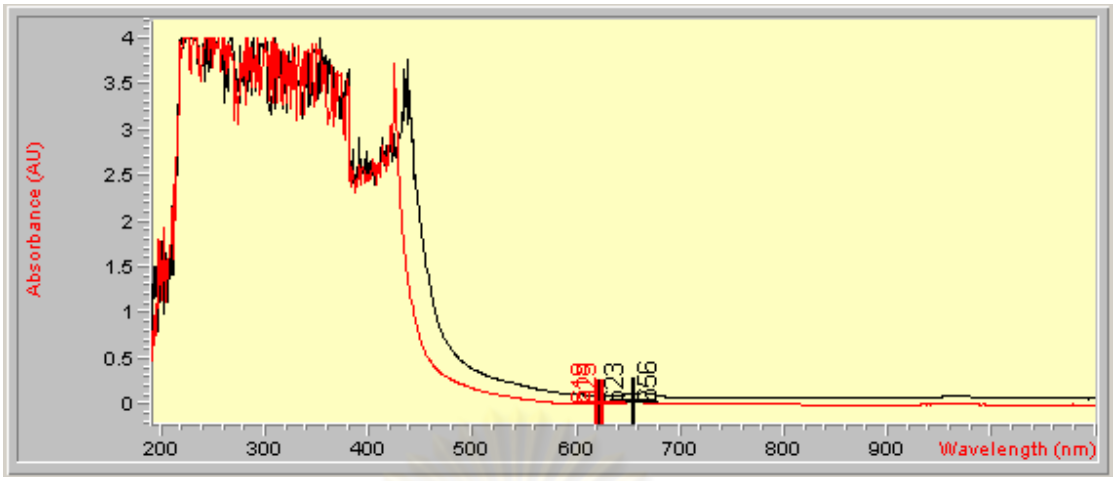


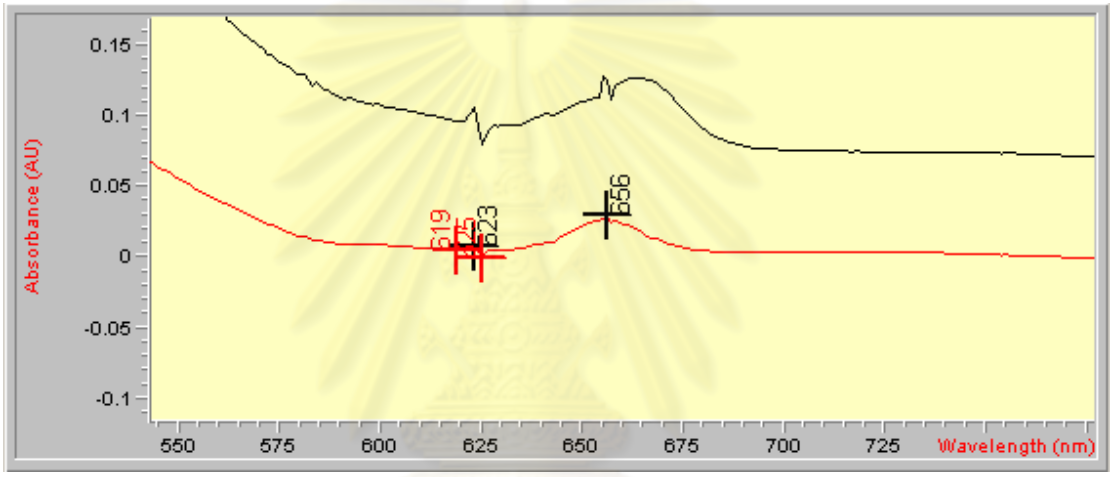
Figure 4.3: Comparison of % residue retains after clean up with various sorbents compared between various sorbent and sample blank mangosteen extract

Sample blank mangosteen extract, magnesium sulphate, MCX, MAX, CM, HLB, Florisil, GCB, PSA, NH_2, Alumina B, Alumina N, SAX, C-18, Silica

Figure 4.4 The colour of 13 mangosteen extracts after dispersive-SPE cleaned up with each sorbent



(a)



(b)

Figure 4.5 UV-VIS spectrums at different wavelenghts of the mangosteen extracts

(a) UV-VIS spectrums 200-800 nm

(b) VIS spectrum in large scale at max wavelength

Table 4.3: The Absorbances of mangosteen extract after d-SPE clean up with different sorbents determined at various wavelengths (623, 655 and 664 nm)

Sorbent	**Absorbance**		
	623 nm	**655 nm**	**664 nm**
Sample blank	0.1060	0.1273	0.1265
MCX	0.0074	0.0263	0.0178
MAX	0.0847	0.1065	0.1063
CM	0.0316	0.0574	0.0657
HLB	0.0771	0.0865	0.0808
Florisil	0.0781	0.1083	0.1094
GCB	0.0826	0.0774	0.0753
PSA	0.0509	0.0809	0.0919
NH_2	0.0739	0.1025	0.1036
Alumina B	0.0832	0.1149	0.1153
Alumina N	0.0525	0.0807	0.0831
SAX	0.0190	0.0504	0.0557
C-18	0.0345	0.04490	0.3556
Silica	0.0953	0.1246	0,1248

The samples of mangosteen extracts after d-SPE cleaned up with 13 sorbents were analyzed with GC/FID and GC-MS using the same condition. All samples showed interference peaks at high temperature (290^{o}C) **(**figure 4.6) as compared with the peak area from the extracts after d-SPE 13 sorbents. The interference peaks after clean up at temperature below 290 ^{o}C (retention time (RT) 0-18 min) were compared with interference peak of sample blank mangosteen extract in term of peak area and no of total peak from running time 30 min, the results were shown in figure 4.7 and table 4.4. The sorbent type such as PSA, florisil, alumina N&B, NH_2, MCX and SAX gave low interference peak area. Nevertheless, in the reagent blank of alumina B and MCX sorbents interferent peaks were founded and chromatograms were shown in figure 4.8. The mangosteen extract analyzed by GC-MS at the same condition,

chromatograms obtained were shown in figure 4.9 and library search reports were shown in table 4.5.

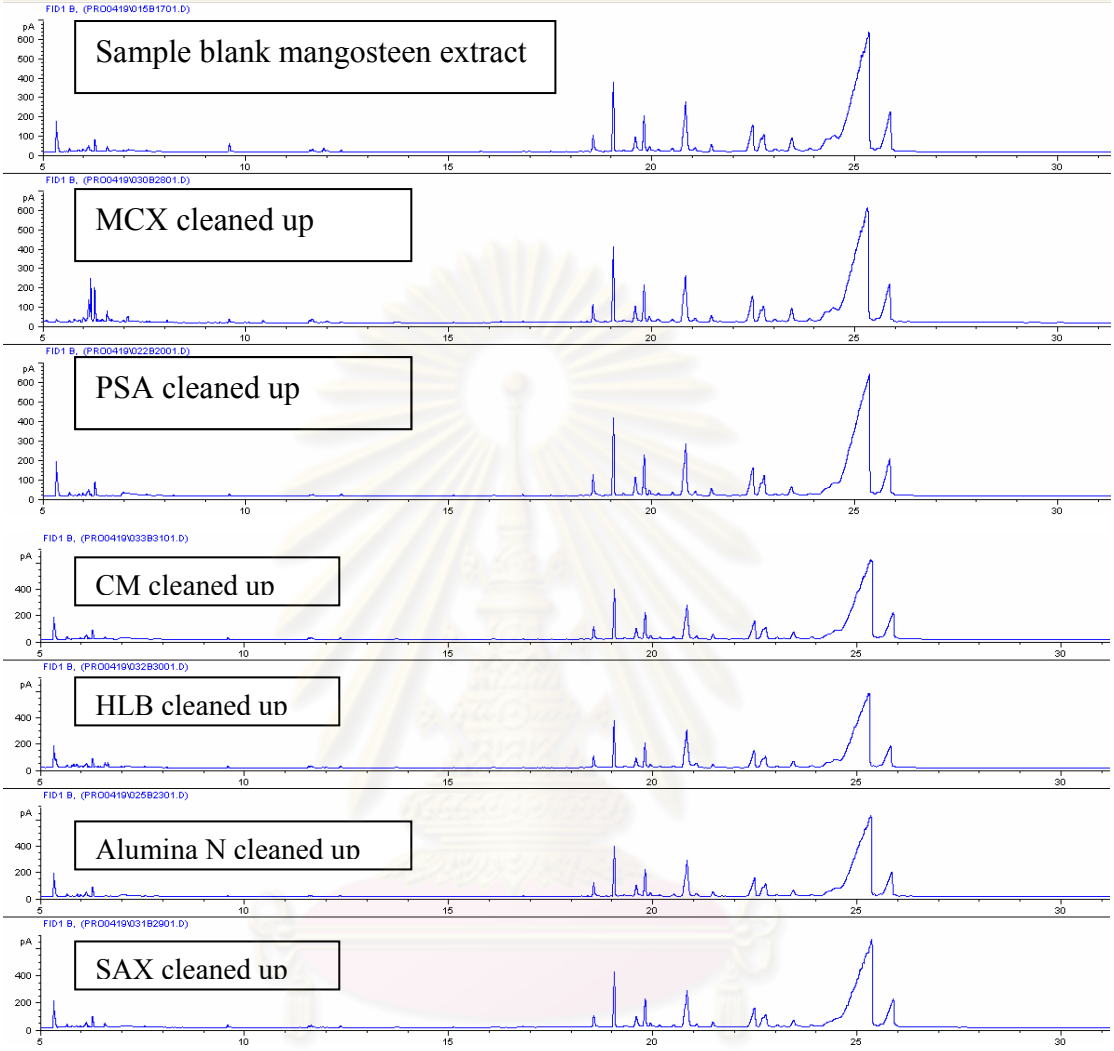


Figure 4.6 Typical chromatograms of sample blank mangosteen extract and mangosteen sample extract after d-SPE cleaned up with MCX, PSA, CM, HLB, alumina N and SAX

