IDENTIFICATION AND QUANTIFICATION OF PESTICIDES RESIDUE IN VEGETABLES BY LIQUID CHROMATOGRAPHY QUADRUPOLE-TIME-OF FLIGHT MASS SPECTROMETRY (LC-Q-TOF MS)

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CHEMISTRY DEPARTMENT

FACULTY OF SCIENCE

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ABSTRACT

A liquid chromatography tandem mass spectrometry (LC-Q-TOF MS) technique was used to simultaneously determine six types of carbamates and organophosphorus pesticides residue in vegetables. The pesticides were extracted by ultrasonic solvent extraction method using ethyl acetate as the extraction solvent followed by Florisil column clean up. Four types of common consumed vegetables from local hypermarket were used to study the recovery. The accurate mass measurement and retention time were used to identify and determine the empirical formula of the pesticides. The mass accuracy errors were less than 7 ppm for all the investigated pesticides. The limit of detection (LOD) were 10 ppb for all the pesticides. Calibration curves were obtained from the pesticides in pure methanol solvent from the range of 0.01ppm to 0.40 ppm. The linearity coefficient, R^2 of the calibration curve for each type of pesticides was > 0.99. The recoveries of acephate and profenofos were 80–120%, and 50-130% for dimethoate, diazinon and quinalphos. All the recoveries of pesticides were in the acceptable range except for carbaryl which was less than 30%.

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Chapter 1

Introduction

1.1 Pesticides

Pesticides mainly used in agricultural during harvest to control pests and diseases. Pesticides also used after harvest or during storage to ensure the quality and requirements of the consumers. There are more than 800 active compounds that have registered to legislation bodies used worldwide nowadays.[1-4] There are many ways to classify pesticides. Pesticides can be classified according to their active ingredient into different chemical classes such as Carbamates, Organochlorines, Organophosphates, Pyrethroids and Thiocarbamates. All the pesticides have different physical and chemical properties, polarity and volatility.[5] Besides the different chemical classes, pesticides can also group as broad spectrum pesticides and narrow spectrum pesticides. Broad spectrum pesticides are used for general purposes and a wide range of pest will be killed, whereas, the narrow spectrum pesticides are specified in one species.

Pesticides are one of the groups of chemical compound that need to be controlled due to their toxicity and threats to human health. Some of the pesticides residue may break down into non-toxic byproduct quickly but some may not. The period of pesticides residue leave on crops are varied, it may leave for weeks, months and even though years. These pesticides residue may cause health problems to human. The effects of pesticides depend on the active ingredients in the pesticide compounds, for example, the organophosphates and carbamates will affect the nervous system. Other effects on human health area such as irritate the skins or eye, affect the hormone or endocrine system and some of the pesticides even are carcinogens.

Vegetables are consumed daily because they contain a lot of vitamins and minerals that are important to maintain human's health, therefore, the concentration of

pesticides residue on the vegetables are very important to be monitored to ensure consumers' safety. Legislation bodies in many countries had established the Maximum Residue Limits (MRLs) to control and monitor the pesticide residue on food.[1,2]

MRLs are defined as the maximum concentration of pesticide residue in milligrams of residue per kilogram of food (mg/kg). MRLs are established to ensure the pesticides are used correctly, to ensure the minimum exposure or intake by the consumers but not as an indicator for the approval of pesticides to use on a particular crop. Powerful, sensitive and selective analytical techniques are required for identifying and characterizing targeted pesticide compounds that fulfill the low MRL.[1,6]

1.2 Analytical Techniques Used For Pesticides Residues

For many years, the widely used techniques for pesticides residue analysis are gas chromatography (GC) with electron capture detection (ECD), nitrogen-phosphorus detection (NPD), mass spectrometry (MS) detection and other GC techniques.[2,7,8,9] Recently, liquid chromatography has been used increasingly for the pesticides residue analysis due to its suitability for most of the pesticides which are thermally labile and non volatile that difficult to be analysed by gas chromatography techniques. Liquid chromatography coupled to mass spectrometry (LC-MS) or liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has become a powerful tool for the pesticides residue analysis. Many multi-residue methods for pesticides residue have been developed by using LC-MS/MS.[10-13]

Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are used as the interfaces for LC-MS. ESI has been the mainly used interface in the pesticides residue analysis because of the high polarity and ionization characteristics of the pesticides.[13] LC-MS/MS provides a very good sensitivity and selectivity in

pesticides residue analysis. Triple quadrupoles (QqQs) and hybrid systems quadrupole-time-of-flight (Q-TOF) are among the LC-MS/MS techniques that are used nowadays for pesticide residue analysis.[14]

QqQs provides high sensitivity and good quantitative capabilities in the multiple reactions monitoring (MRM) mode. QqQ is best suited to obtain the strict MRLs regulation as a very low limit of detection (LOD) can be obtained in MRM mode. Unfortunately, for confirmation purposes, at least two transitions must be recorded and the LOD will be increase as the second transition is less abundant in MRM mode. Accurate identification and quantitation of target analytes at trace levels are required to establish MRLs regulations; the European Union (EU) Council Directive regarding analytical method and interpretation of results required three identification points for correct LC-MS/MS confirmation. [15]

Q-TOF provides both MS and tandem MS (MS/MS) acquisition mode with high scanning speed, high resolution and accurate mass measurement for both parent and product ions. Q-TOF has lower sensitivity compare to QqQ but it is able to provide parent-product ion scans which are more sensitive compare to QqQ. Another advantage of Q-TOF is the possibility of confirming the molecules that subjected to collision-induced dissociation (CID) by generate only one product ion. Q-TOF allows an unequivocal confirmation of the compound detected. Q-TOF can eliminate false positive and avoid interpretation uncertainty because it can generate full scan product ion spectra with exact masses that enable to identify the correct empirical formulas of a compound. In the product ion scan mode of Q-TOF, a precursor ion with a mass selected is chosen in quadrupole and the collision cell will generate its fragment ions. The fragment ions are then analyzed by time-of-flight (TOF). Q-TOF is able to measure accurate mass of the fragment ions that passed the first quadrupole and this is useful for ensure the correct

identification of unknowns. The accurate mass measurement can therefore used to generate empirical formula of the compound. Besides that, high mass resolution in Q-TOF can minimize the possibility overlap of two mass peaks, and the background or contaminant ions can be avoided since a clean and informative product ion spectra can be obtained for a better qualitative analysis when using Q-TOF.[5,16,17] LC-Q-TOF MS are mainly used for qualitative analysis of pesticides residue and not for quantitative analysis because of the narrow dynamic range in linear calibration.[14] A range of two orders of magnitude has been reported but it still has potential for quantitative analysis. An analog to digital converter is used in Q-TOF nowadays to enhance the linear dynamic range and makes it possible for quantitative analysis.[18,19]

1.3 Sample Preparation

1.3.1 Sample Extraction

Quantitative results are highly depends on sample preparation steps besides analytical techniques. The extraction method and the solvents chosen to extract pesticides residue in vegetables have to ensure all the pesticides residue has extracted from the sample matrices to obtain a good recovery.

There are studies showed that ultrasonic solvent extraction (USE) method was effectively used in pesticides analysis of leafy vegetables, soil and honey. It was found that the USE method for multi-residues pesticide analysis in leafy vegetables is more effective than homogenized method without further sample clean-up.[20] Ethyl acetate, acetonitrile, methanol, dichloromethane, acetone and cyclohexane are commonly used solvents for pesticide extraction from vegetables. Ethyl acetate was one of the most effective solvent used in pesticides analysis.[21] Acetonitrile has strong dissolving ability with water, it can be used to extract most of the polar pesticides but it is highly toxic

compare to ethyl acetate. Ethyl acetate can used to extract most of the polar pesticide also and it has lower vapour pressure compare to acetone. The low vapour pressure causes more violent acoustic cavitation in USE method to increase the efficiency of the extraction by USE method. Less co-extracted compounds are found when using ethyl acetate as the extraction solvent in pesticides analysis for vegetables.[20]

Most of the studies showed that it is not necessary to have further clean-up process after sample extraction.[7] This is because only a small amount of sample will be injected into liquid chromatography column and the specificity of LC-MS/MS (ability of to distinguish the analyte being measured from other substances).[5] It was found that raw extracts with methanol-water and acetonitrile can be analysed without clean up as they show insignificant matrix effects whereas ethyl acetate extraction will have lipid coextract and show matrix effects. Even though sample clean-up is not necessary after sample extraction, the clean-up process still required to concentrate the pesticides residue in the samples, to enhance the sensitivity, to reduce matrix effects in certain cases such as ethyl acetate extraction and to extend the life time of the LC column.[22]

1.3.2 Matrix Effects

Matrix effects may reduce or enhance the response of the pesticides compare to the pesticides in pure solvent. ESI interface in positive mode always show high signal suppression or enhancement compares to ESI in negative mode.[14] Matrix effects can be expressed as the ratio of signal of the substances in matrix to the signal of the substances in the solvent. The matrix effects depend on the nature of sample and pesticides, although many hypotheses have been proposed in the literature, still no definitive explanation. A few methods can be used to correct the matrix effects, for examples, by addition of isotopically labeled substances or internal standard[23] (but use of such internal standard is

difficult for multi-residues method) and used of matrix matched calibration (need blank sample extracts).[11,12] As reported, among 13 matrices have been tested for matrix effect, it was found that only lemon show significant different response for several pesticides due to signal suppression or stability problem in very acidic samples.[2] In most case, no significant differences in response for pesticides in samples matrix from the pesticides standard in pure solvent calibration,[7,24] therefore, solvent based standards could be used for accurate quantitation of real sample.[14]

1.3.3 Sample Clean-up

The cleanup step is the most tedious step in sample preparation, because it generally consumes a long time compare to other steps, restricts the number of pesticides that are recovered causes the low recovery in certain cases. Florisil, silica gel, charcoal-celite, alumina and gel permeatation chromatography are the commonly used column for sample clean up after sample extraction.

Alumina can be used for the cleanup of fatty foods but will decompose some of the organophosphates pesticides; it does not effectively separate some plant materials from the pesticide. Silica gel is particularly useful for isolation of certain polar pesticides without losses but will not adequately separate some plant coextractives from some pesticides. Gel permeation chromatography (GPC) cleanup is able to remove oils and pigments from the sample matrix and large molecule of the sample matrix but cannot remove completely the non-polar interferences. Florisil is a diatomaceous earth adsorben, it can retain some lipids so it is particularly suited for cleanup of fatty foods. Florisil is good for cleanup of nonpolar pesticides, such as the chlorinated hydrocarbons. It produces very clean eluants by removing most of the interferences when eluted with nonpolar solvents but difficult to use for fruits and vegetables when moderately polar to

polar pesticides are present. The recoveries of organophosphates and carbamates pesticides are quite low when Florisil clean-up is used.[22,25] Diethyl ether in petroleum ether and acetonitrile are the solvents that usually used to elute moderate polar to polar organophosphate and organochlorine pesticides in Florisil column.

1.4 Rationale

Vegetables are consumed daily as vegetables contain a lot of vitamins and minerals that are essential for our health, unfortunately, these vegetables may contaminate with pesticides residue which are hazardous to our health. Frequent consumed vegetables which are available in local hypermarket in Malaysia such as *Sawi hijau (leafy vegetables), Kailan (Kale), Yau Mak (Romaine Lettuce) and Tai Pak Choy (Chinese Cabbage)* was used in this study to investigate the pesticides residue. Six types of pesticides from different groups of pesticides are used (five organophosphates and one carbamates) in this study. Acephate, carbaryl, dimethoate, diazinon, quinalphos and profenofos are broad spectrum and general used pesticides in agricultural. Among the pesticides, organophosphates pesticides were frequently detected in Malaysia. Acephate, dimethoate, quinalphos and profenofos were found exceed the MRL in Sarawak in the year 2000 to 2006, 5.6 % of the vegetable samples analyzed were found to contain pesticide residues exceeding the Maximum Residue Limit (MRL) as set in Food Regulation 1985.[26] Various formulations of these pesticides will have varied toxicity classes.

A simple, rapid and efficient method was developed to identify and quantify the pesticide residue in local vegetables. LC-Q-TOF MS was used because it can provide accurate mass measurement which are important to identify the empirical formula

correctly of the pesticides and allow simultaneously identification of six types of pesticides in a matrix sample.

1.5 Objectives

The objectives of this study are:

(a) To develop the sample extraction and sample clean-up procedure for six types of different groups of pesticides in four types of local vegetables.