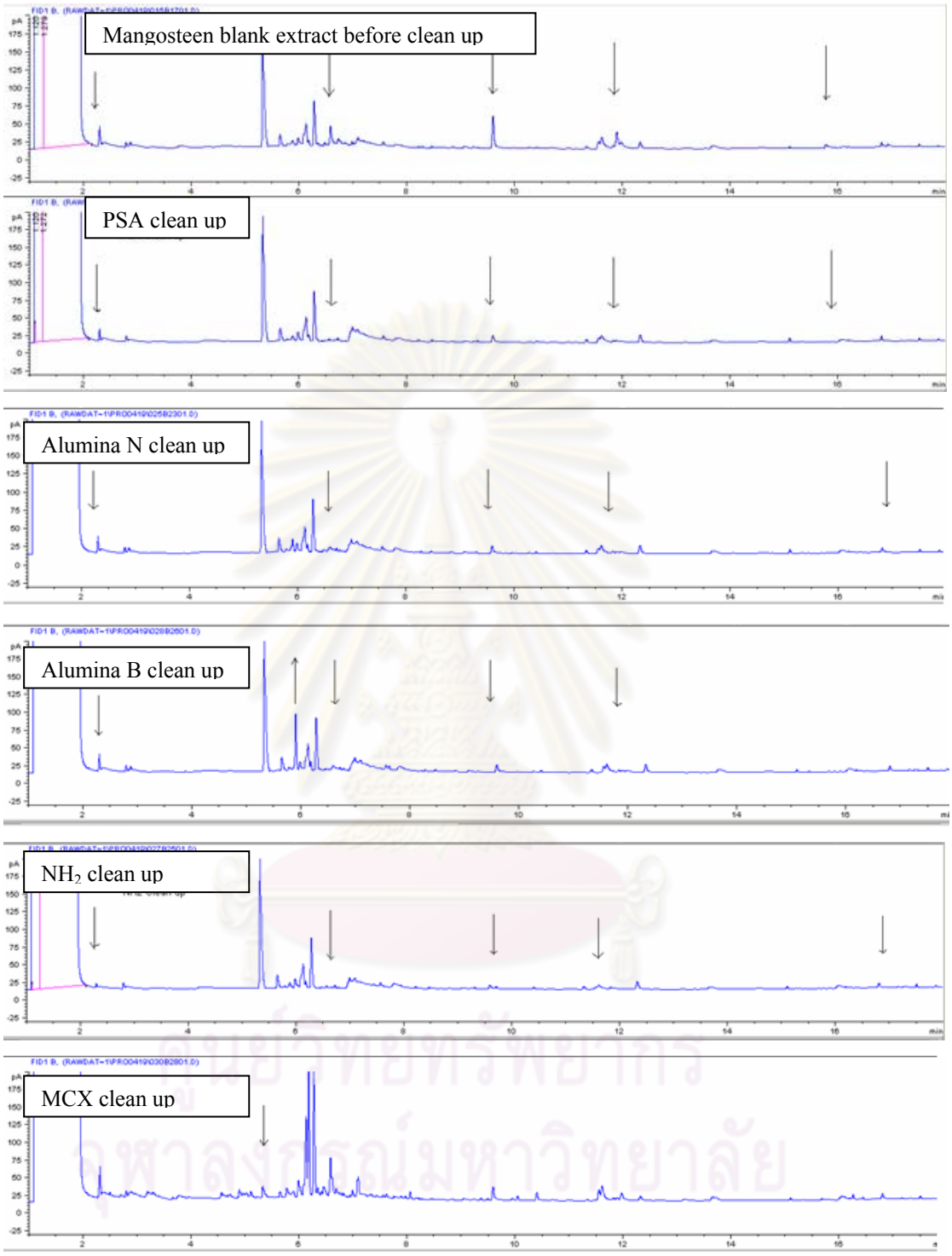


Figure 4.7 Typical chromatogram of sample clean up with PSA, alumina N, alumina B, NH_2, MCX sorbents

Table 4.4 Peak area at different retention times of the mangosteen extracts after clean up compared with various sorbents

RT	Peak Area													
	Sample	**GCB**	**PSA**	**Florisil**	**Silica**	**Alumina N**	**Alumina B**	**C-18**	**NH2**	**MAX**	**MCX**	**SAX**	**HLB**	**CM**
2.301	45.87	61.13	20.12	44.36	58.79	30.65	31.19	51.51	14.11	84.57	63.18	59.52	53.63	45.44
5.342	371.62	411.45	428.82	421.96	425.72	412.27	432.25	368.00	424.00	479.73	46.62	438.19	345.47	422.00
6.142	105.76	112.63	108.17	108.77	111.81	109.69	118.98	102.23	108.63	251.89	263.30	114.30	105.46	110.25
6.293	131.61	163.88	148.73	145.67	149.08	148.73	151.84	135.98	155.61	183.88	333.12	157.08	141.44	148.49
6.595	74.46	96.20	2.45	44.43	85.01	36.08	38.73	77.01	3.38	160.82	150.06	79.87	85.92	73.14
9.612	100.19	38.25	24.65	34.74	40.09	25.18	26.31	32.71	16.22	36.76	44.11	43.98	39.45	36.41
11.629	64.23	57.88	38.28	54.85	62.92	40.90	42.56	47.29	32.24	60.78	72.87	68.45	63.55	61.08
11.906	61.41	7.70	3.76	0.00	6.80	0.00	0.00	6.31	0.00	9.25	7.34	8.61	16.48	7.58
11.989	31.82	20.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18.545	265.52	243.00	289.10	276.52	275.98	262.54	267.35	258.84	279.21	281.31	254.23	264.37	237.27	256.30
No of peak	74	63	35	42	46	38	39	43	38	82	77	52	62	52

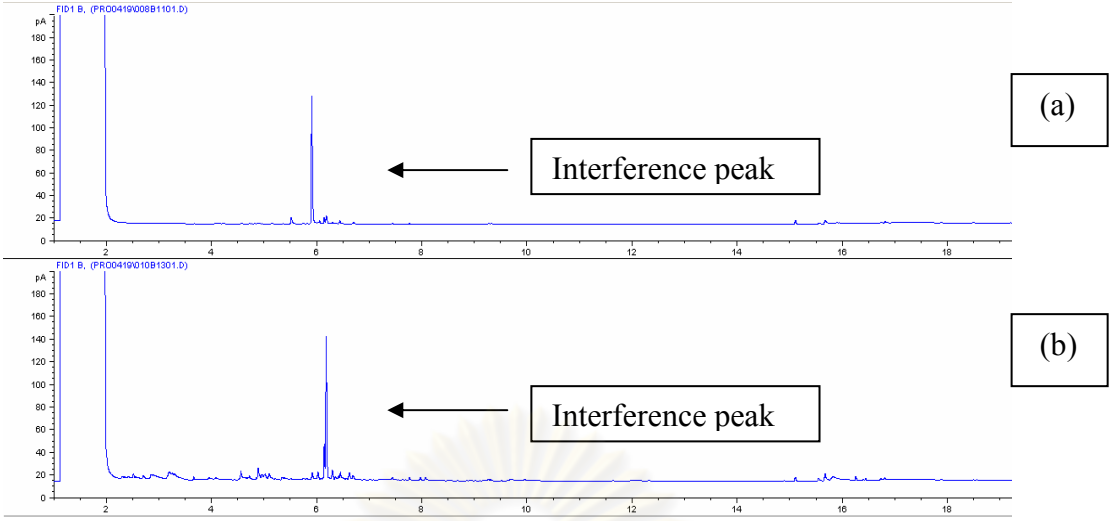


Figure 4.8 Typical chromatogram of reagent blank
(a) reagent blank of Alumina B sorbents
(b) reagent blank of MCX sorbents

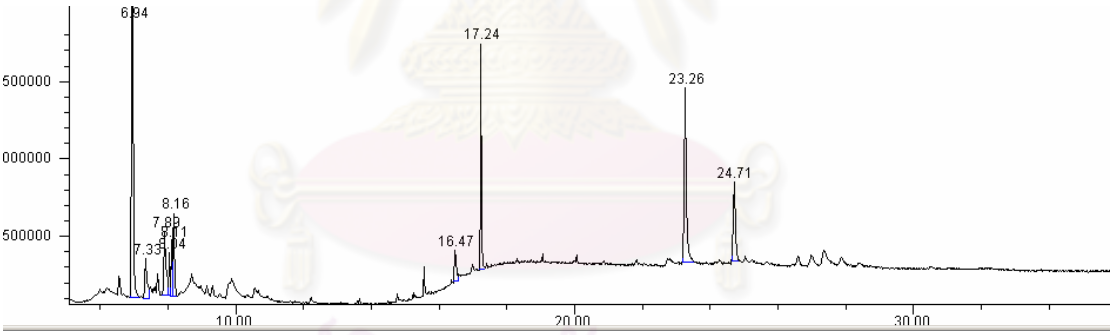


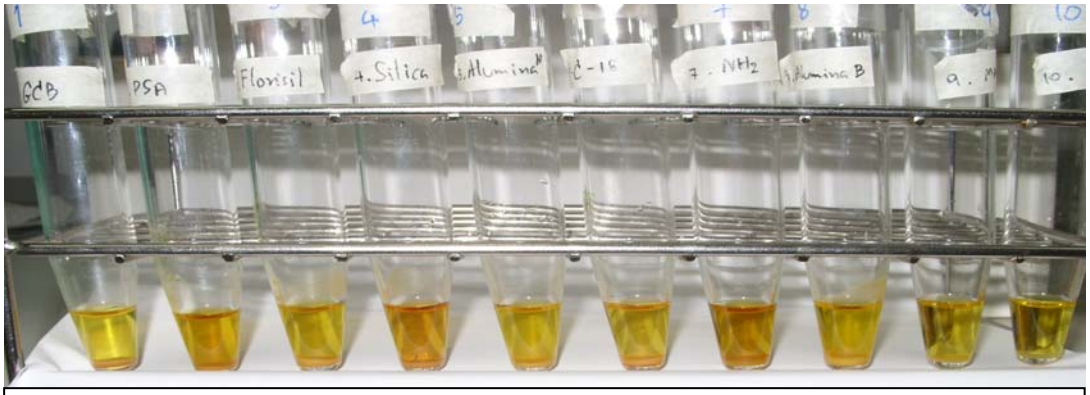
Figure 4.9 Typical chromatogram of mangosteen extract analyzed by GC-MS

Table 4.5 Library search reports of mangosteen extract analyzed by GC/MS

RT (min)	Matching Compounds found	% Match
6.94	Alpha.-Copaene	99
7.33	Trans-Caryophyllene	97
7.89	Valencene	99
8.04	Delta-Cadinene	99
8.15	7-epi-.alpha.-selinene	98
15.55	Trans-Farnesol	93
17.23	Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	95
23.26	2-(Geranylgeranyl)-6-methyl-1,4-benzohydroquinone	50
24.71	4-[.beta.-[p'-(Di-n-butylamino)-p-stilbenyl] vinyl]pyridine	90

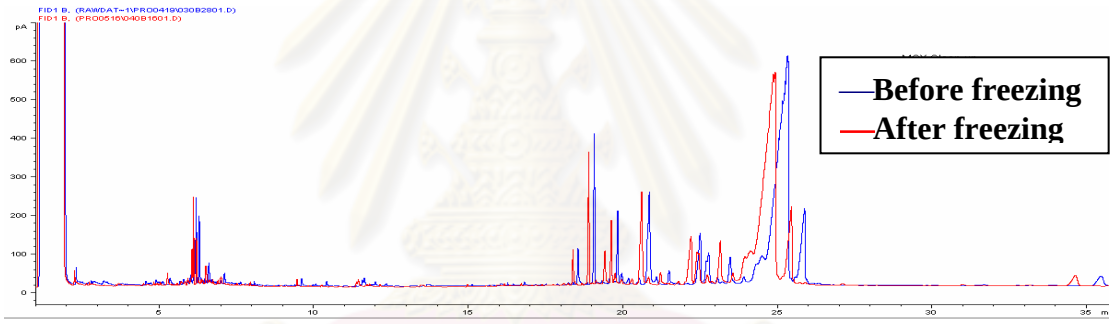
4.4.2 The Effect of Temperature for Clean up

Using temperature to removed matrix interference by freezing out. The extract after cleaned up was freezed at -20^{o}C overnight and centrifuged at low temperature -4 ^{o}C the results were shown in figure 4.10. The results showed some precipitate in tube's bottom except the extract sampled after d-SPE cleaned up with MCX showed the clearest solution. The clear solution injected to GC/FID, chromatogram of MCX which no precipitate showed interference peak as the same chromatogram of PSA which have precipitate, the chromatogram were shown in figure 4.11. The centrifuge at the low and ambient temperatures showed quite the same chromatograms. The chromatogram was shown in figure 4.12.

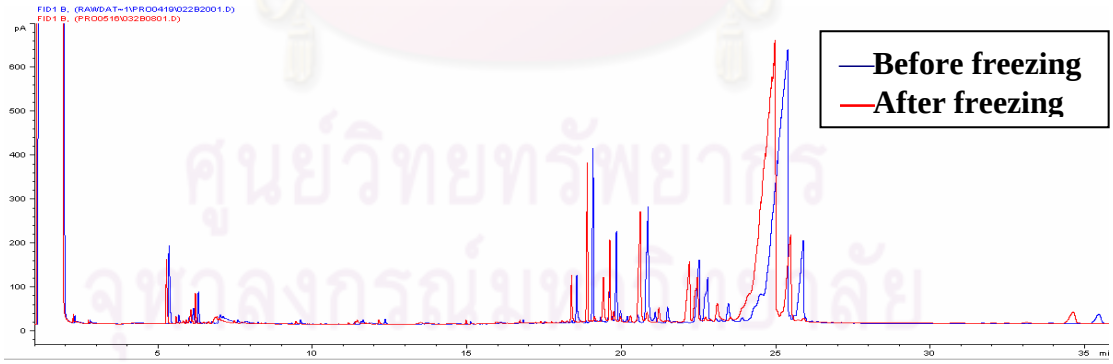


GCB, PSA, Florisil, Silica, Alumina N, C-18, NH_2, Alumina B, MAX, MCX

Figure 4.10 The extract samples after cleaned up and freezed at -20°C overnight



(a)



(b)

Figure 4.11 Typical chromatogram of the extract samples after cleaned up and freezed at -20°C overnight

(a) MCX cleaned up before and after freezing

(b) PSA cleaned up before and after freezing

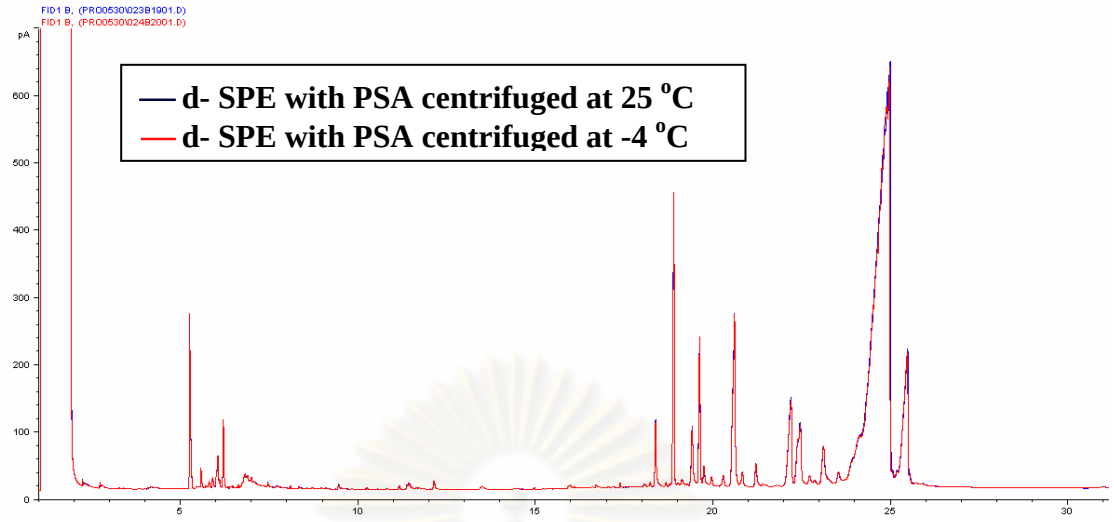


Figure 4.12 Typical chromatograms of the extract samples after cleaned up and centrifuged at 25°C and -4 °C

4.4.3 The Study of Effect of Sorbent Type

Polar interferences and large molecules such as fatty acids and wax were further removed by dispersive-SPE adding sorbent. Five different dispersive-sorbent types of primary secondary amine (PSA), alumina N, florisil, MCX, SAX were tested at 0.10 mg/Kg spiked level in three replicates. PSA, alumina N and florisil worked very well for removing interferences of fatty acids and wax in the matrix, theirs recovery data were shown in table 4.6 and figure 4.13.

Table 4.6 Mean recovery (n=3) data of 22 pesticides after clean up with 5 sorbents

Compound	Sorbent Type				
	PSA	**Alumina N**	**Florisil**	**MCX**	**SAX**
Oxamyl	113	89	87	166	163
Methomyl	168	145	166	228	215
Carbofuran-3-OH	145	82	143	192	67
Carbaryl	104	226	88	106	121
Carbofuran	178	134	110	159	101
Isoprocarb	120	197	88	107	131
Fenobucarb	167	145	95	124	155
Methiocarb	126	143	97	150	141
Bendiocarb	120	213	80	114	138
Propoxur	120	168	98	116	117
Propham	146	79	102	115	97
Carbosulfan	138	84	73	0	23
Alanycarb	337	44	43	0	0
Benfuracarb	150	11	79	1	0
Omethoate	86	94	93	301	109
Methamidophos	84	95	86	86	96
Mevinphos	93	113	126	144	108
Dimethoate	109	140	133	155	150
Monocrotophos	193	257	196	230	217
Dicrotophos	128	209	164	193	179
Diazinon	133	73	87	47	82
DDVP	91	138	103	110	139

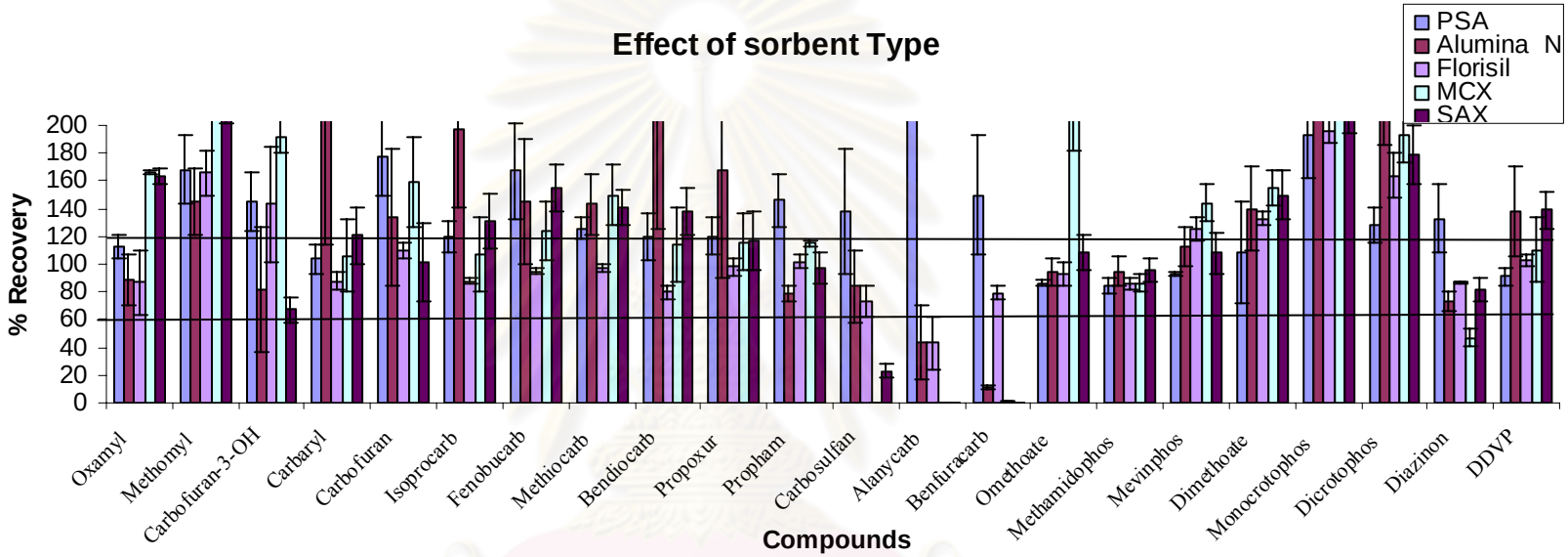


Figure 4.13 Effect of sorbent types: Mean recovery (n=3), of 22 pesticides spiked at 0.10 mg/Kg and cleaned up with PSA, alumina N, florisil, MCX, SAX.

4.5 The Study of Effect of Mixed and Weight of Sorbent

From tha data above, PSA, alumina N and florisil exhibited well for removing interferences of fatty acids and wax in the matrix. PSA with effective sorbents from QuEChERS method was combided with alumina N and florisil for study. Ratio of mix sorbent was evaluated to the optimum quantity by using 3 ratios: at 25: 25 mg, 50: 50 mg and 75: 75 mg at 0.10 mg/Kg spiked level in 3 replicates. Ratio at 25: 25 mg of both mixed sorbents gave the best recovery (range recovery data 60-120 %) of all compounds the results were shown in table 4.7-4.8 and figure 4.14. .

Table 4.7 Recovery (R) data of mixed sorbent PSA: alumina N at various ratios (25:25, 50:50, 75:75 mg)

Compound	**PSA : Alumina N 25 : 25mg**		**PSA : Alumina N 50 : 50mg**		**PSA : Alumina N 75 : 75mg**	
	% R	**SD**	**% R**	**SD**	**% R**	**SD**
Oxamyl	83	2.80	69	5.29	105	15.87
Methomyl	103	4.38	116	9.57	130	5.17
Carbofuran-3-OH	95	8.90	105	12.77	133	14.20
Carbaryl	102	3.20	110	11.13	123	4.25
Carbofuran	108	8.77	128	12.83	136	3.09
Isoprocarb	96	16.83	84	2.85	91	8.76
Fenobucarb	94	15.43	86	9.97	162	40.13
Methiocarb	96	25.89	70	19.84	86	11.21
Bendiocarb	91	2.32	93	3.20	97	5.76
Propoxur	82	4.52	97	3.14	101	4.60
Propham	90	6.87	98	9.13	111	18.58
Carbosulfan	90	28.43	135	52.16	148	54.67
Alanycarb	54	43.59	45	25.14	137	94.57
Benfuracarb	82	15.57	114	22.57	127	19.51
Omethoate	84	4.10	79	9.44	84	4.12
Methamidophos	72	2.26	78	2.93	78	3.53
Mevinphos	97	4.95	116	2.76	119	3.31
Dimethoate	107	11.54	112	3.77	133	14.85
Monocrotophos	95	5.38	104	8.25	105	2.52
Dicrotophos	89	1.88	105	3.47	108	3.45
Diazinon	86	9.94	97	9.15	143	13.87
DDVP	66	22.86	67	21.46	52	10.13

Table 4.8 Recovery (R) data of mixed sorbent PSA: florisil at various ratios (25:25, 50:50, 75:75 mg)

Compound	**PSA : Florisil 25:25mg**		**PSA : Florisil 50:50mg**		**PSA : Florisil 75:75mg**	
	% R	**SD**	**% R**	**SD**	**% R**	**SD**
Oxamyl	75	2.58	84	11.95	73	9.76
Methomyl	99	6.47	122	8.80	116	4.73
Carbofuran-3-OH	93	6.93	120	21.15	122	19.05
Carbaryl	76	49.38	109	4.59	122	3.87
Carbofuran	136	8.59	123	11.25	130	3.84
Isoprocarb	94	5.14	74	8.52	89	1.24
Fenobucarb	106	21.02	83	19.51	101	6.20
Methiocarb	73	2.85	117	21.06	75	5.73
Bendiocarb	80	6.46	107	3.07	85	1.46
Propoxur	83	6.37	87	7.83	91	2.74
Propham	84	12.89	87	6.49	75	5.91
Carbosulfan	96	23.28	61	13.19	68	1.17
Alanycarb	105	50.76	63	47.98	105	16.48
Benfuracarb	77	19.73	83	19.06	94	11.61
Omethoate	74	5.13	87	3.58	80	3.71
Methamidophos	68	1.36	77	2.12	74	1.95
Mevinphos	88	3.58	110	10.43	116	8.32
Dimethoate	90	4.99	112	7.41	120	5.78
Monocrotophos	109	4.59	114	9.97	106	3.64
Dicrotophos	95	5.78	101	9.17	99	2.26
Diazinon	104	4.12	85	13.38	85	15.00
DDVP	72	19.97	61	9.03	65	14.81