(b) To optimize the parameters of liquid chromatography to obtain better separation of co-eluting peaks in chromatogram for six types of pesticides.

(c) To optimize the parameters of mass spectrometry analyzer (Q-TOF) to obtain accurate mass measurement of the precursor and product ions of individual pesticides.

(d) To identify pesticides from the empirical formula based on the accurate mass measurement.

(e) To quantify the concentration of pesticides from the calibration curves.

(f) To determine the Lower Detection Limits (LOD) of each pesticides in LC-Q-TOF-MS

CHAPTER 2

Experimental

2.1 Chemicals and Materials

The reagents that had been used in this study:

(a) Solvents

- Methanol, Purity (GC): ≥99.9%, hypergrade for LC-MS LiChrosolvLCMS grade from Merck
- Ethyl acetate, A. R. grade analytical reagent, from J.T.Baker, Inc., Pillipsburg,NJ, USA.
-Acetonitrile, A. R. grade analytical reagent, from J.T.Baker, Inc., Pillipsburg,NJ, USA.

(b) Anhydrous sodium sulphate – GR for analysis, from Merck.

(c) Florisil – Magnesium silicate, activated – pesticide residue grade (60—100 Mesh) 100 Mesh), from Sigma Chemical Co.,St.Louis, MO, USA

(d) Pestanal – acephate, carbaryl, dimethoate, quinalphos, diazinon and profenofos, from Sigma-Aldrich.

(e) Deionised water

All the deionised water and methanol were filtered through 0.2 µm nylon filter from Merck when used as mobile phase in LC-MS-MS analysis. All pesticide standards were obtained from Sigma-Aldrich, Pestanal, analytical standard and of purity ranging from 98-100% except acephate at 96%. Acephate, carbaryl and dimethoate are in the solid form while quinalphos, diazinon and profenofos are in the liquid form. Other chemicals were used without any further purification. The used of high purity reagents and solvents were to minimize interference problems. All the standard solutions were

stored in the dark at 4 °C when not in used. **Table 1** shows the molecular formulas and the molar mass of the pesticide standards used in this study.

Group of pesticides	Pesticides	Molecular Formula	Molar Mass (g/mol)
Organophosphate	Acephate	$C_4H_{10}NO_3PS$	183.17
	Dimethoate	$C_5H_{12}NO_3PS_2$	229.26
	Quinalphos	$C_{12}H_{15}N_2O_3PS$	298.30
	Diazinon	$C_{12}H_{21}N_2O_3PS$	304.35
	Profenofos	$C_{11}H_{15}BrClO_3PS$	373.63
Carbamates	Carbaryl	$C_{12}H_{11}NO_2$	201.22

Table 1:The molecular formula and molar mass of pesticides standard used in this study.

2.2 Glassware

All the glassware was cleaned with cleaning detergent and tap water. The glassware was then soaked in chromic acid bath which was prepared by adding potassium dichromate, $K_2Cr_2O_7$ to concentrated sulphuric acid, H_2SO_4 until saturated. The glassware was soaked overnight and rinsed with distilled water and dried in an oven at 105°C. The cleaned glassware was then capped with aluminium foil and stored in a cupboard to protect from any other contaminants. The glassware was rinsed with acetone before used.

2.3 Apparatus

(a) Food processor - Philip

(b) Weighing instrument- Denver Instrument XL-610

(c) Rotary vacuum evaporator – Buchi Rotavapor R-114, Buchi Waterbath B-480, from Switzerland.

(d) Ultrasonicator – Branson 3200

(e) Whatman No.1 filter paper

(f) Nylon Syringe Filter, 13mm, 0.2um, Cronus

(g) 5mL Disposable Syringe with Needle, Luer Lock, Needle: 21 G x 1½ "

2.4 Instrumentation

The liquid chromatography (LC) tandem mass spectrometry (LC-Q-TOF MS) analyses were performed with an Agilent, model 1200 Series High Performance Autosampler SL+ instrument comprising degas-unit, Agilent 1200 Series Binary Pump SL, autosampler, and Agilent 1200 Series Thermostatted Column Compartment SL. A 2.1 x 100 mm i.d., 600 bar LC column (Zorbax Eclipse, 1.8 μm XDB-C18, Agilent,USA) maintained at 40^{o}C was coupled to Agilent G6530A Quadrupole-Time Of Flight Detector.

2.5 Samples

4 types of vegetables, *Sawi hijau (leafy vegetables), Kailan (Kale), Yau Mak (Romaine Lettuce) and Tai Pak Choy (Chinese Cabbage)* were purchased from a local hypermarket.

2.6 Standard Stock Solution

Each stock standard solution was prepared in methanol and the working standard solutions prepared by serial dilution with methanol. The mixture of standard stock solution containing all the 6 pesticides was prepared by adding the aliquots of the individual working standard solutions and diluting with methanol. The concentration of each individual pesticide in the mixed standard stock solution was 1 ppm. The mixed working standard solutions in an interested range were then prepared by serial dilution of the mixed standard stock solution with methanol. This mixed working standard solution

was prepared for calibration and recovery test. The concentration of mixed working standard solution was in the range of 0.01 – 0.40 ppm. The mixed working standard solution was then analysed by LC-Q-TOF-MS. Calibration curves for each individual pesticide were plotted. The peak area for each pesticide in the chromatogram was used for calibration curve plotting and recovery calculation. The fragments of the precursor ion (product ions) for each pesticide in mass spectrum were used as quantifier ions.

2.7 Samples Preparation

2.7.1 Sample Extraction

The vegetables samples used in this study were purchased from a local hypermarket. The samples were cut, chopped and thoroughly blended using a food processor. Two 5g portions of the vegetables samples were weighed into two different Mason jar. 1 ml of 0.2 ppm spiking mixed working standard solution was added into one of this jar for recovery study. The spiked solution was allowed to stand in the samples for 20 minutes before 10 g of anhydrous sodium sulphate was added into the Mason jar. The samples were then extracted with 40 ml ethyl acetate in an ultrasonic bath for 35 min. After sonicfication, the samples were filtered through a filter funnel fitted with Whatman No.1 filter paper into a 250 ml round-bottomed flask. 10 ml of ethyl acetate was used to rinse the Mason jar and decanted through the filter funnel into the same round-bottomed flask. The filtrate was then evaporated to just dryness by a vacuum rotary evaporator using 40^{o}C water bath. The above procedures were repeated for the other sample.**[10]**

2.7.2 Sample Clean-up

The extracted sample was cleaned up by activated Florisil column. The glass column (25 cm x 10 mm i.d.) was slurry packed with 2 g of Florisil. The column was pre-

eluted with 10 ml of acetonitrile to wet, rinse and saturated the Florisil. The elution of acetonitrile was then stopped just prior to the exposure of the Florisil layer to air. This eluted acetonitrile was discarded. About 0.5 cm height of anhydrous sodium sulphate was added on the top of the Florisil to protect the Florisil layer from disturbance and absorb water in the extracted samples.**[27,28]**

The 250ml round-bottomed flask with extracted sample was rinsed three times with 1ml of acetonitrile each. The extract was then transferred quantitatively into the Florisil column. The pestiside residues were eluted with 20 ml of actonitrile and collected in a 100 ml round bottomed flask. The eluant was then evaporated to just dryness using a vacuum rotary evaporator in 40^{o}C water bath. The dried residue in the 100ml round-bottomed flask was rinsed 3 times with 1 ml of methanol each into a 5 ml volumetric flask and top up to the mark with methanol. 1ml of the pesticide residues samples was filtered through a 0.2µm nylon filter into a vial for LC-Q-TOF-MS analysis.

Figure 1shows the flow chart of sample preparation in this study.

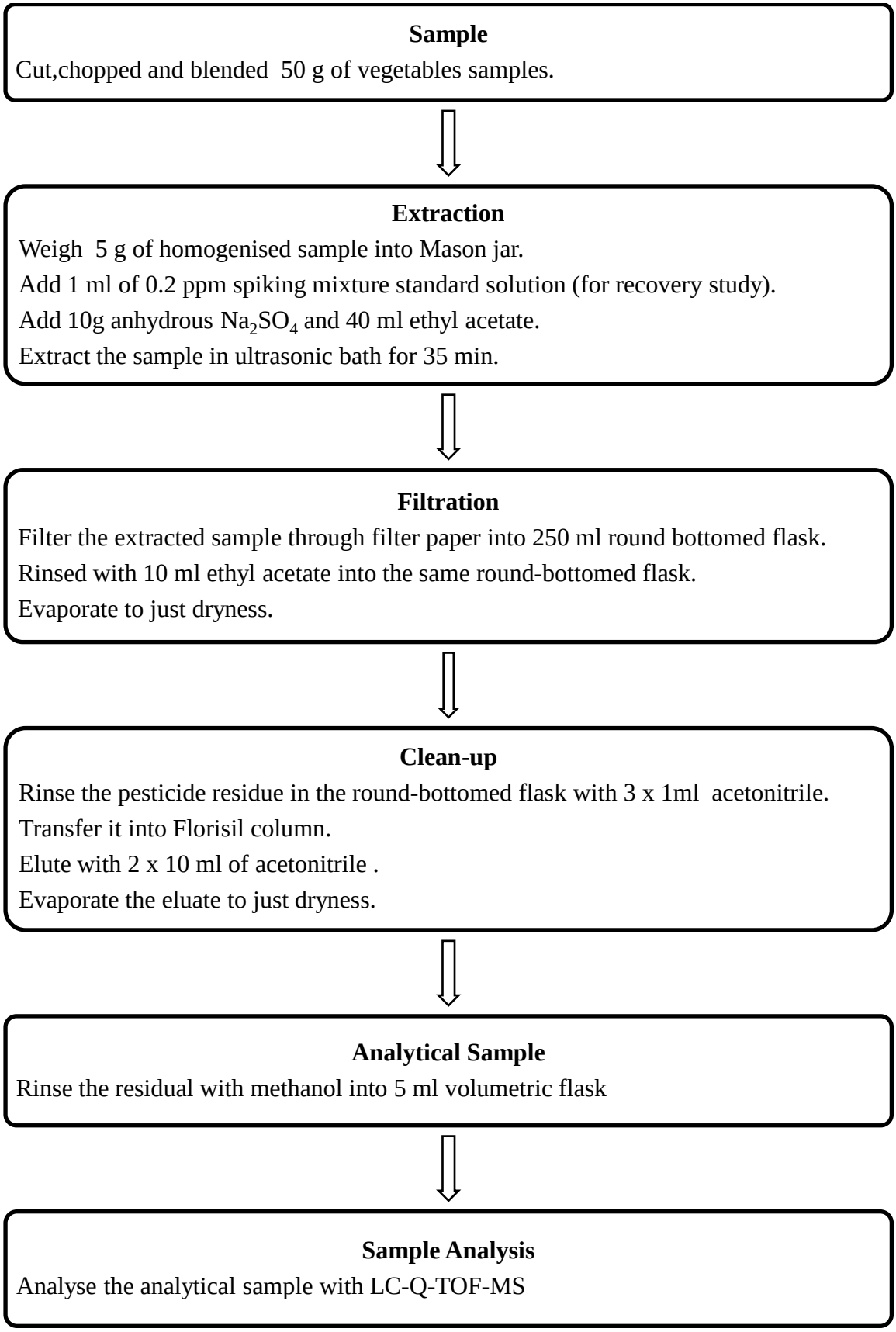


Figure 1: Flow Chart of Multiresidue Method for Vegetables

2.8 Recovery Study

The recovery study of pesticide was carried out by fortifying selected vegetables purchased from a local hypermarket. The pesticide residue in the original vegetables samples was analysed before the fortification. A 5 g amount of the homogenized sample was fortified with 1ml of 0.2 ppm and was processed as described in Section 2.7.1 and 2.7.2 to obtain a final fortification level of 0.04 ppm. The percentages of the recovery were then calculated.

2.9 Limit of Detection

The Limit of Detection (LOD) of pesticides was estimated from the standard mixtures with the minimum detectable quantities. Calibration curves for each pesticide were plotted using the response (peak area) of chromatogram against concentration of pesticide in standard mixture of pesticides. The known concentration of standard mixture of pesticide was prepared by appropriate serial dilution of the mixed standard stock solution. The LOD was first estimated from chromatograms of the pesticides at signal to noise (S/N) = 3, then, the linear equations of the calibration curves and the standard deviation of response were used to calculate to estimate a more accurate LOD for each pesticides.