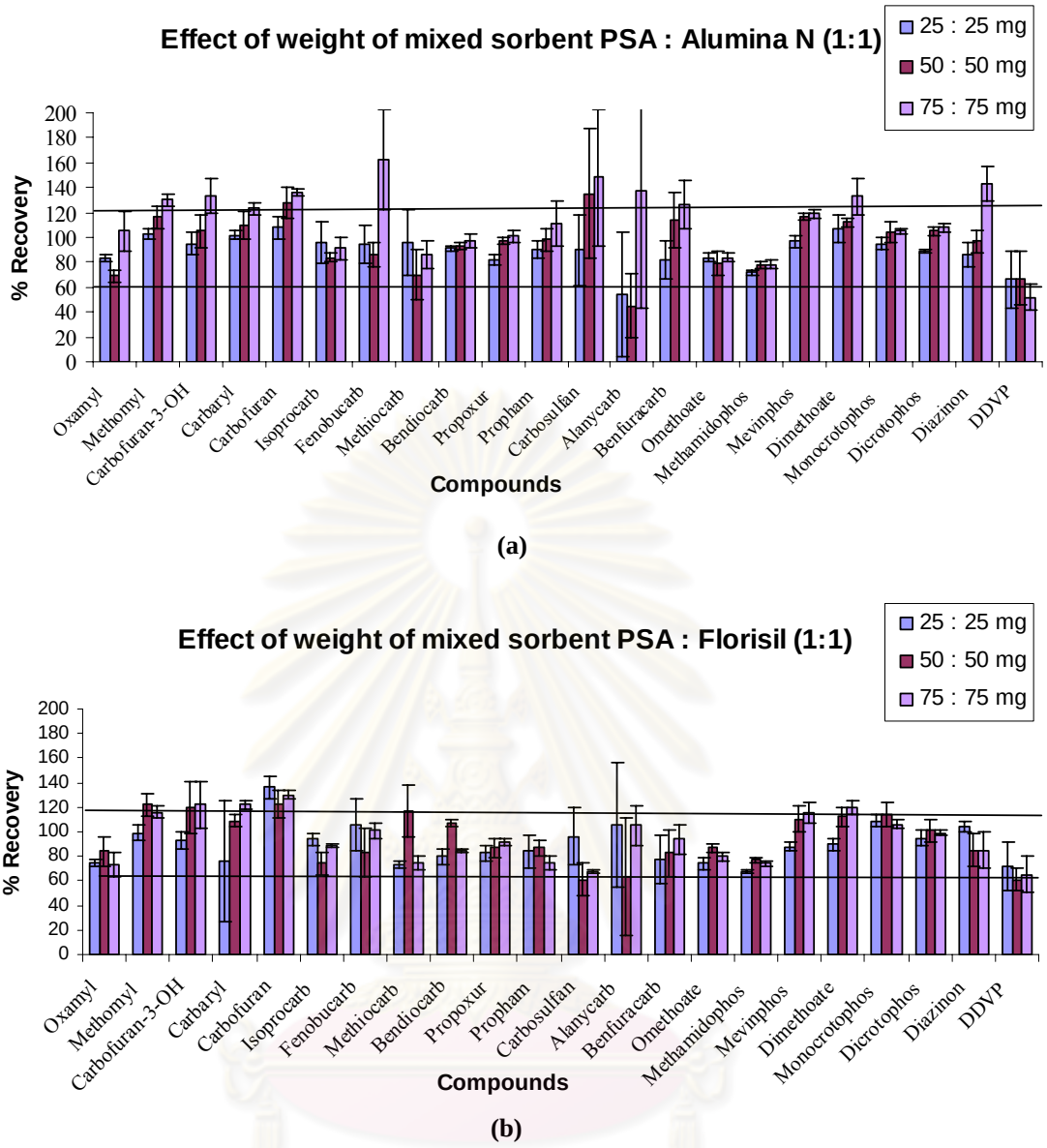


Figure 4.14: Extraction efficiency of mixed sorbents and theirs amount employed. Mean recovery (n=3) of 22 pesticides spiked at 0.10 mg/kg and cleaned up with the mixture of PSA: alumina-N (a) and PSA: florisil (b) at 3 levels**.**

4.6 Study of Effect of Mixed Sorbents in Term of Precision

The data obtained from the mixture of PSA & alumina-N and PSA & florisil sorbents were evaluated in term of precision at 10 replicates and 0.01 mg/kg spiked level. PSA and alumina N mixed sorbents gave higher precision at RSD 5.98-25.52 %, where PSA: alumina-N (25:25mg) and RSD gave 4.74-39.08 % for PSA: florisil (25:25mg) mixed sorbents. The precision data were shown in table 4.9 and 4.10. Mixture of PSA and alumina-N gave good precision at mixed ratio 25: 25 mg so it

was proved to be very good for cleaning up to remove the interferents of mangosteen matrix

Table 4.9 Accuracy and precision data of 22 pesticides at 0.01 mg/Kg spiked level (Mean, SD and %RSD) treated by PSA: alumina N mixed sorbent (n=10).

Compound	% Recovery										Mean	SD	% RSD
	1	2	3	4	5	6	7	8	9	10			
Oxamyl	67	67	74	74	74	55	73	79	58	39	66	12.14	18.39
Methomyl	96	131	81	79	60	91	67	67	64	87	82	21.00	25.52
Carbofuran-3-OH	127	104	105	121	85	124	114	49	85	90	100	23.88	23.79
Carbaryl	100	90	83	81	90	86	105	81	60	103	88	13.24	15.06
Carbofuran	140	100	104	106	117	98	96	119	112	109	110	13.03	11.83
Isoprocarb	120	116	114	112	125	119	115	108	94	90	111	11.21	10.07
Fenobucarb	128	98	103	117	107	100	108	89	106	101	106	10.71	10.13
Methiocarb	133	123	116	110	111	118	98	94	110	96	111	12.41	11.19
Bendiocarb	144	104	108	122	123	112	126	124	96	104	116	14.14	12.16
Propoxur	100	101	116	93	86	80	83	104	84	74	92	12.97	14.09
Propham	98	104	98	68	79	71	76	85	59	55	79	16.84	21.24
Carbosulfan	102	83	81	94	84	86	76	87	85	81	86	7.34	8.54
Alanycarb	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Benfuracarb	92	82	90	82	112	76	91	67	92	82	87	11.99	13.85
Omethoate	120	74	88	89	119	79	82	67	84	95	90	17.56	19.58
Methamidophos	81	77	78	81	70	71	73	74	70	70	75	4.45	5.98
Mevinphos	102	83	82	68	73	105	87	102	63	75	84	14.91	17.76
Dimethoate	104	113	110	110	94	83	105	86	67	98	97	14.66	15.11
Monocrotophos	100	84	83	90	88	78	85	79	75	81	84	7.15	8.48
Dicrotophos	105	97	93	92	78	88	86	88	81	86	89	7.81	8.73
Diazinon	91	78	71	73	74	80	75	81	79	70	77	6.14	7.96
DDVP	112	89	102	90	86	72	83	80	81	74	87	12.29	14.14

Table 4.10 Accuracy and precision data of 22 pesticides at 0.01 mg/Kg spiked level (Mean, SD and %RSD) treated by PSA: florisil mixed sorbent (n=10).

Compound	% Recovery										Mean	SD	% RSD
	1	2	3	4	5	6	7	8	9	10			
Oxamyl	53	26	74	88	87	21	66	81	78	56	63	23.95	38.01
Methomyl	75	50	67	62	80	31	47	56	66	66	60	14.44	24.06
Carbofuran-3-OH	66	54	77	87	79	91	99	66	58	114	79	18.98	23.99
Carbaryl	126	70	95	106	95	75	72	80	59	102	88	20.32	23.09
Carbofuran	55	71	70	79	88	96	106	97	94	99	86	16.15	18.89
Isoprocarb	95	103	103	108	82	77	95	92	84	82	92	10.57	11.47
Fenobucarb	99	58	80	87	102	96	77	83	87	88	86	12.65	14.76
Methiocarb	112	148	120	104	102	108	105	119	108	97	112	14.45	12.86
Bendiocarb	74	72	96	72	137	69	110	108	109	91	94	22.44	23.92
Propoxur	121	104	100	127	105	102	117	93	98	96	106	11.43	10.75
Propham	222	121	117	106	74	114	98	109	88	62	111	43.41	39.08
Carbosulfan	67	73	73	80	83	70	85	84	86	87	79	7.36	9.34
Alanycarb	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Benfuracarb	67	55	59	80	75	51	84	85	108	101	77	18.99	24.82
Omethoate	87	64	100	79	62	70	68	78	76	98	78	13.27	16.97
Methamidophos	115	78	78	70	69	62	68	65	68	66	74	15.33	20.74
Mevinphos	86	70	76	93	81	81	51	86	73	87	78	11.89	15.17
Dimethoate	85	127	112	91	115	56	81	64	86	95	91	22.22	24.36
Monocrotophos	77	72	76	88	77	72	88	82	70	83	79	6.50	8.28
Dicrotophos	80	71	77	78	75	74	80	78	84	80	78	3.68	4.74
Diazinon	76	91	92	106	93	95	104	110	107	107	98	10.59	10.79
DDVP	104	88	91	85	81	84	82	96	98	86	90	7.60	8.50

4.7 Method Validation

The performances of method were studied at concentration range from 5-125 μg/L. Standard calibration curve was used to determine analytes concentration in matrix-match standard to compensate matrix effect and interference. The coefficient of determination (R^2) for all compounds was shown in table 4.11 and appendix A. The propuxur obtained was low coefficient of determination (R^2) as shown in figure A-10 appendix A. The limit of detections (LODs) was observed at lowest calibration level (LCL) spiked level 0.005 mg/Kg in 10 replicates. The chromatograms were shown in figure 4.15. The accuracy and precision data at spiked level 0.01, 0.02, 0.05 and 0.10 mg/Kg and ten replicates were shown in table 4.11. Working range of method was 0.01-0.10 mg/Kg. The validation data of 20 compounds obtained demonstrated good method performance with satisfactory recovery and RSD range from 0.01 - 0.10 mg/Kg and proved to be accurate and precise since 0.01 mg/Kg spiked level as well as the limited of quantification (LOQ). This method can be analyzed for 12 compounds of carbamates and 8 polar organophosphates, expected 2 compounds as alanycarb and propuxor having low recovery and low coefficient of determination (R^2), respectively.

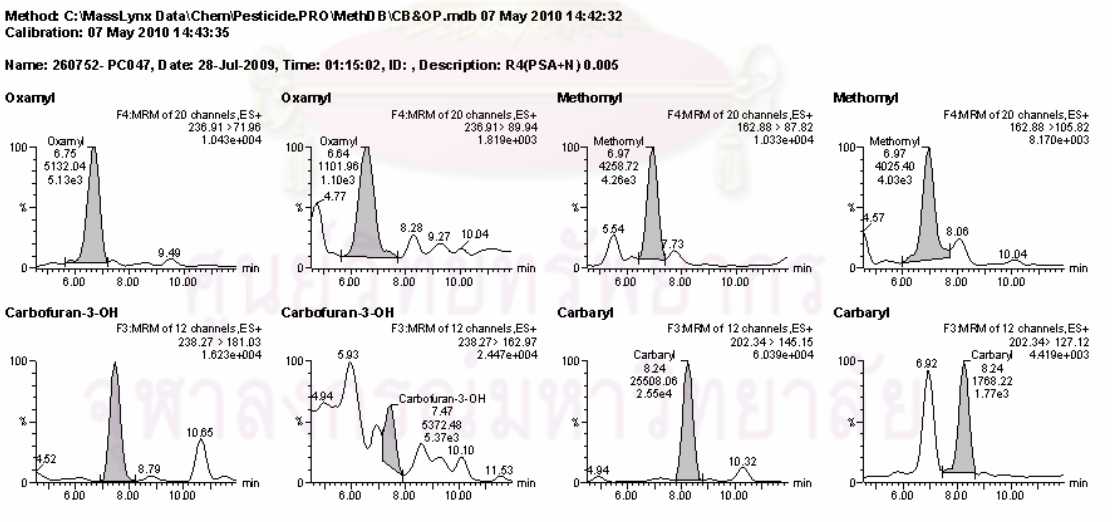


Figure 4.15 Chromatogram at spiked level 0.005 mg/ Kg of carbamate and polar organophosphate (LOD)

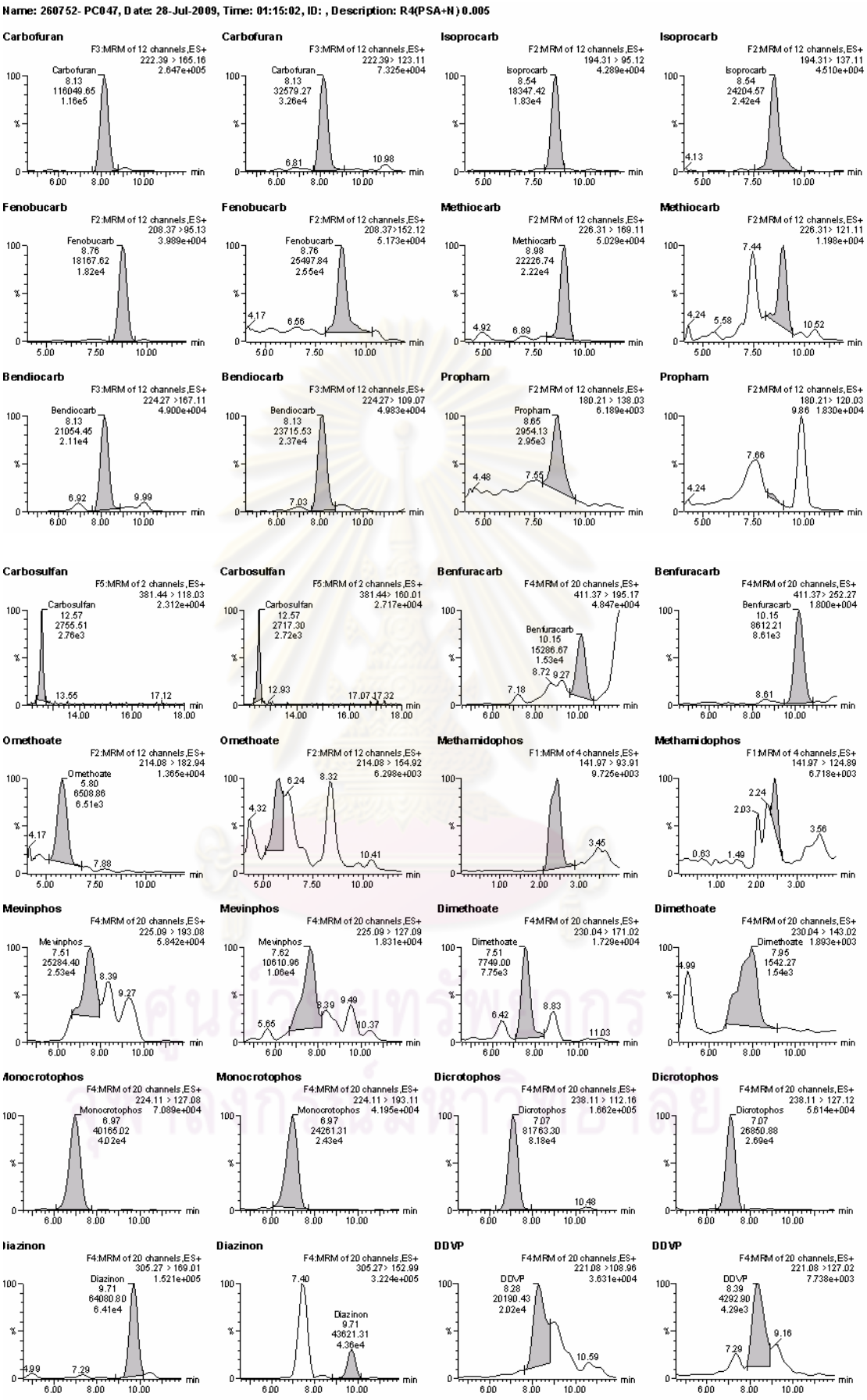


Figure 4.15 Chromatogram at spiked level 0.005 mg/ Kg (LOD) (continued)

Table 4.11 Performance of the method obtained for pesticide residuess in the mangosteen matrix.

Pesticide	R^2	The performance of method								LOD (mg/Kg)	LOQ (mg/Kg)
		0.01 mg/Kg		0.02 mg/Kg		0.05 mg/Kg		0.10 mg/Kg			
		* R	%RSD	* R	%RSD	* R	%RSD	* R	%RSD		
Oxamyl	0.9609	66	18.39	134	19.94	83	15.84	76	10.58	0.005	0.01
Methomyl	0.9773	82	25.52	90	25.48	90	7.71	101	6.32	0.005	0.01
Carbofuran-3-OH	0.9921	100	23.79	75	22.15	78	12.99	88	7.12	0.005	0.01
Carbaryl	0.9339	88	15.06	98	17.14	86	8.01	94	16.05	0.005	0.01
Carbofuran	0.9749	110	11.83	101	19.59	101	7.82	98	7.10	0.005	0.01
Isoprocarb	0.9662	111	10.07	108	9.05	75	24.42	87	5.01	0.005	0.01
Fenobucarb	0.9711	106	10.13	99	19.50	83	19.79	106	12.46	0.005	0.01
Methiocarb	0.9396	111	11.19	95	12.66	78	13.45	89	9.23	0.005	0.01
Bendiocarb	0.9911	116	12.16	118	11.55	75	22.98	93	11.00	0.005	0.01
Propham	0.9559	79	21.24	113	8.98	79	18.86	88	9.34	0.005	0.01
Carbosulfan	0.9918	86	8.54	89	9.58	108	31.06	70	6.50	0.005	0.01
Benfuracarb	0.9849	87	13.85	76	19.96	74	15.58	75	10.52	0.005	0.01
Omethoate	0.9947	90	19.58	68	5.98	76	14.98	76	8.81	0.005	0.01
Methamidophos	0.9887	75	5. 98	63	6.23	70	6.03	69	3.54	0.005	0.01
Mevinphos	0.9843	84	17.76	75	16.74	102	13.15	78	6.46	0.005	0.01
Dimethoate	0.9847	97	15.11	97	19.96	81	15.91	100	10.41	0.005	0.01
Monocrotophos	0.9930	84	8.48	80	7.30	89	7.02	81	3.75	0.005	0.01
Dicrotophos	0.9963	89	8.73	104	7.55	93	9.02	93	5.13	0.005	0.01
Diazinon	0.9773	77	7.96	82	5.87	85	20.67	93	5.97	0.005	0.01
DDVP	0.9701	87	14.14	73	13.01	95	8.64	93	7.28	0.005	0.01

*R Mean recovery (n = 10)

CHAPTER V

CONCLUSIONS AND SUGGESTIONS FOR FUTHER STUDY

A modified QuEChERS procedure was proved to be satisfactory for the extraction of carbamate and polar organophosphate residues in whole mangosteen. The optimum method employed acetonitrile added with combination of salt magnesium sulfate and sodium chloride and sodium acetate buffering agent to induce liquid phase separation as well as stabilize acid and base labile pesticides. Five sorbents such as PSA, Alumina N, Florisil, MCX and SAX were suitable for mangosteen matrix, MCX sorbent provided most clear colour but recovery data was not good in recovery studied as SAX. sorbent. PSA, Alumina N, Florisil provided good recovery but not best recovery for all compounds, then, three solvents were evaluated in mixed sorbents with ratio 1:1 and evaluated in term of weight of sorbent at 25, 50, 75mg. PSA and alumina-N in ratio 25 : 25 mg showed better result than PSA and florisil mixed sorbents in term of precision data. PSA and alumina-N mixed sorbents in ratio 25: 25 mg (1:1) were used as dispersive mixed sorbent to clean-up the polar interferences and fatty acids in the mangosteen matrix. The validation data demonstrated good method performance with satisfactory recovery range from 66-116 % and RSD 9-26 % for carbamate and recoveries 75-97% and RSD 5-20 % for polar organophosphate. The limit of detection (LOD) was 0.005 mg/Kg and limited of quantification (LOQ) was 0.01 mg/Kg. Range of method were at 0.01-0.10 mg/Kg. When the method was analyzed in optimized condition, details were as follow:

7. Weighed: 10g of homogenized mangosteen were weight into 50 mL centrifuge tube.
8. Spiked: Mix standard were spiked into sample. The tubes containing spiked samples were vortexed for 30 sec and left standing for 10 min to allow timing for pesticides interact with matrix.
9. Add extracting solution: 10 mL of acetonitrile were added into spiked sample.
10. Extraction/Partition: 4g of anhydrous $MgSO_4$, 1g of NaCl and 1g of anhydrous sodium acetate were added into centrifuge tube.After that, cap tightly and mix

on vortex mixer immediately for 1 min. then centrifuged the extract for 10 min at 3500 rpm.

11. Cleaned up: 1 mL of the upper extract was cleaned up with mix sorbent between PSA:Alumina N (25:25mg) After that, cap tightly, shaked and vortex 30 sec then centrifuge the extract for 5 min at 10000 rpm.
12. The clear extract was transfered into 2 mL amber vial for inject LC-MS/MS and quantitative analysis by bracketing calibration curve.

The method is safe and quick for simultaneous determination of 20 compounds of carbamate and polar organophosphate residues in mangosteen with QuEChERS method which stands for quick, easy, cheap, effective, rugged and safe.

Quick

- The method was streamlined extensively by avoiding or redesigning various inconvenient analytical steps such as clean up with LLE or SPE and pre concentration step that complicate traditional multi residues method.
- Carbamate and polar organophosphate residues can analyzed in the same instrument and analysis in qualitative and quantitative in one run, which different that conventional method carbamate analyzed by HPLC/Post column derivatizer (pickering) or LC-MS and organophosphate analyzed by GC/FPD.

Easy

- A single person performed the method without much training or technical skill.
- The method can be done in a small mobile or field laboratory.
- The method avoided glassware and cleaning and storage and rinsing.