2.10. Identification and Quantification of Pesticides

Agilent MassHunter Workstation Sofware - Qualitative Analysis version B.02.00 and Agilent MassHunter Workstation Sofware - Quatitative Analysis (QTOF) version B.01.04 were used for data processing. The characteristic fragment ions and the $[M+H]^+$ ion were used as quantification and qualification ion. The concentration of pesticide in vegetables sample was then calculated from the linear equation of the calibration curves. The recoveries, linearity of the method were examined. This multi-residue method was demonstrated on 4 types of vegetables samples.

CHAPTER 3

Results and Discussion

3.1 Sample extraction

The aim of sample extraction is to obtain as much as possible the pesticides residue from the samples without the interference from sample matrixes. The samples were cut and blended to obtain a large total surface area and homogenized sample so that the extraction of pesticides would be more complete.

Ethyl acetate was chosen as the extraction solvent because of its high polarity, less toxicity and lower vapor pressure. Ethyl acetate is immiscible with water, therefore, no need to salt out the aqueous phase but anhydrous sodium sulphate was added to force polar pesticides compound into the organic phase.[12] The mixture of the vegetables samples, 40 ml of ethyl acetate and 10 g of anhydrous sodium sulphate was then put into the ultrasonic water bath for 35 minutes. The volume of ethyl acetate and the time for sonification was chosen as above to obtain an optimum recovery for the pesticides.[20] The extraction was filtered through a filter funnel fitted with Whatman No.1 filter paper into a 250 ml round-bottomed flask and was evaporated to just dryness by rotary vacuum evaporator in the water bath at 40^{o}C. The dried pesticides residue was dissolved in acetonitrile for further sample clean-up.

3.2 Sample Clean-up

Since the compound will be analysed in Targeted MS/MS mode, a specific m/z value of the targeted compound was set, therefore, sample clean-up process was not necessary in the LC-MS-MS analysis. The pesticides can be detected even if there are interfering peaks from the co-extracted matrices in the samples due to the selectivity in LC tandem mass spectrometry (targeted MS/MS mode). However, sample cleaned-up by Florisil still carried on in this study to reduce the unwanted matrices in the samples,

reduce the maintenance of the instrument, especially the LC column and enhance the sensitivity of the chromatography and mass spectrometry.

Florisil clean-up is an adsorption type clean-up,[22] it is used to remove the interferences in the sample which may overlapped with the interested analytes. The interferences may cause the low recovery in the recovery study. The analytes cannot be identified if the interference peaks overlapped with the peaks of analytes in the same retention time and may also cause the retention time shifted from the original position. The particles of inteferences may clog up the column as well as destroy the column. The samples were cleaned up in this study and filtered through a 0.2µm nylon filter before injected into the LC column to avoid large particles from clogging the LC column and therein reduce the maintenance of the analytical instrument.

Most of the organophospate and carbamate pesticides will be not be recovered from the Florisil column since it is more suitable for non-polar pesticides such as organochlorine pesticides. This is due to most of the polar interference will be removed during Florisil clean-up, therefore, the percentage of the recoveries of organophospate and carbamate pesticides were not in the acceptable range.[24] To overcome this drawback, a very polar solvent, acetonitrile was used in this study to elute pesticide residue during sample clean-up.

The pesticides residue was eluted from the Florisil column by actonitrile and was collected in a 100 ml round-bottomed flask and evaporated to just dryness in a rotary vacuum evaporator. The dried pesticides residue was then dissolved in methanol (which is compatible with the mobile phase in LC analysis) in a 5ml volumetric flask. The concentration of spiked pesticides in the spiked samples was 0.04 ppm. About 1 ml of the samples was filtered through 0.2 µm nylon filter into vials for LC-Q-TOF-MS analyses.

3.3 LC-MS-MS condition

The liquid chromatography-tandem mass spectrometry (LC-Q-TOF-MS) analyses was performed with an Agilent, model 1200 Series High Performance Autosampler SL+ instrument comprising degas-unit, Agilent 1200 Series Binary Pump SL, autosampler, and Agilent 1200 Series Thermostatted Column Compartment SL. A 2.1 x 100 mm i.d., 600 bar LC column (Zorbax Eclipse, 1.8 μm XDB-C18, Agilent,USA) maintained at 40^{o}C was coupled to Agilent G6530A Quadrupole-Time Of Flight Detector. Agilent MassHunter Workstation Software –Data Acquisition for 6500 Series Q-TOF was used for instrument control and data acquisition. Additional data processing was performed by using Agilent MassHunter Workstation Sofware –Qualitative Analysis version B.02.00 and Agilent MassHunter Workstation Sofware –Quatitative Analysis (QTOF) version B.01.04.

Compounds were separated with an isocratic elution of 25:75 of H_2O: Methanol with the flow rate of 0.3 mL min^{-1} for 10 minutes. The injection volume was 2 μL. Data were acquired in Targeted MS/MS mode. Ion source of Q-TOF mass Spectrometer is ESI+ Agilent Jet Stream and the ESI ion source parameters were as follow:

Parameter	Value
Gas temperature	***350 ^{o}C***
Gas flow	***8 l/min***
Nebulizer	***50 psi***
Sheath Gas Temperature	***350 ^{o}C***
Sheath Gas Flow	***11 l/min***

The scan source acquisition parameters were as follow:

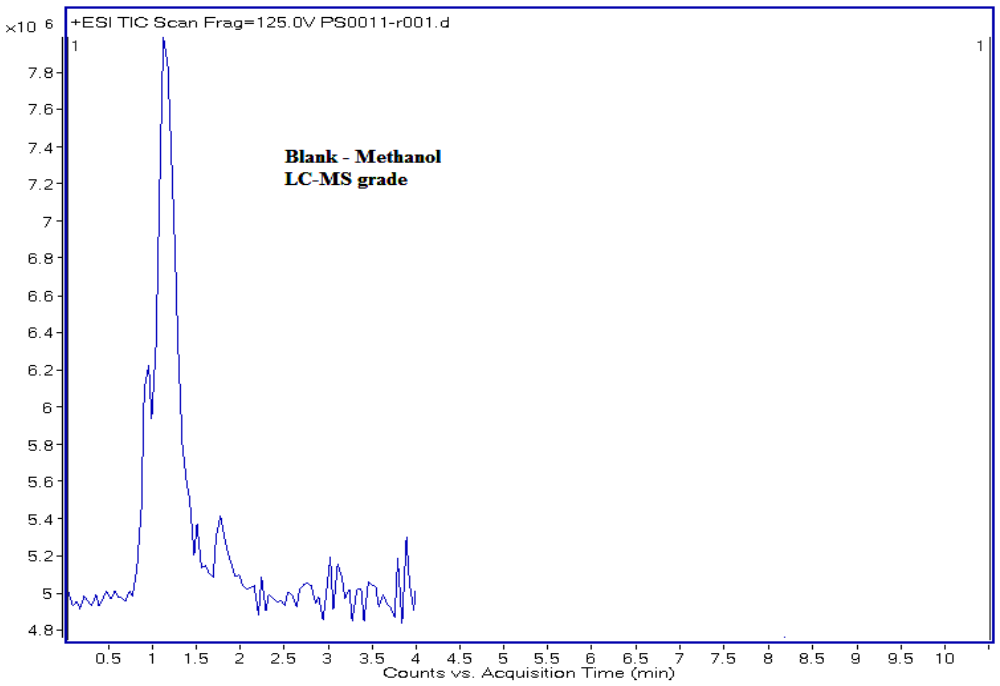
Parameter	Value
Ion polarity	***positive***
VCap	***3500 V***
Corona Positive	***4V***
Fragmentor	***125V***
Skimmer 1	***65V***

The precursor ion and the collision energy for each of the pesticides used in the Targeted MS/MS mode were listed in **Table 2**.

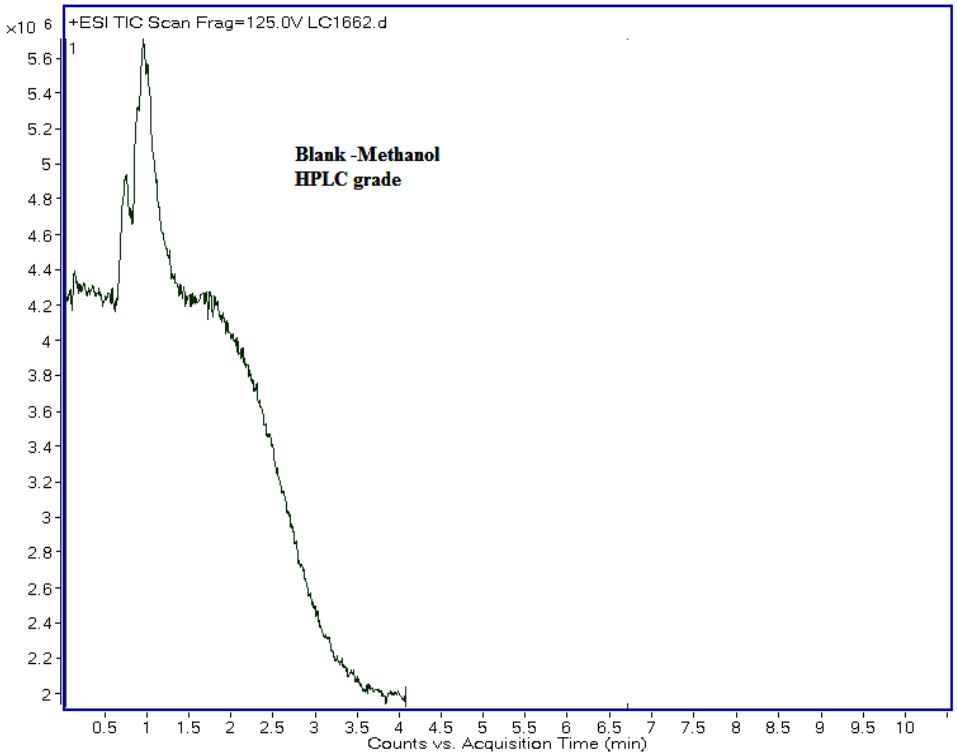
Pesticides	Precursor Ion (m/z)	Collision Energy (V)
Acephate	184.0192	15
Carbaryl	202.0863	12
Dimethoate	230.0069	12
Quinalphos	299.0614	18
Diazinon	305.1083	18
Profenofos	372.9424	15

Table 2: The precursor ions and the collision energy used in Targeted MS/MS mode

The solvents used for the mobile phase were methanol and water. The water used was deionised water and the methanol used was purity (GC): ≥99.9%, hypergrade for LC-MS LiChrosolv LCMS grade from Merck. The higher purity of methanol produced a lower noise background. Both the water and methanol were filtered through a 0.2 µm nylon filter to eliminate the larger particulate which will clog up the LC column. The total ion chromatogram (TIC) of blank – methanol of LCMS grade in **Figure 2(a)** showed a lower background noise compared to blank –methanol of HPLC grade in **Figure 2(b)**.



(a) Methanol in LCMS grade



(b) Methanol in HPLC grade

Figure 2: Total ion chromatograms of blank methanol in (a) LCMS grade and (b) HPLC grade

The composition of the mobile phase was chosen at 25:75 of H_2O: Methanol to obtain well resolved and narrow peaks in the chromatogram. All the pesticides in the solution were well separated in the extracted ion chromatogram (EIC) as shown in **Figure 3**:

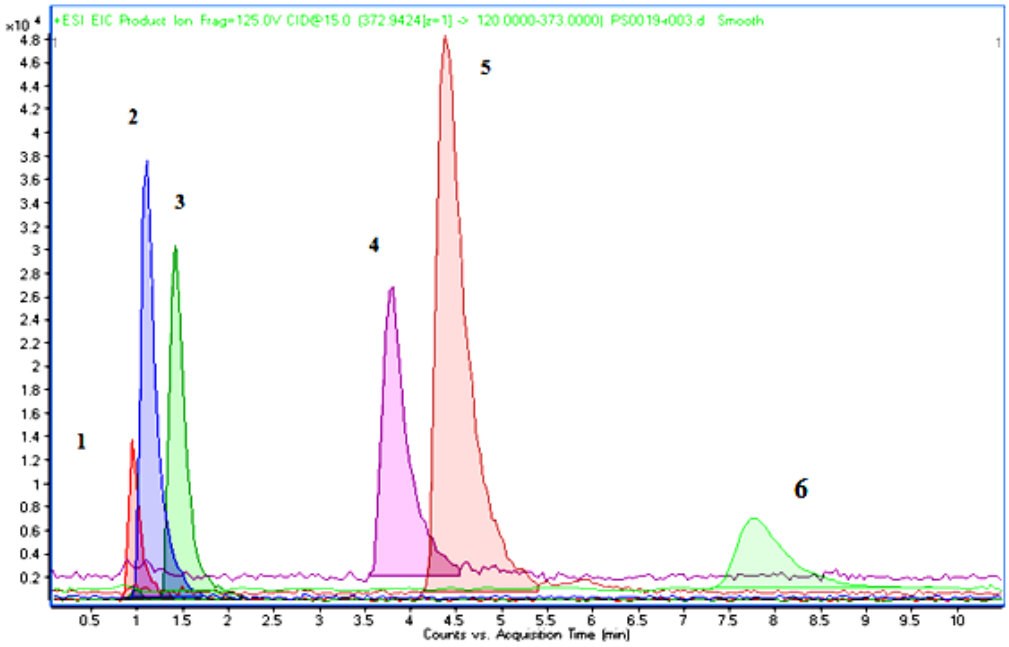


Figure 3: Extracted ion chromatogram of 6 types of pesticides in this study; Peak 1: Acephate, 2: Dimethoate, 3:Carbaryl, 4:Quinalphos, 5:Diazinon and 6:Profenofos

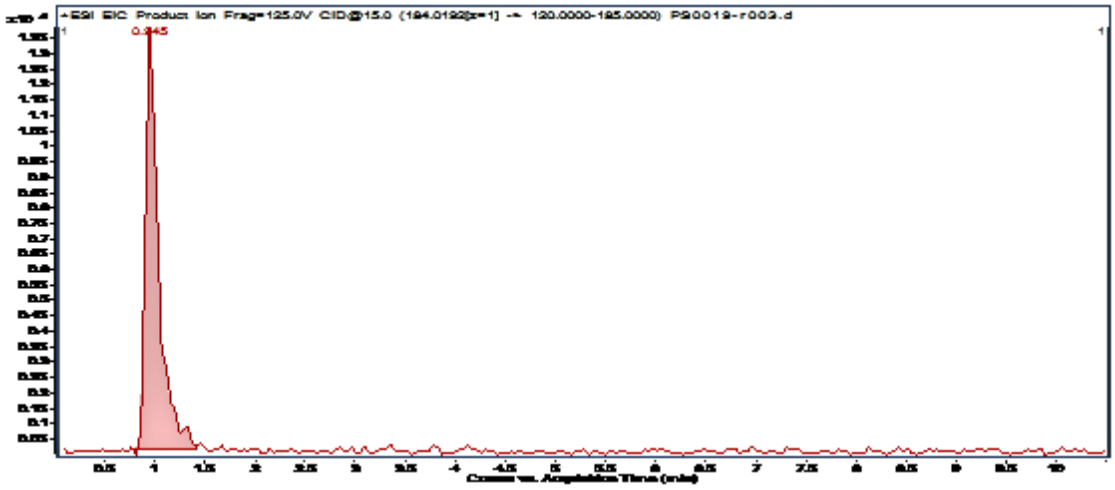
The sample volume used in the LC was 2 µL which was enough to produce an adequate response or abundance in chromatograms and mass spectrums for a low concentration of pesticides. The time required for all the pesticide to elute was less than 8 minutes. The average retention time for each of the pesticide was shown in the **Table 3** below.

Pesticide	Retention time (min)
Acephate	0.945
Carbaryl	1.408
Dimethoate	1.091
Quinalphos	3.784
Diazinon	4.376
Profenofos	7.759

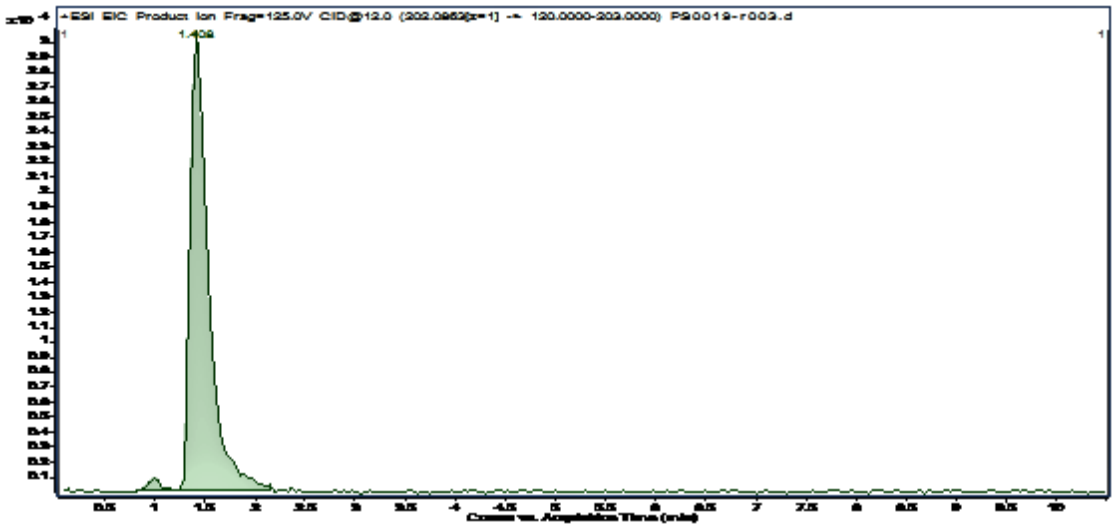
Table 3: Retention time for pesticides

Extracted ion chromatogram (EIC) in **Figure 4** showed that each of the pesticide has narrow peak and low background noise in the chromatograms.