Cheap

- The method was used very little glassware, the mixing on vortex mixer rather than blending. The extraction/partition procedure was performed in a sealed centrifuge tube, which was the only item to be cleaned or reused.
- The method needed very little bench space.
- The reagent costs in the method were not so expensive.
- The little devices were needed to carry out sample preparation.
- No special equipment needed for extraction such as rotary evaporator, and manifold for SPE cleanup
- Time, labor and expense were saved.

Effective

- High recoveries were achieved for a wide polarity and volatility range of pesticides such as carbamate and polar organophosphate.
- The cleanup step was considerably simplified by introducing the dispersive-SPE approach, in which the SPE material is simply mixed with a portion of the extract without dilution of the extract and better cleanup is obtained.
- The method need no concentrating by rotary evaporator or nitrogen evaporator, thus, reduce contaminating from glassware and cross contaminate of sample evaporated. And no evaporating of extract to dryness, that can avoid lost in this way.
- Analyzing carbamate and polar organophosphate residues in mangosteen by LC-MS/MS analyzed for qualitative, quantitative and confirmation in one run at default MRL 0.01 mg/Kg.

Rugged

- The method was quite rugged because extract cleanup was done to remove organic acids from fruit and vetgetable.
- The selectivity of the partitioning step has been optimized by addition of salts and further selectivity is achieved with cleanup using PSA and alumina N mixed sorbents.

Safe

- The acetonitrile was added by dispenser to an unbreakable vessel that was immediately scaled, thus minimizing solvent exposure to the chemist.
- Solvent usage and waste were very small and no chlorinated solvent were used, which was safe to the environment.

This method determined pesticide residues in mangosteen such as carbamate and polar organophosphate by LC-MS/MS. Tandem mass (MS/MS) was operated in multiple reaction monitoring mode with the most two sensitive transitions used for both quantification and confirmation. Additionally, the work can analyzed residues at low concentration level of 0.01 mg/Kg which is compliance to the benchmark parameters of Directive EC 396/2005. This study can also be extended to detect other pesticide residues in mangosteen, especially for compound analyzed by GC such as pyrethroid, endosulfan and other organophosphates monitoring for Thailand export. The QuEChERS technique provided cheap and effective method for analysis at trace level and could be further applied with other matrices or analytes such as pesticide residues in durian, antibiotics in animal tissue and mycotoxin in cereal.