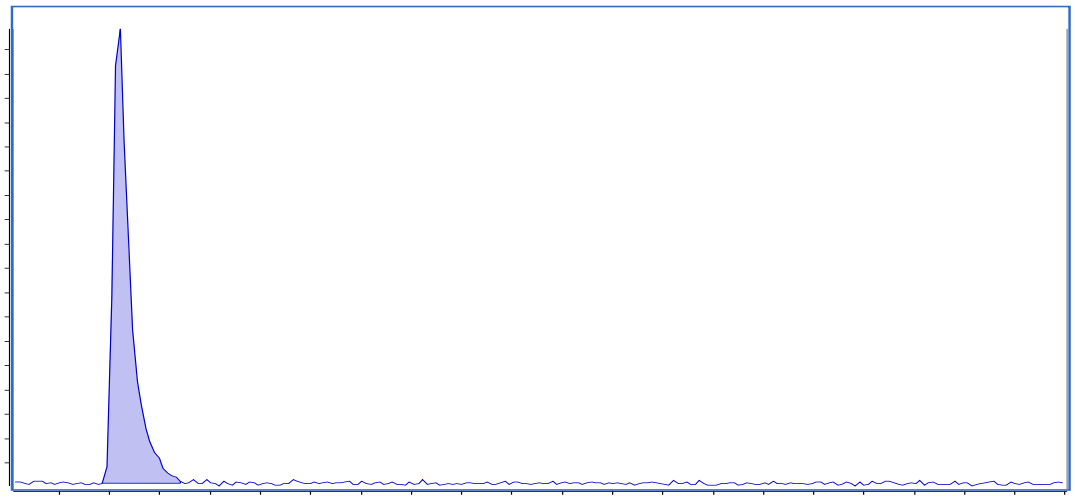
4(a) Acephate



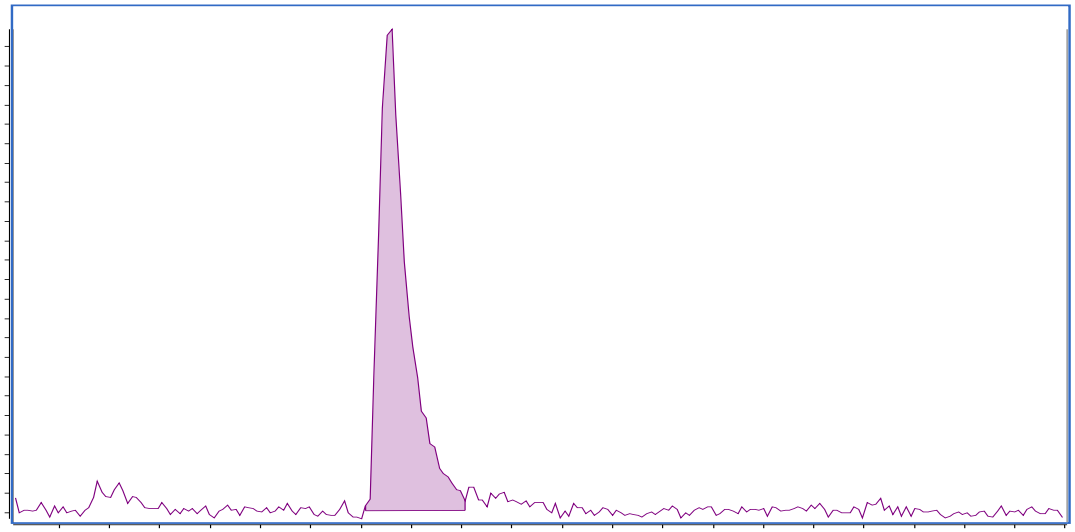
4(b) Carbaryl



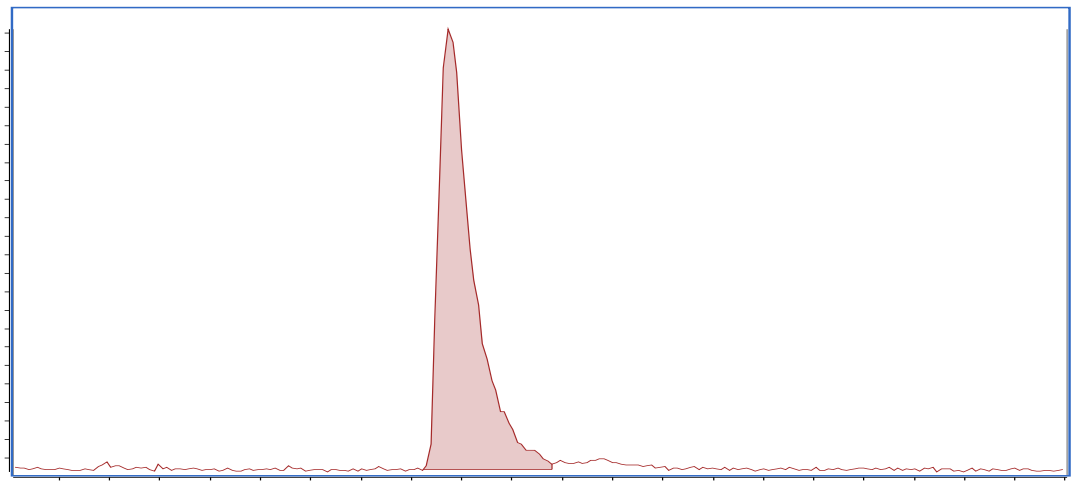
4(c) Dimethoate



4(d) Quinalphos



4(e) Diazinon



4(f) Profenofos

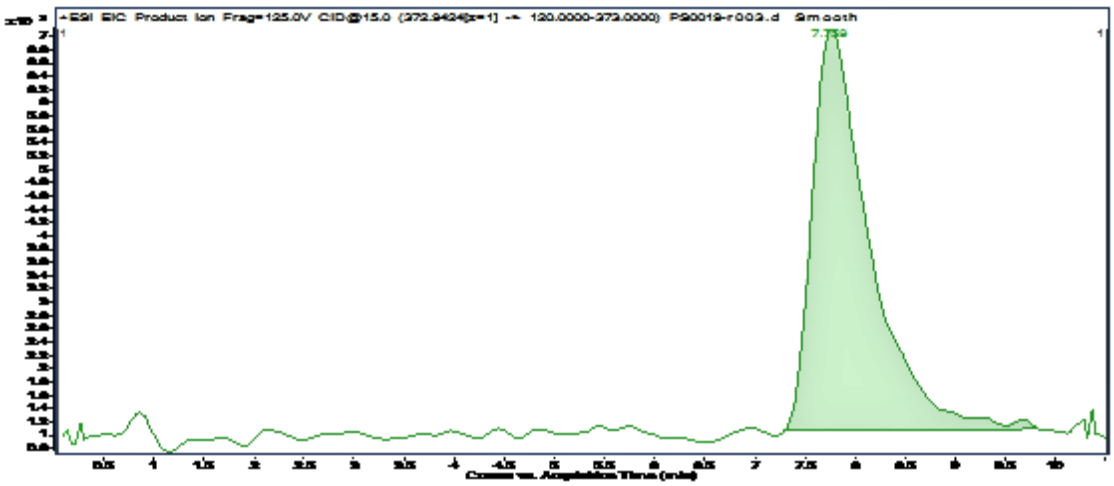


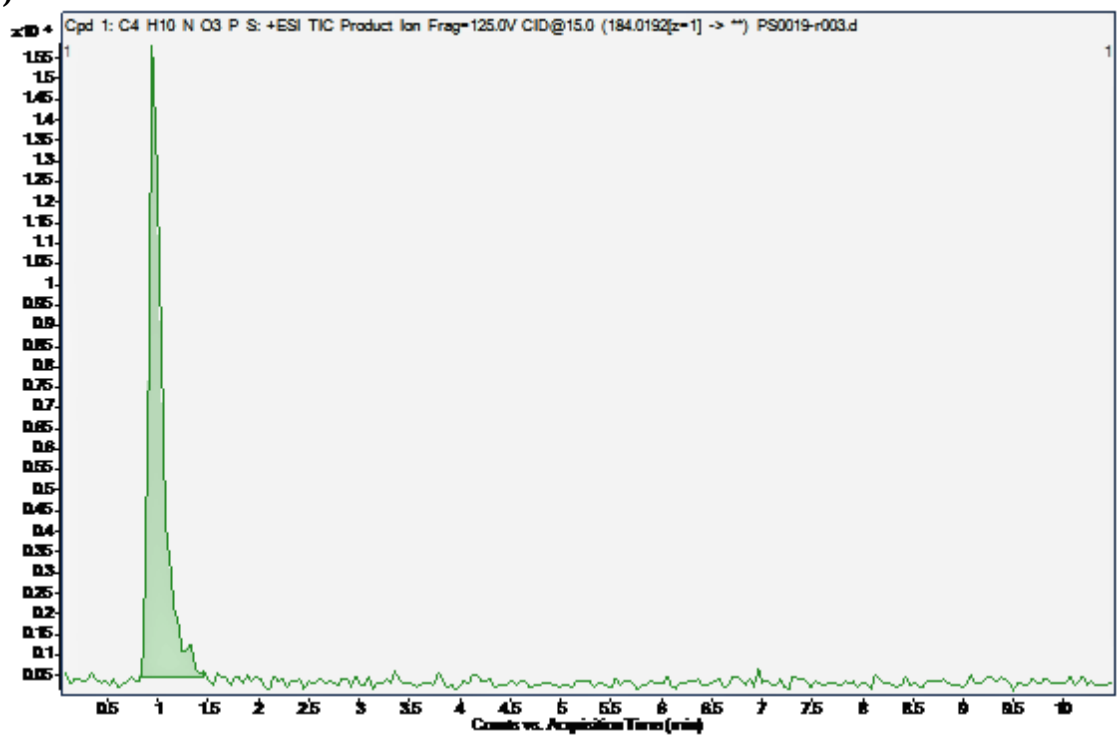
Figure 4 (a) to (f): The individual EIC of pesticides with the retention time show on the top of the peaks

The fragmentor voltage used in this study was 125 V but different collision energy in the quadrupole was chosen to obtain a good abundance for the characteristic fragments ion (product ion) for each of the pesticide as shown in **Table 2** in page 19.

The precursor ions were chosen and set in the Targeted MS/MS mode. Agilent MassHunter Workstation Sofware –Qualitative Analysis version B.02.00 was used to find the compound from the chromatogram in targeted MS/MS mode. The results of chromatogram and the mass spectrum of the individual compounds are shown in the **Figure 5**.

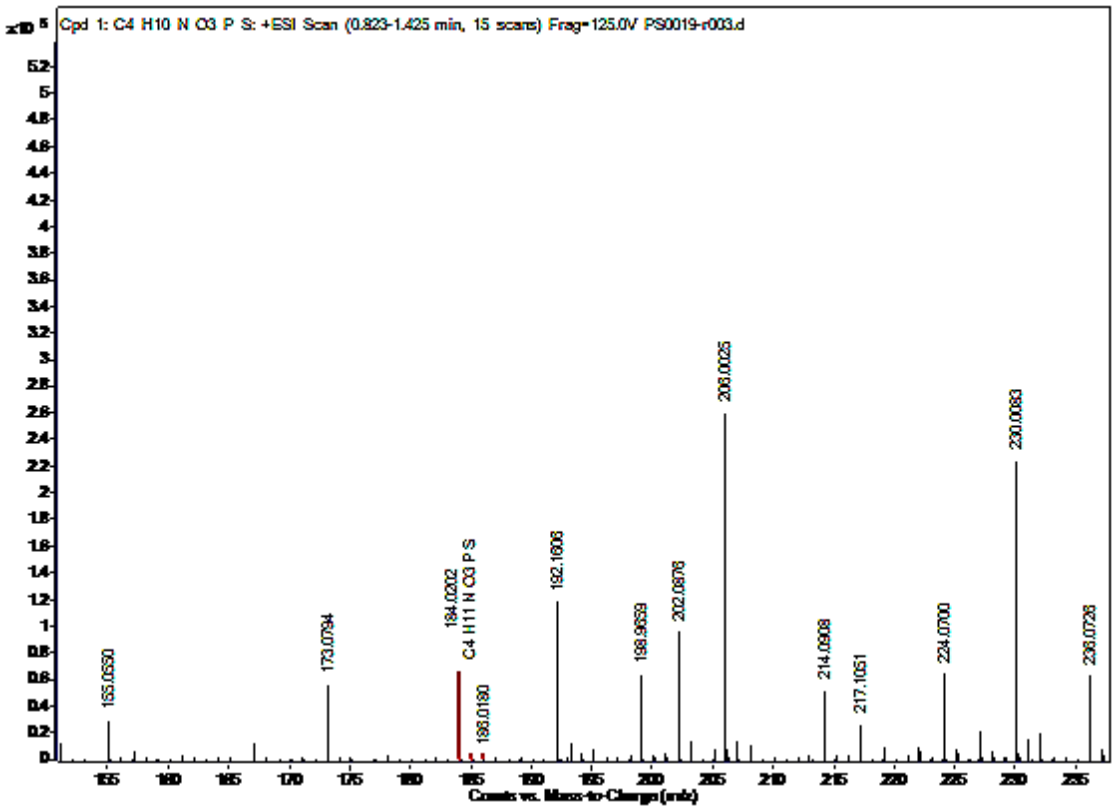
5 (a) Acephate

(i)



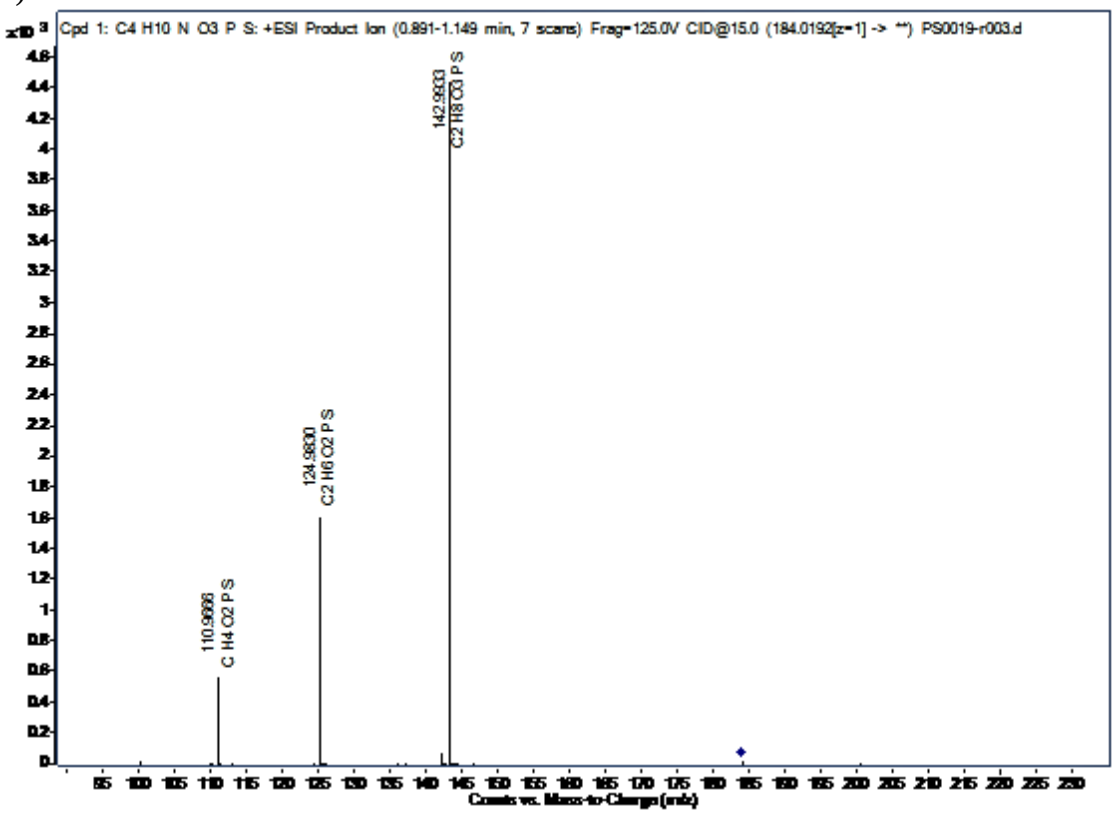
Extracted Ion Chromatogram (EIC) of Acephate

(ii)



Mass Spectrum of Acephate with the molecular formula, $[M+H]^+$

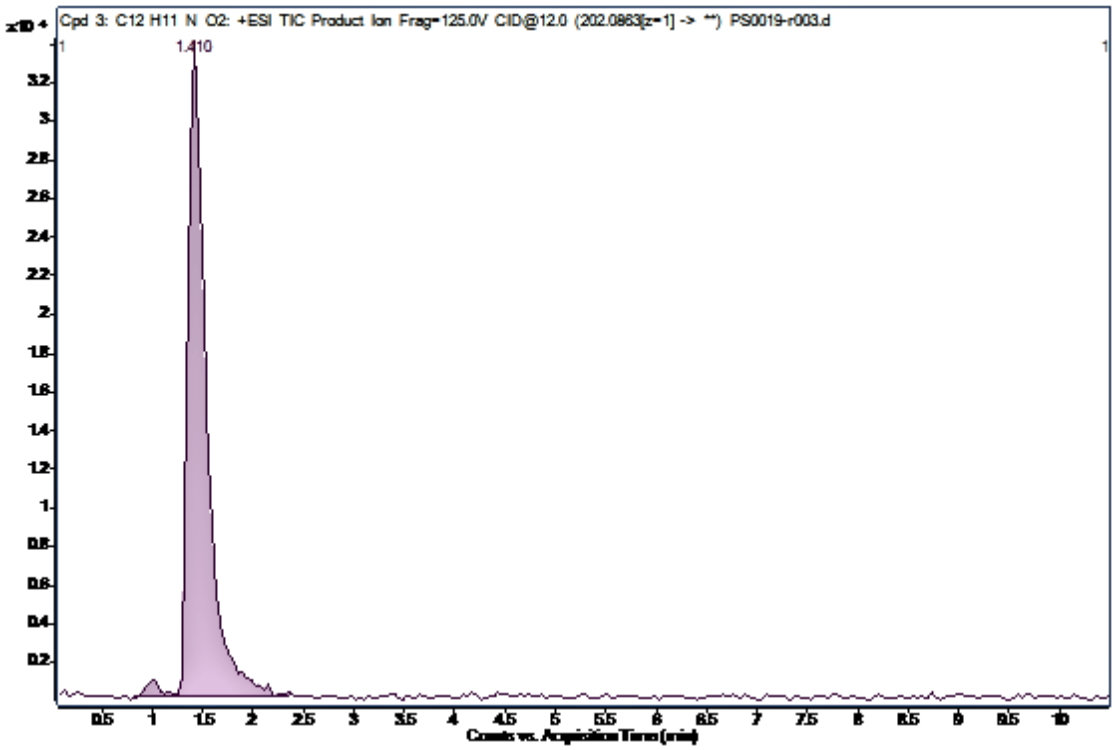
(iii)



Mass spectrum of Acephate with the molecular formula of product ion, $[M+H]^+$

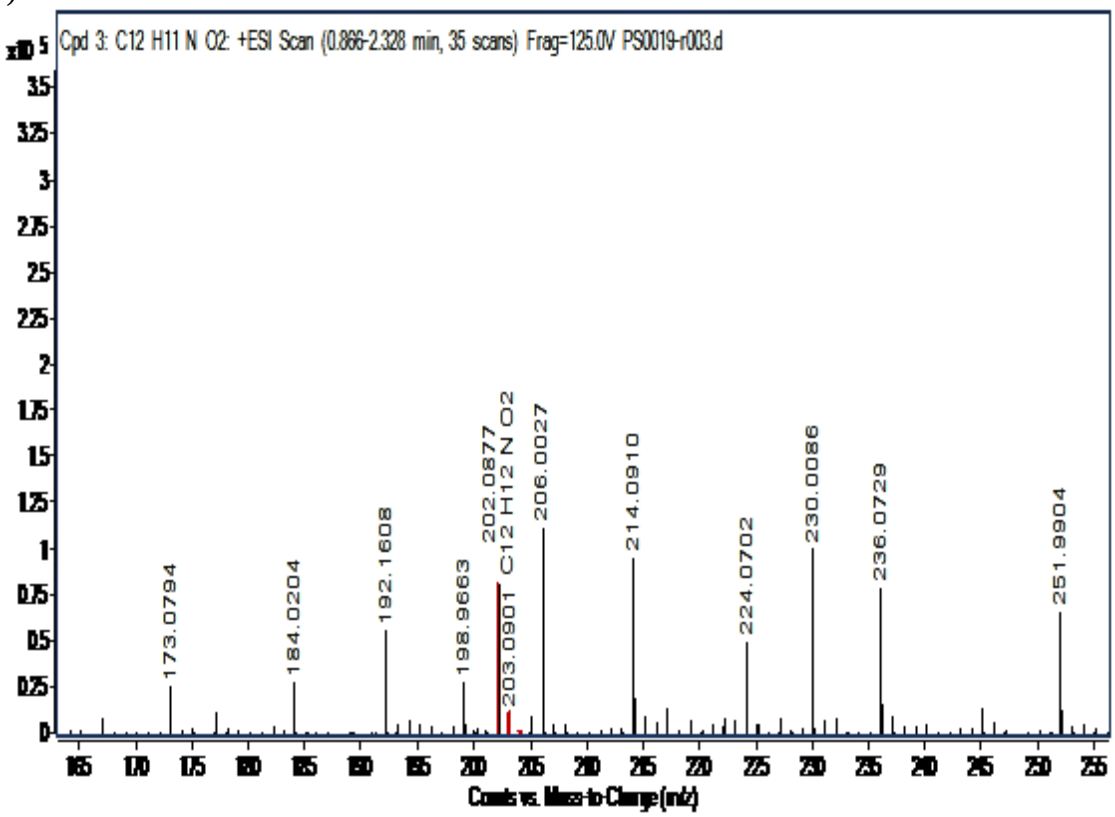
5(b) Carbaryl

(i)



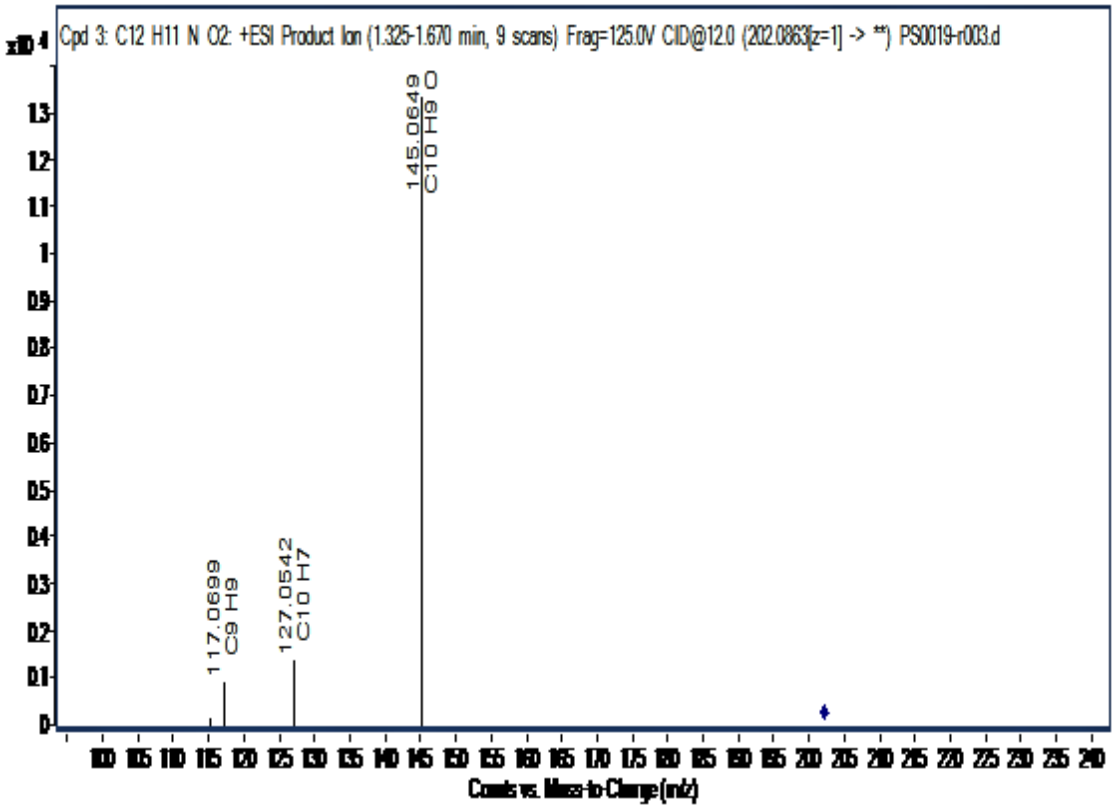
Extracted Ion Chromatogram (EIC) of Carbaryl

(ii)



Mass Spectrum of Carbaryl with the molecular formula, $[M+H]^+$

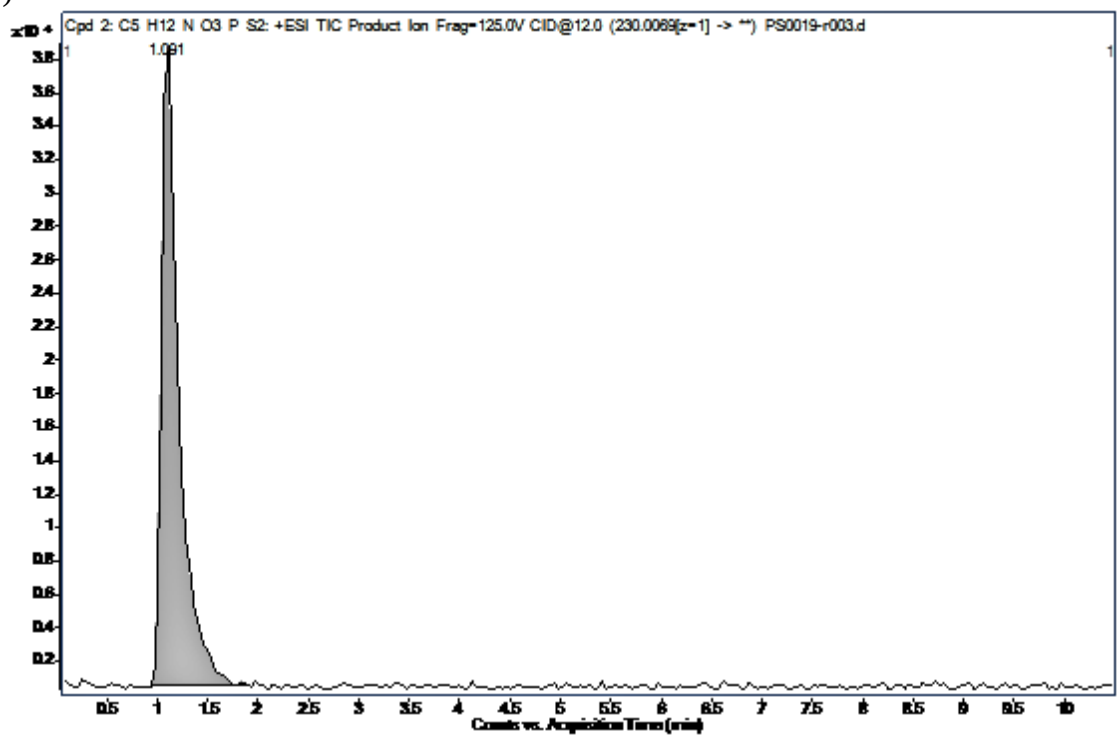
(iii)



Mass spectrum of Carbaryl with the molecular formula of product ion, $[M+H]^+$

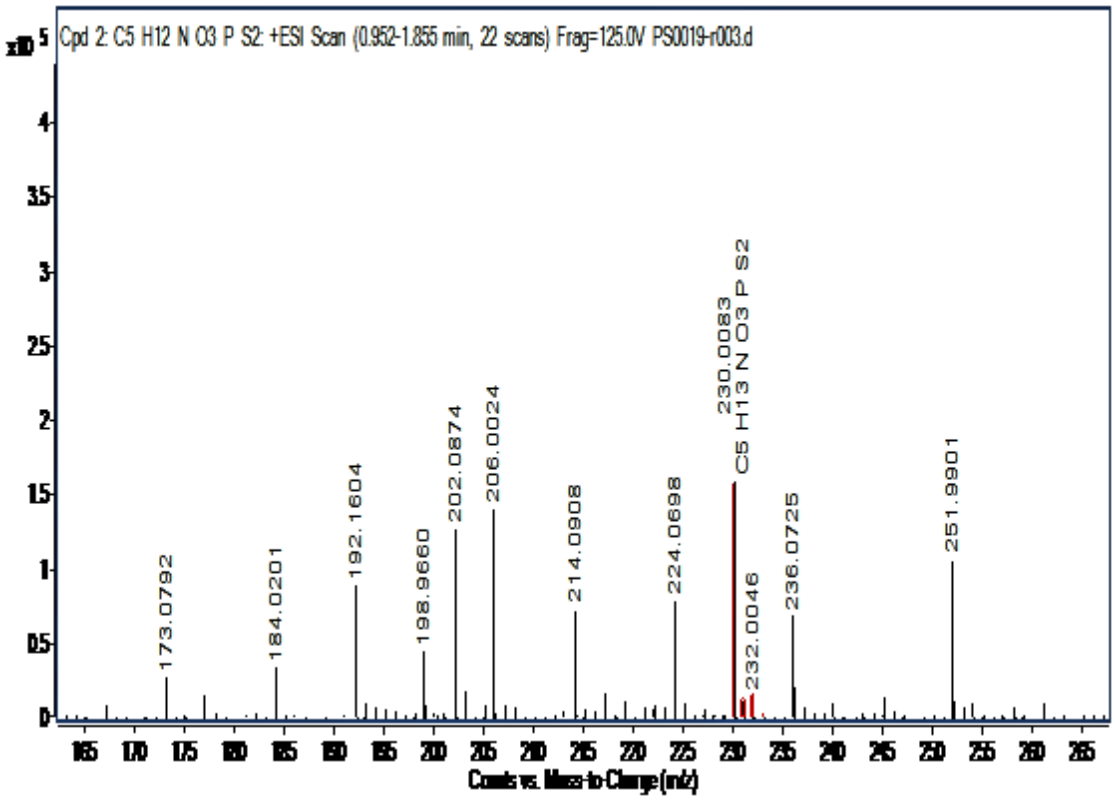
5(c) Dimethoate

(i)