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APPENDICES

APPENDIX A

A. Matrix Matched Standard Calibration Curve

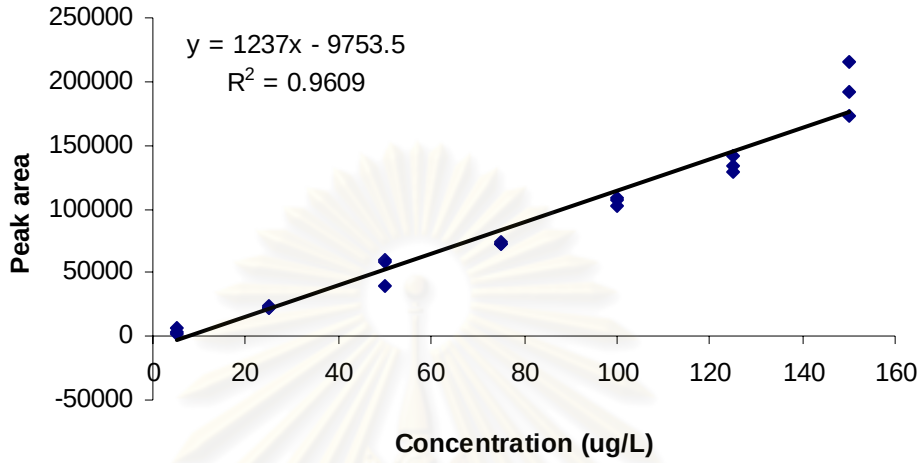

Figure A-1 Matrix-matched standard calibration curve of oxamyl

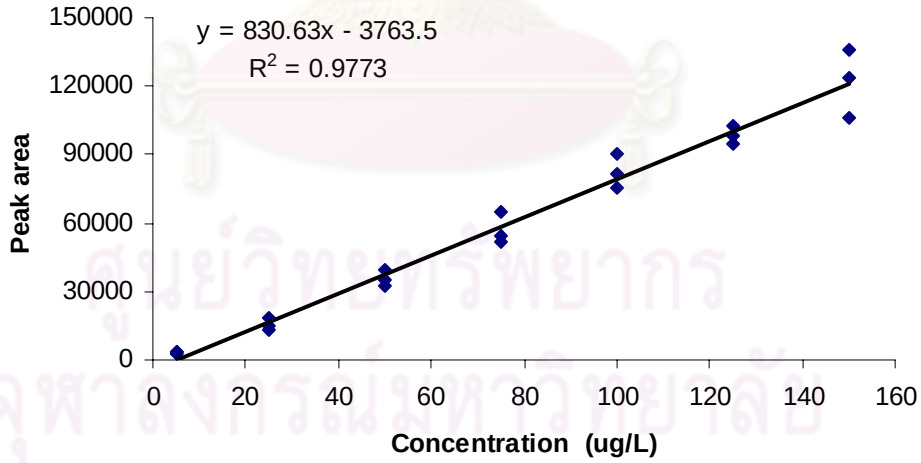

Figure A-2 Matrix-matched standard calibration of methomyl

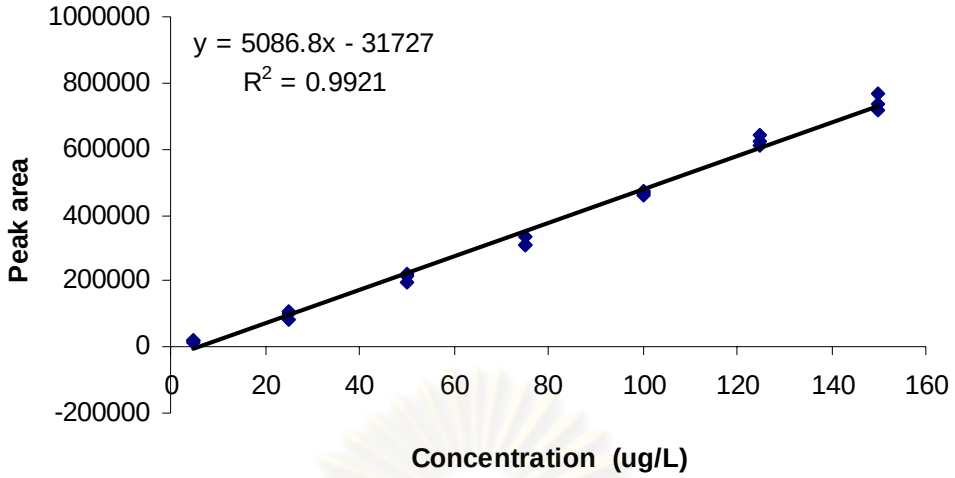

Figure A-3 Matrix-matched standard calibration of carbofuran-3-hydroxy

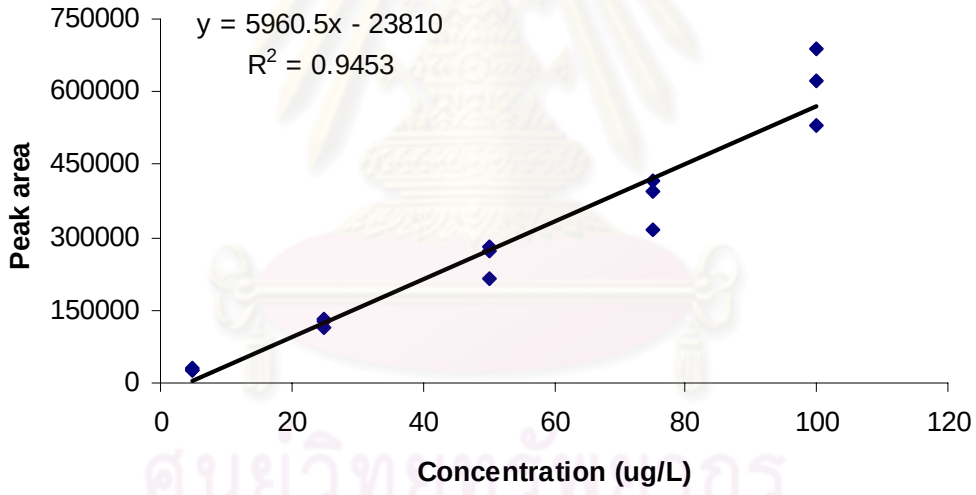

Figure A-4 Matrix-matched standard calibration of carbaryl

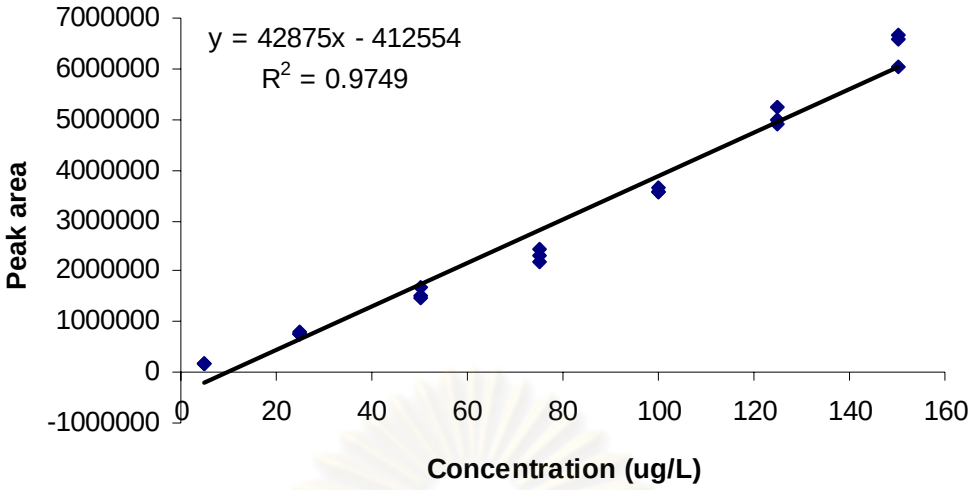

Figure A-5 Matrix-matched standard calibration curve of carbofuran

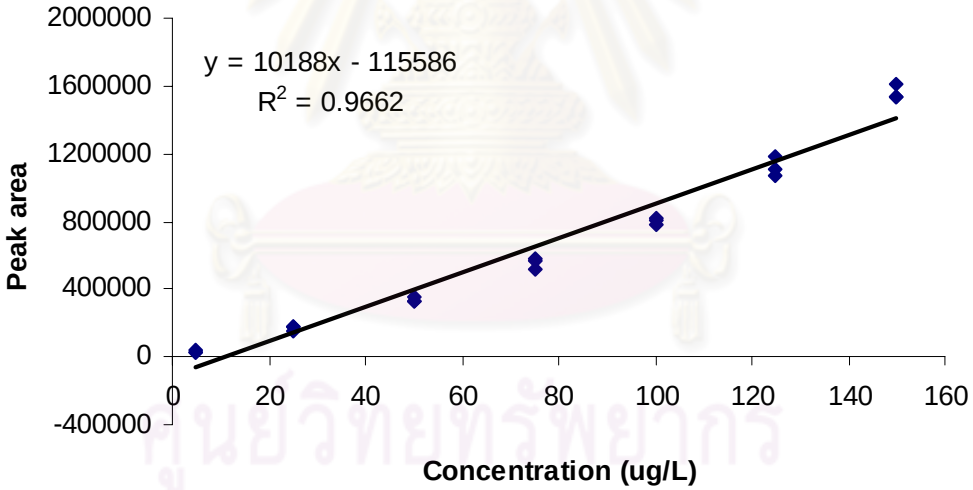

Figure A-6 Matrix-matched standard calibration curve of isoprocarb

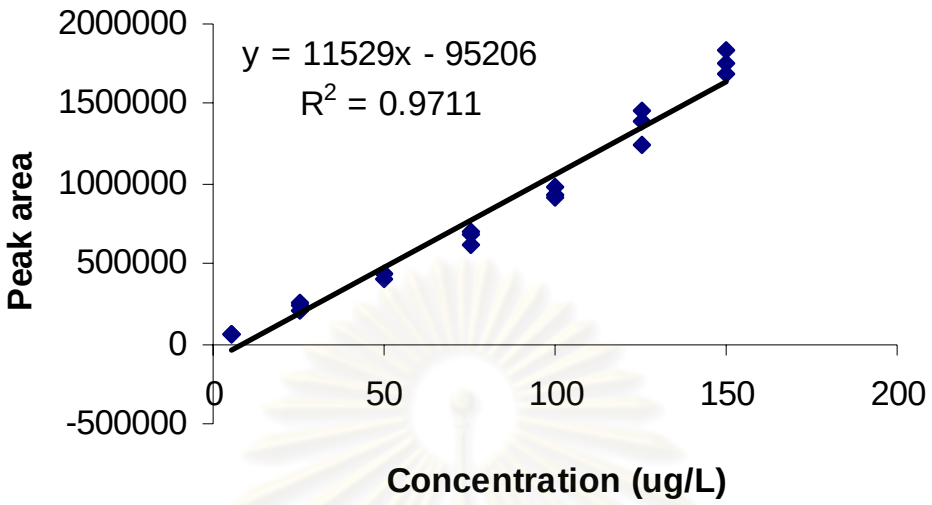

Figure A-7 Matrix-matched standard calibration curve of fenobucarb

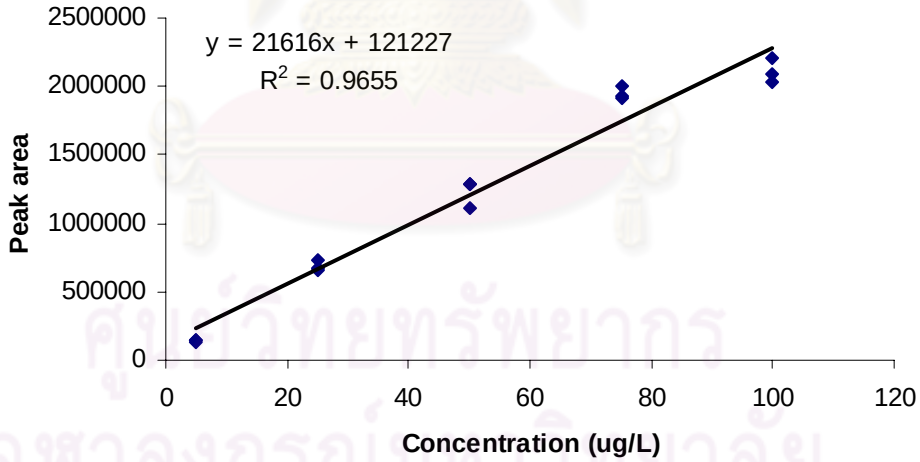

Figure A-8 Matrix-matched standard calibration curve of methiocarb

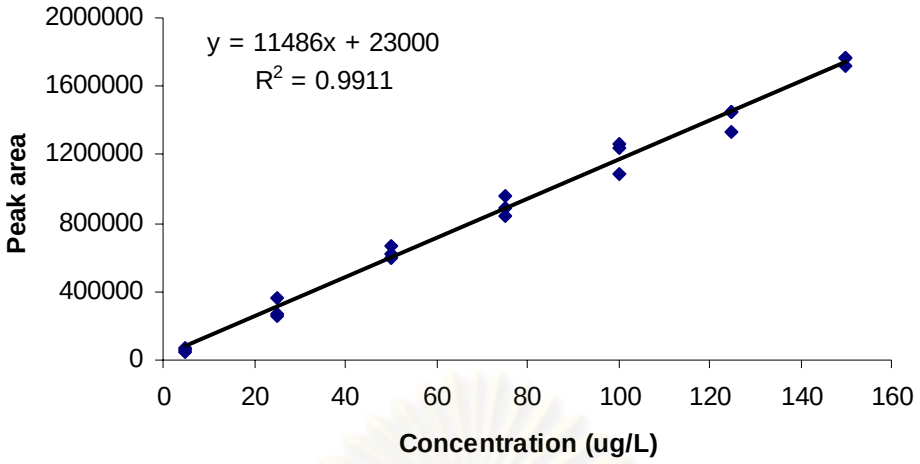

Figure A-9 Matrix-matched standard calibration curve of bendiocarb

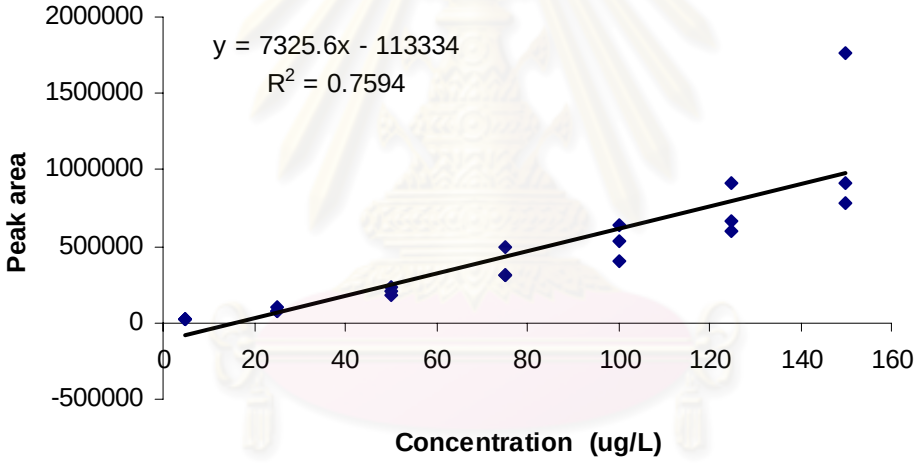

Figure A-10 Matrix-matched standard calibration curve of propoxur

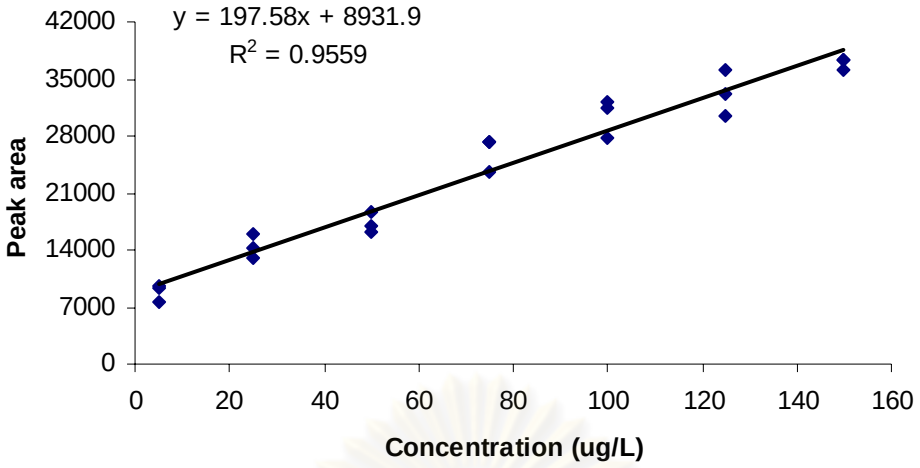

Figure A-11 Matrix-matched standard calibration curve of propham

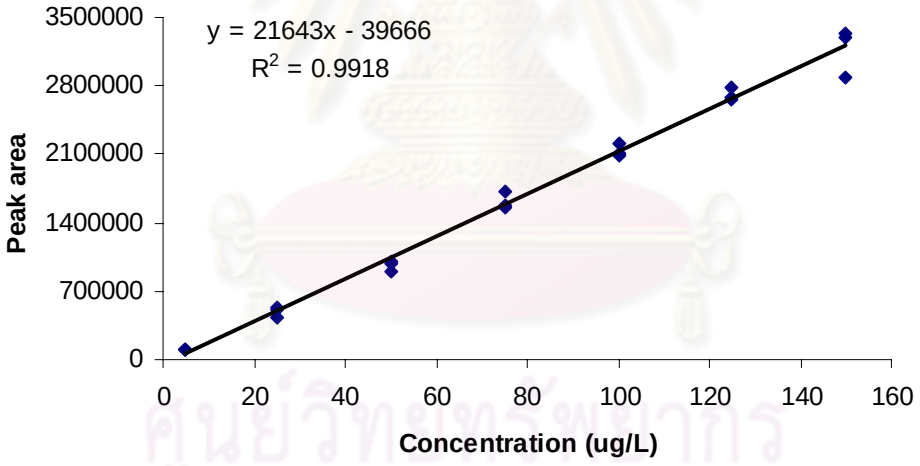

Figure A-12 Matrix-matched standard calibration curve of carbosulfan

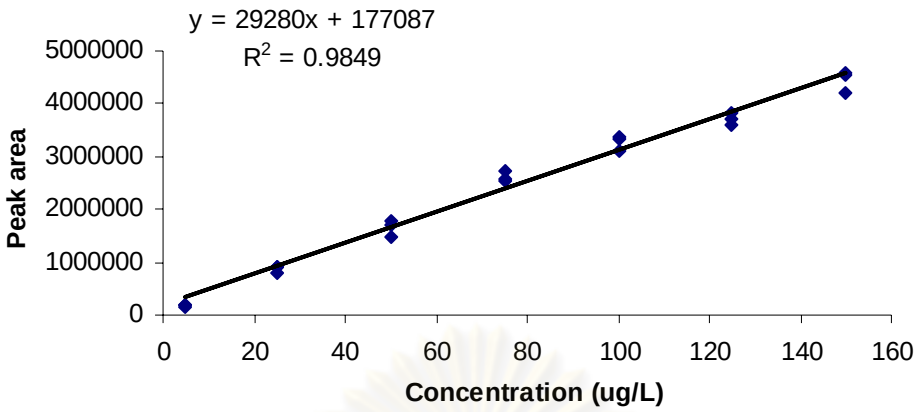

Figure A-13 Matrix-matched standard calibration curve of benfuracarb