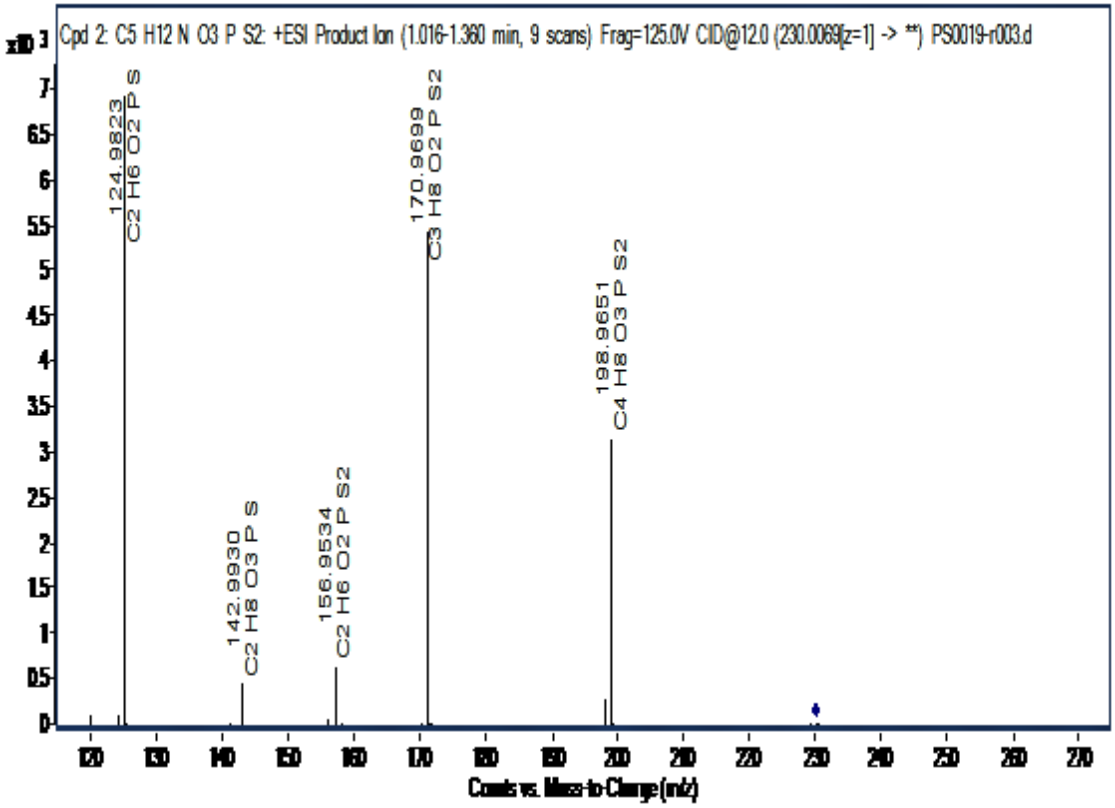
Extracted Ion Chromatogram (EIC) of Dimethoate

(ii)



Mass Spectrum of Dimethoate with the molecular formula, $[M+H]^+$

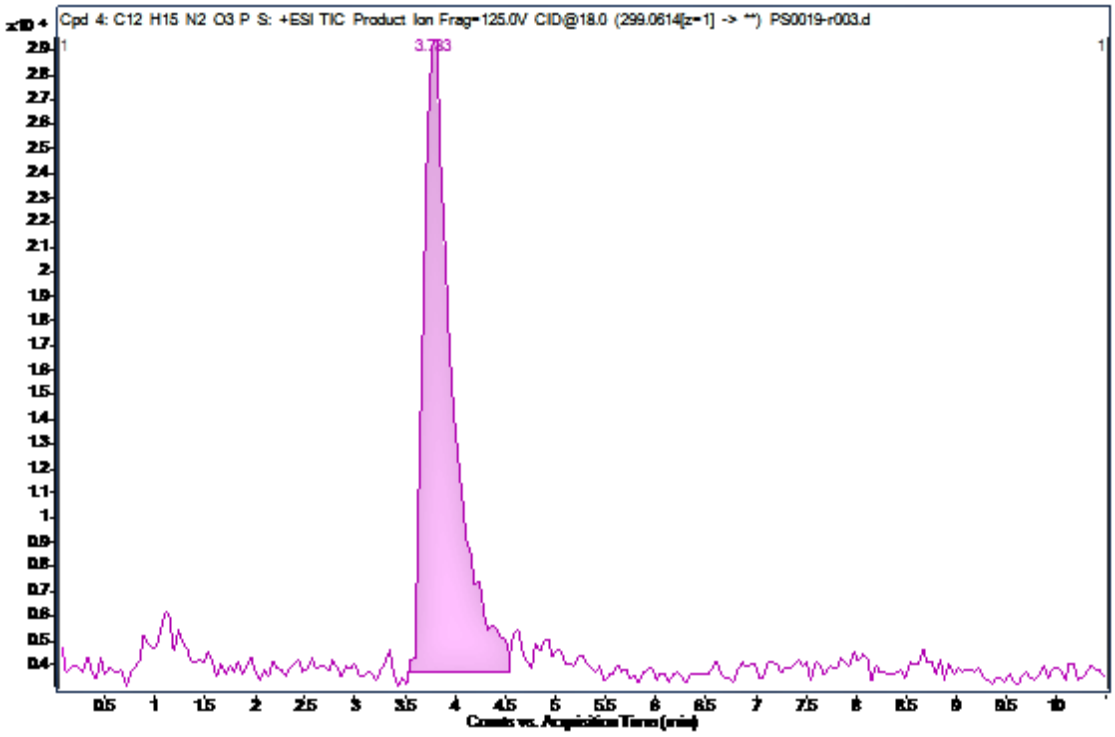
(iii)



Mass spectrum of Dimethoate with the molecular formula of product ion, $[M+H]^+$

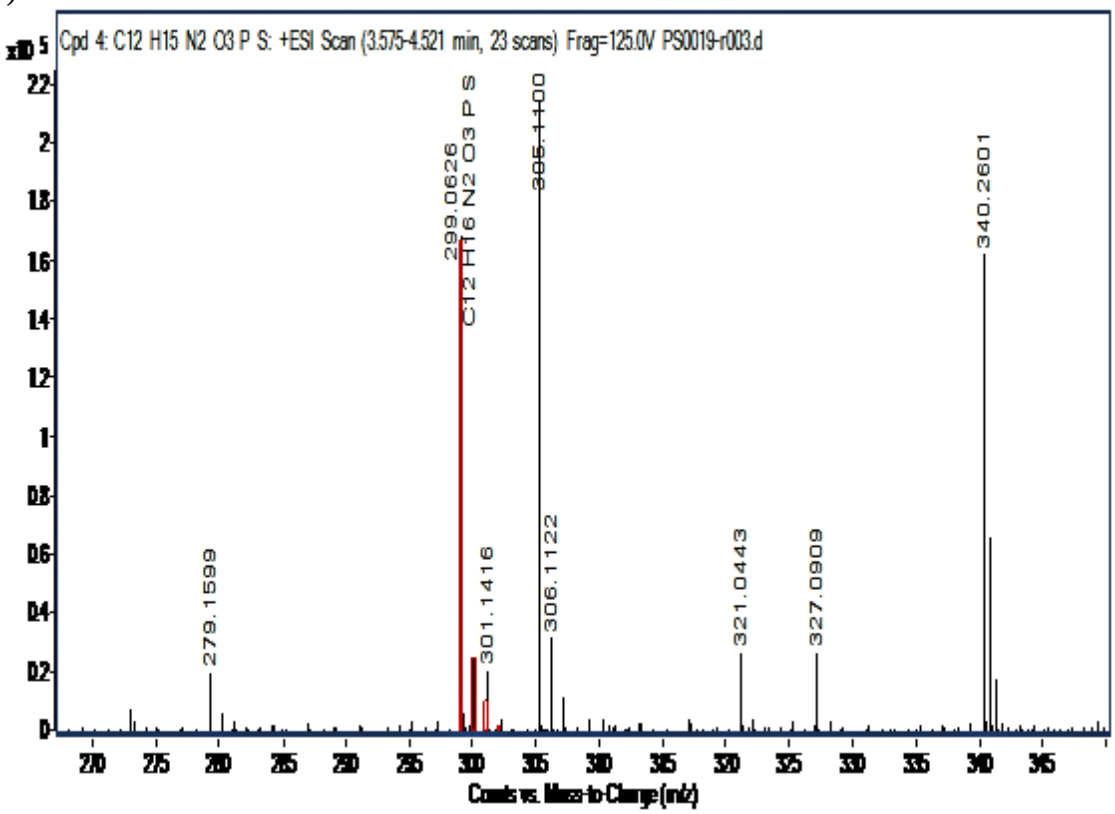
5(d) Quinalphos

(i)



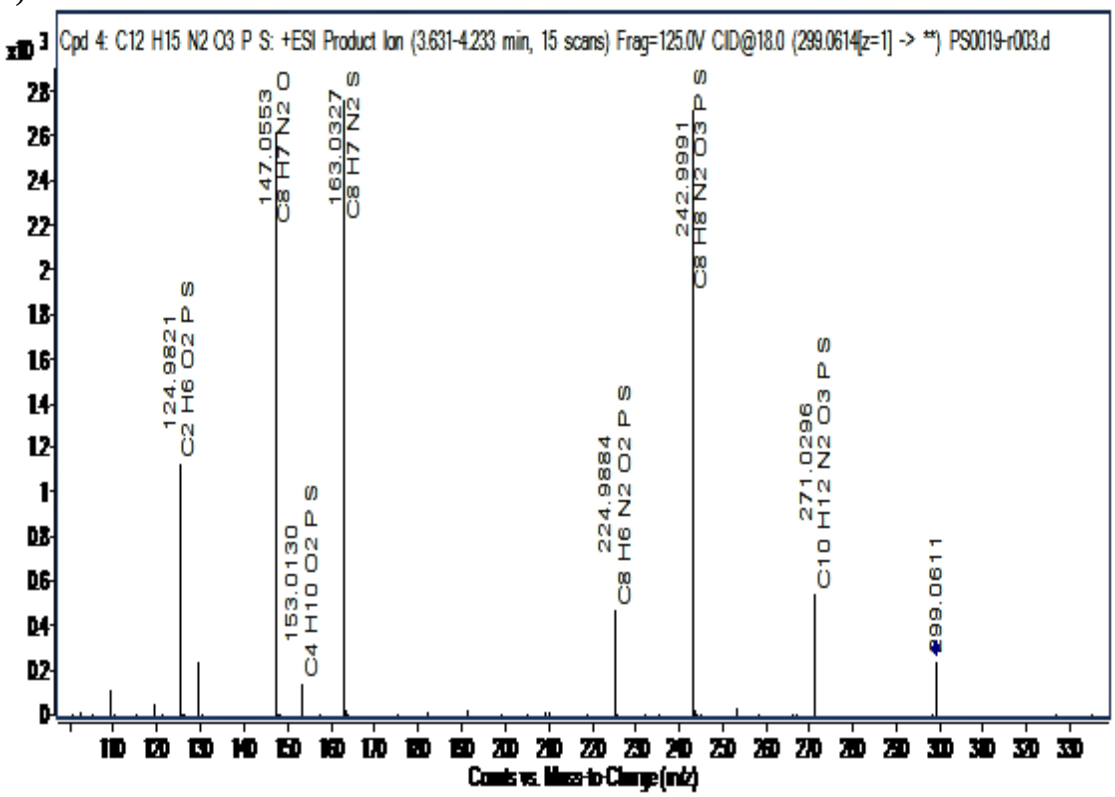
Extracted Ion Chromatogram (EIC) of Quinalphos

(ii)



Mass Spectrum of Quinalphos with the molecular formula, $[M+H]^+$

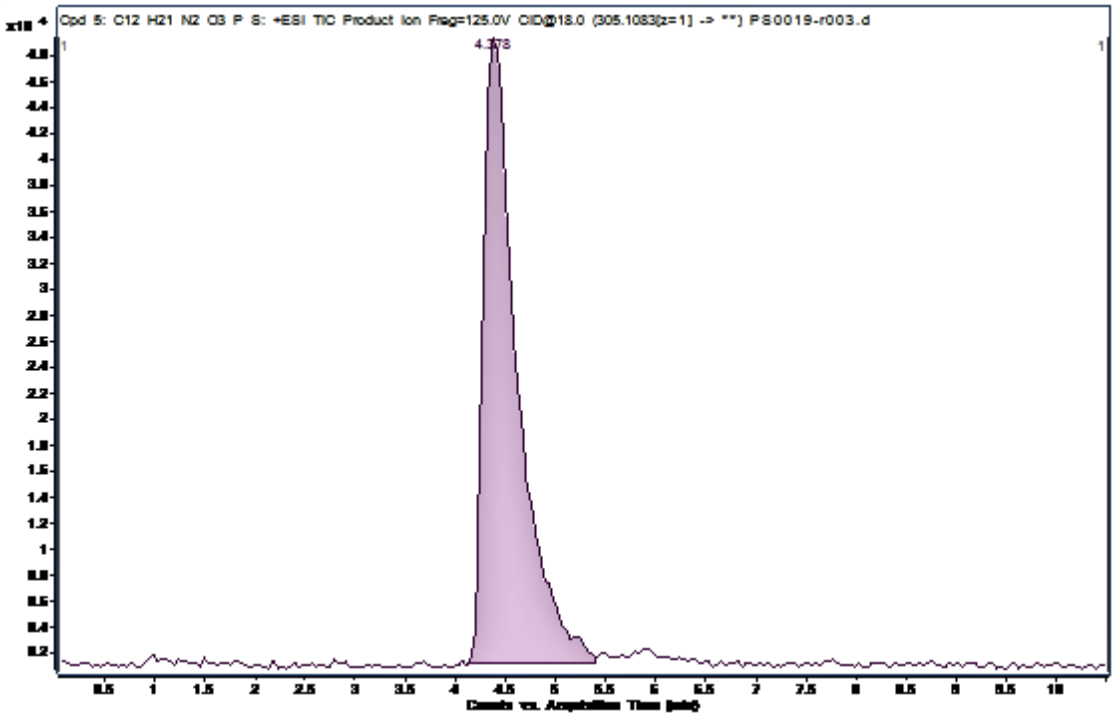
(iii)