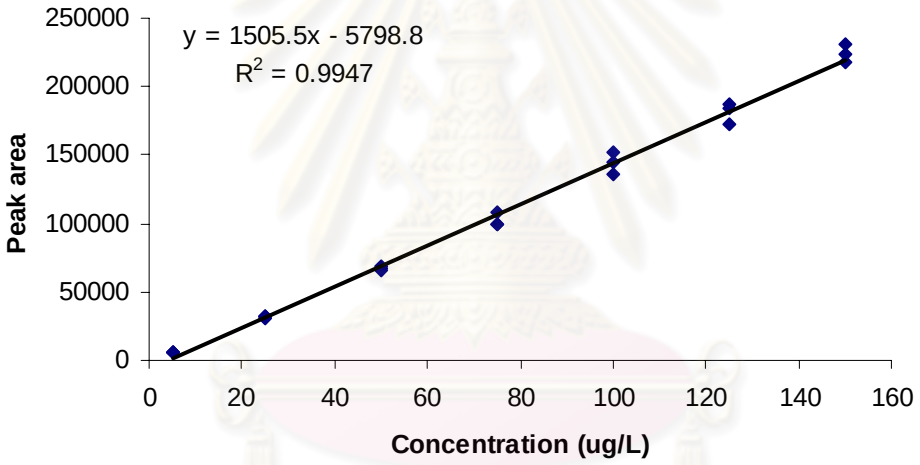

Figure A-14 Matrix-matched standard calibration curve of omethoate

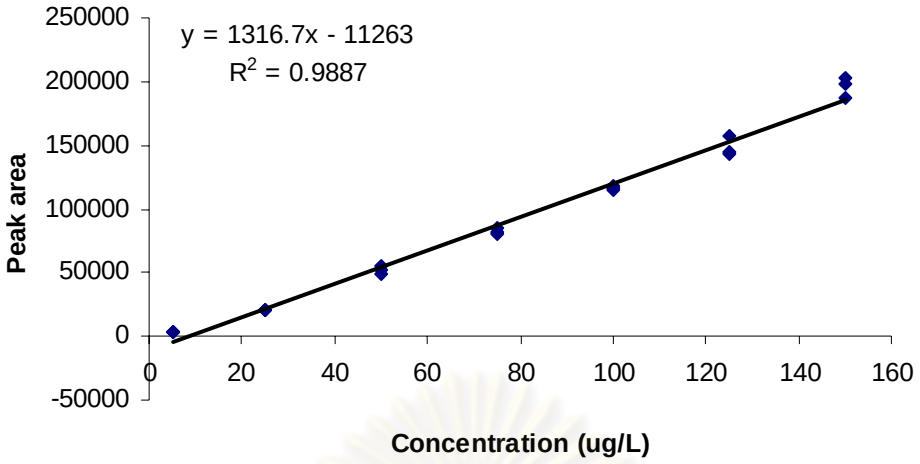

Figure A-15 Matrix-matched standard calibration curve of methamidophos

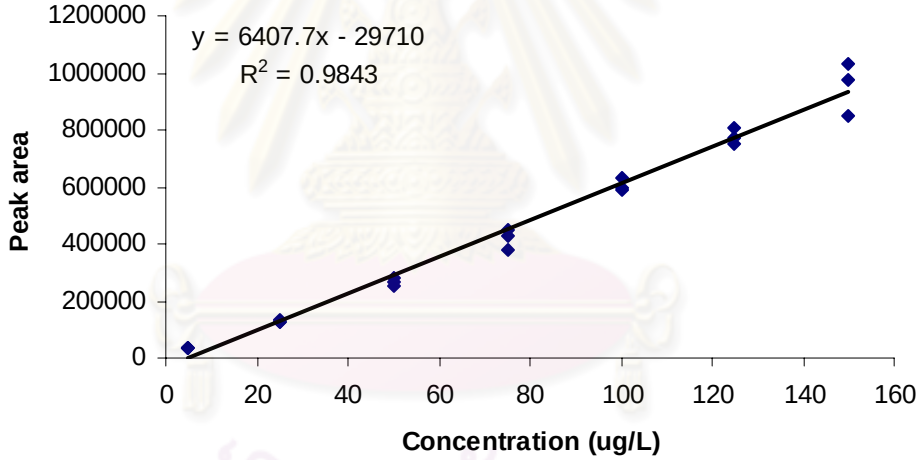

Figure A-16 Matrix-matched standard calibration curve of mevinphos

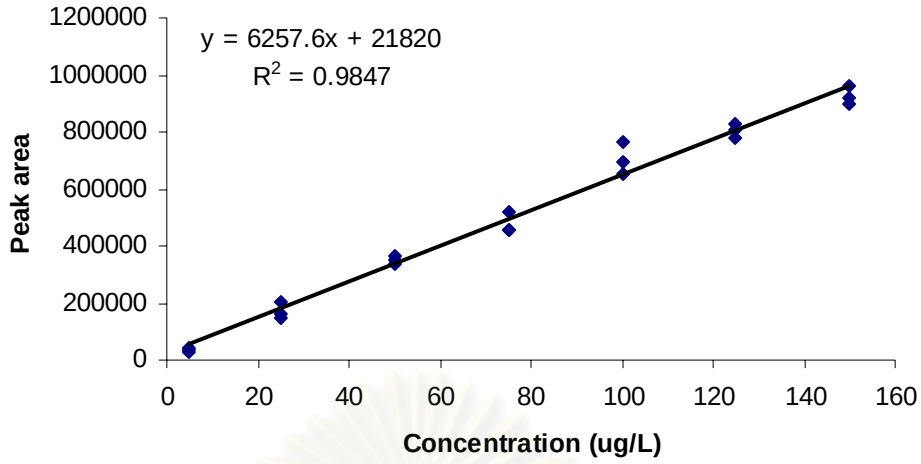

Figure A-17 Matrix-matched standard calibration curve of dimethoate

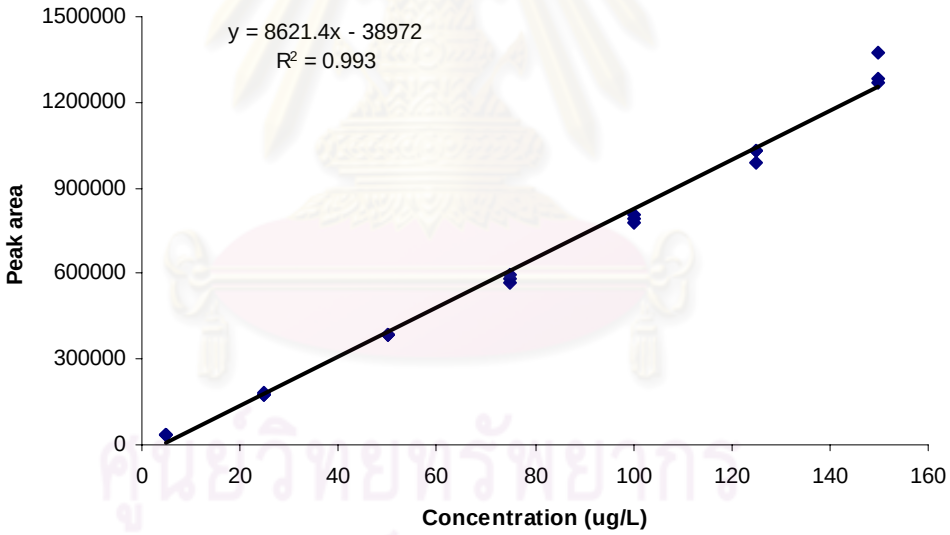

Figure A-18 Matrix-matched standard calibration curve of monocrotophos

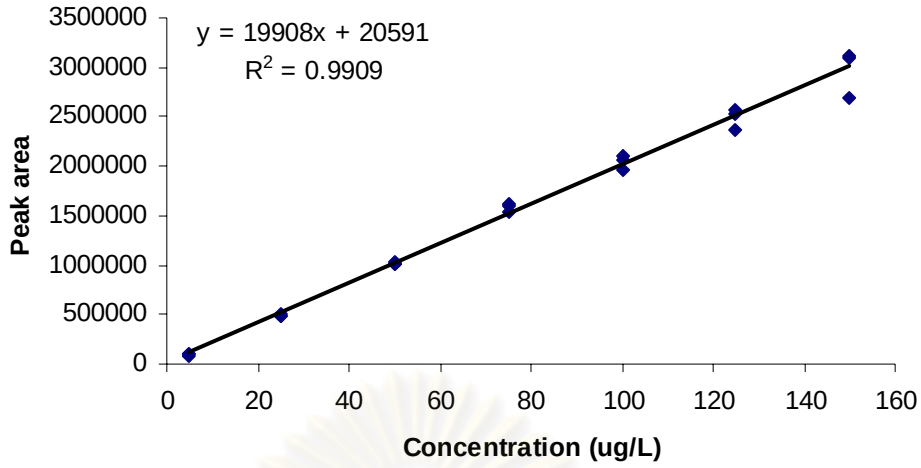

Figure A-19 Matrix-matched standard calibration curve of dicrotophos

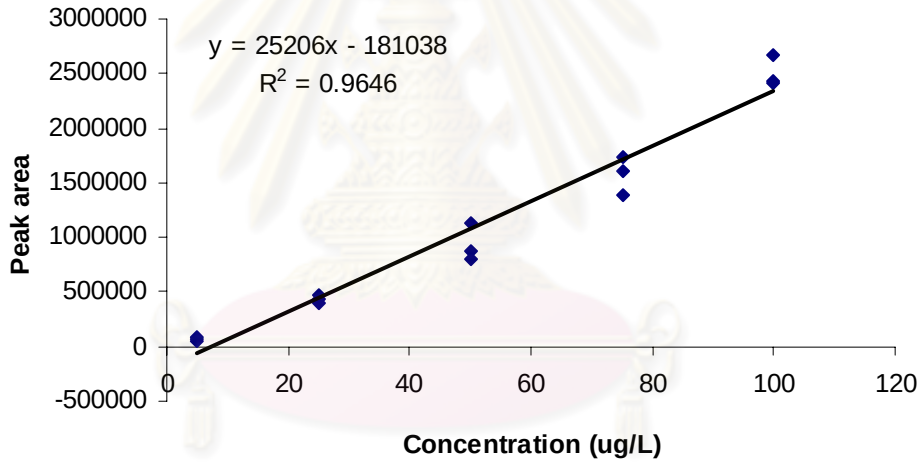

Figure A-20 Matrix-matched standard calibration curve of diazinon

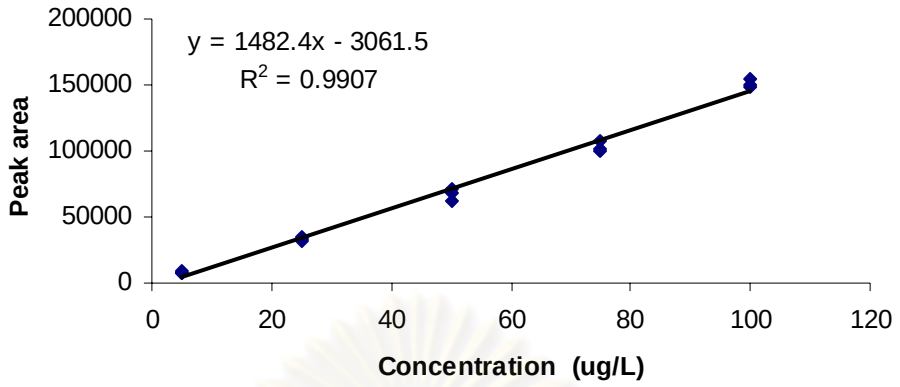

Figure A-21 Matrix-matched standard calibration curve of dichlorvos (DDVP)

VITA

Ms. Wanisa Meecharoen was born on March 23, 1974 in Hua Hin, Prachuap Khiri Khan, Thailand. She gradruated with a Bachelor's degree of Science in Chemistry from Rajabhat Suandisit University in 1994. She had worked on pesticide residues analysis in food at OMIC Company from 1994 until 2004. Since April 2004 until present she has been working at Central Laboratory (Thailand) Company as a chief chemist for pesticide residues and mycotoxin analysis. In 2007, she continued her academic education for master degree at Department of Chemistry, Faculty of Science, Chulalongkorn University.