Mass spectrum of Quinalphos with the molecular formula of product ion, $[M+H]^+$

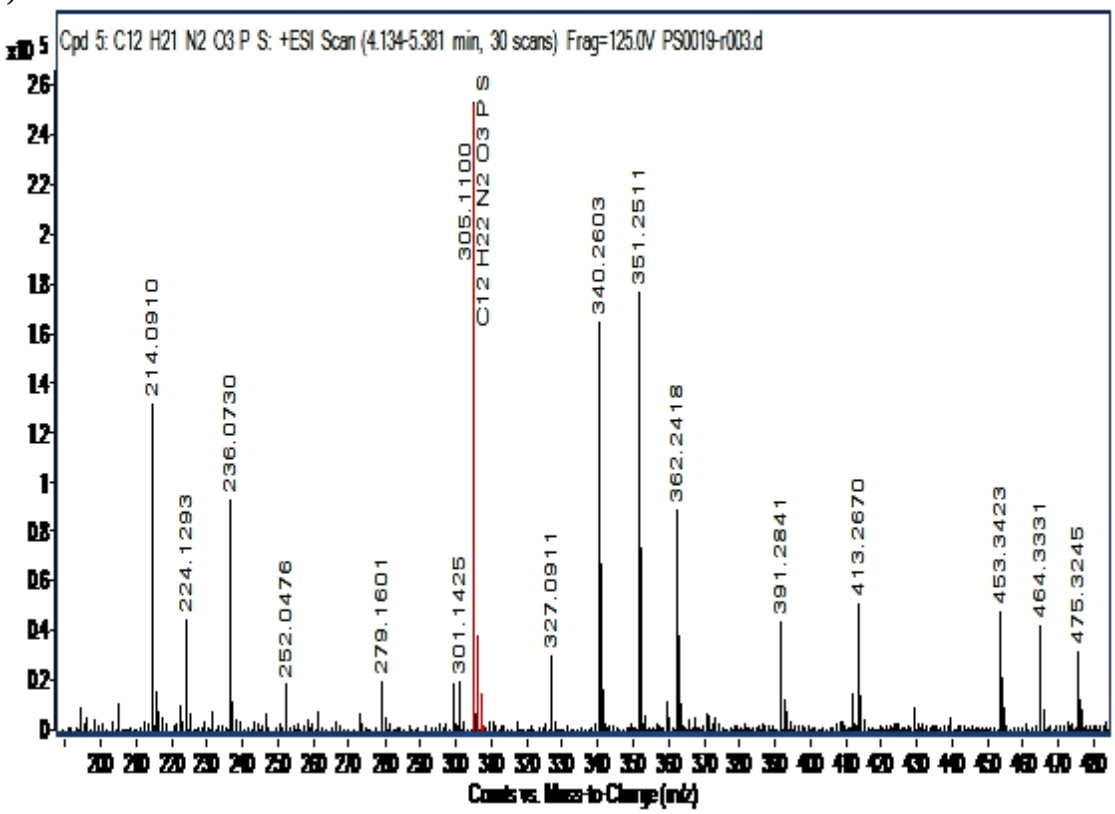
5(e) Diazinon

(i)



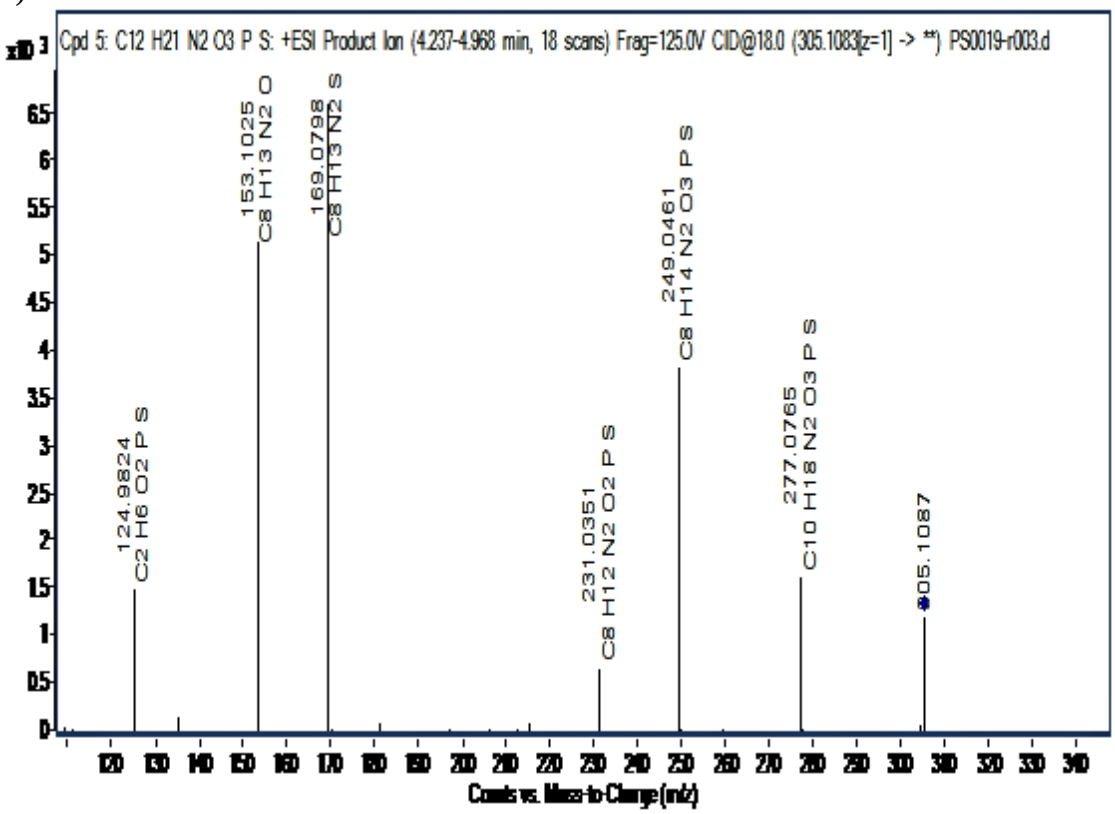
Extracted Ion Chromatogram (EIC) of Diazinon

(ii)



Mass Spectrum of Diazinon with the molecular formula, $[M+H]^+$

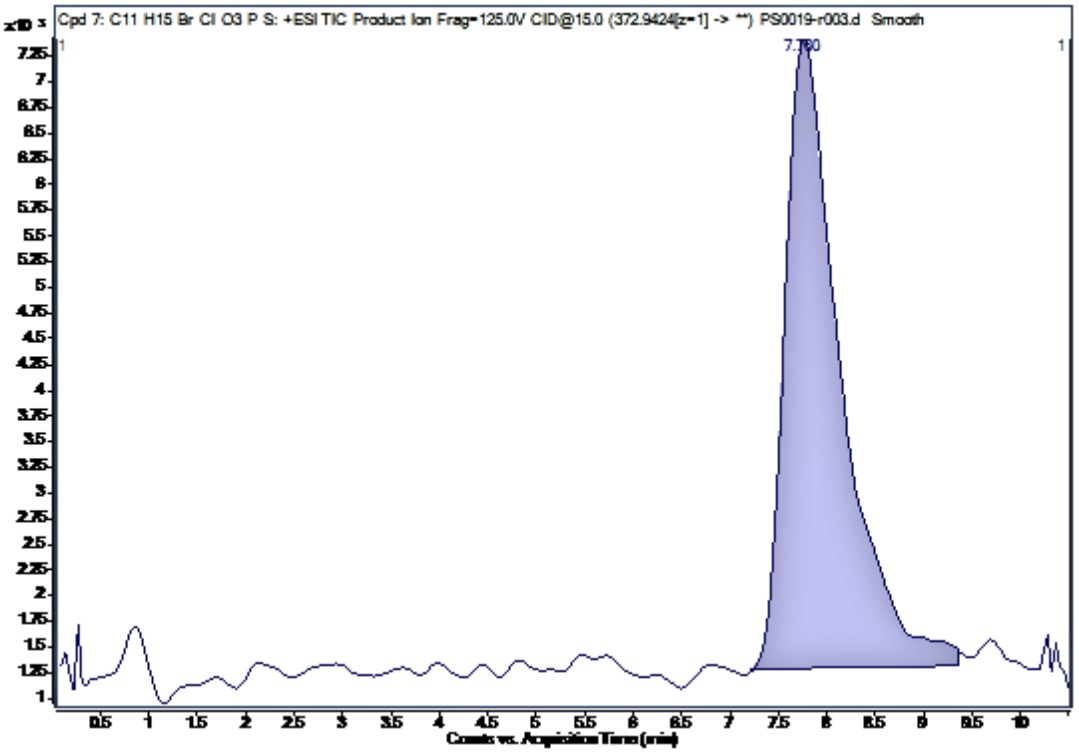
(iii)



Mass spectrum of Diazinon with the molecular formula of product ion, $[M+H]^+$

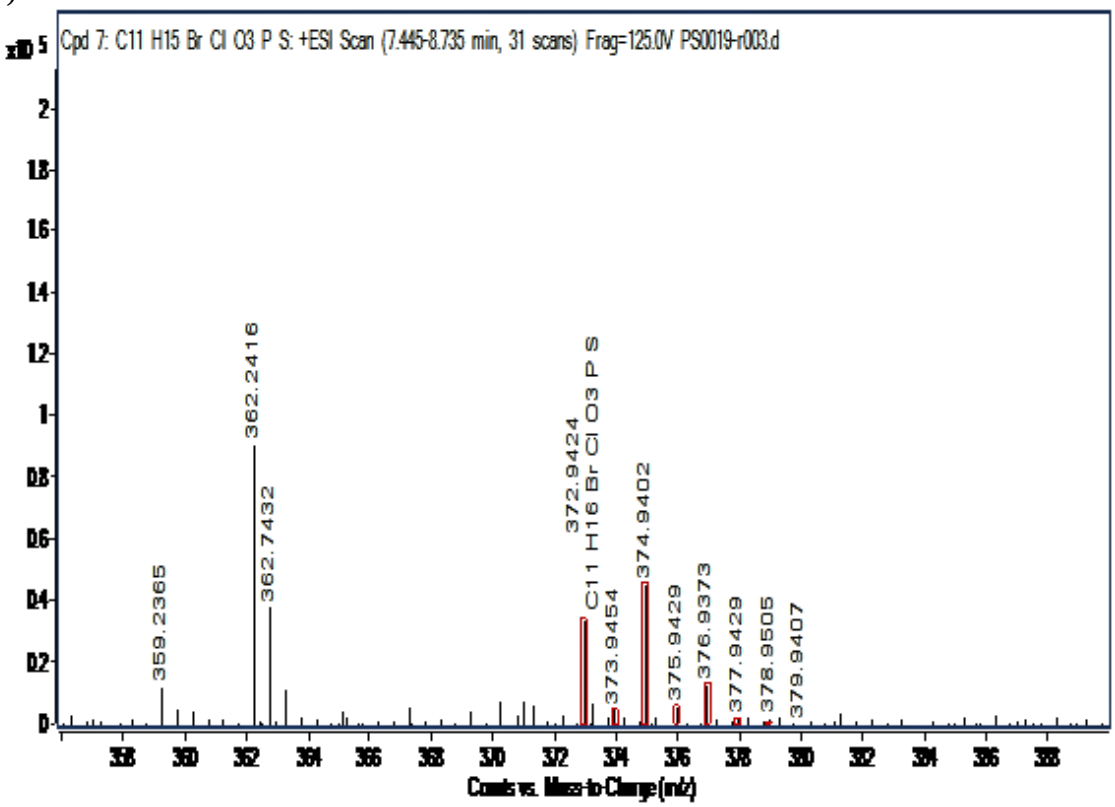
5 (f) Profenofos

(i)



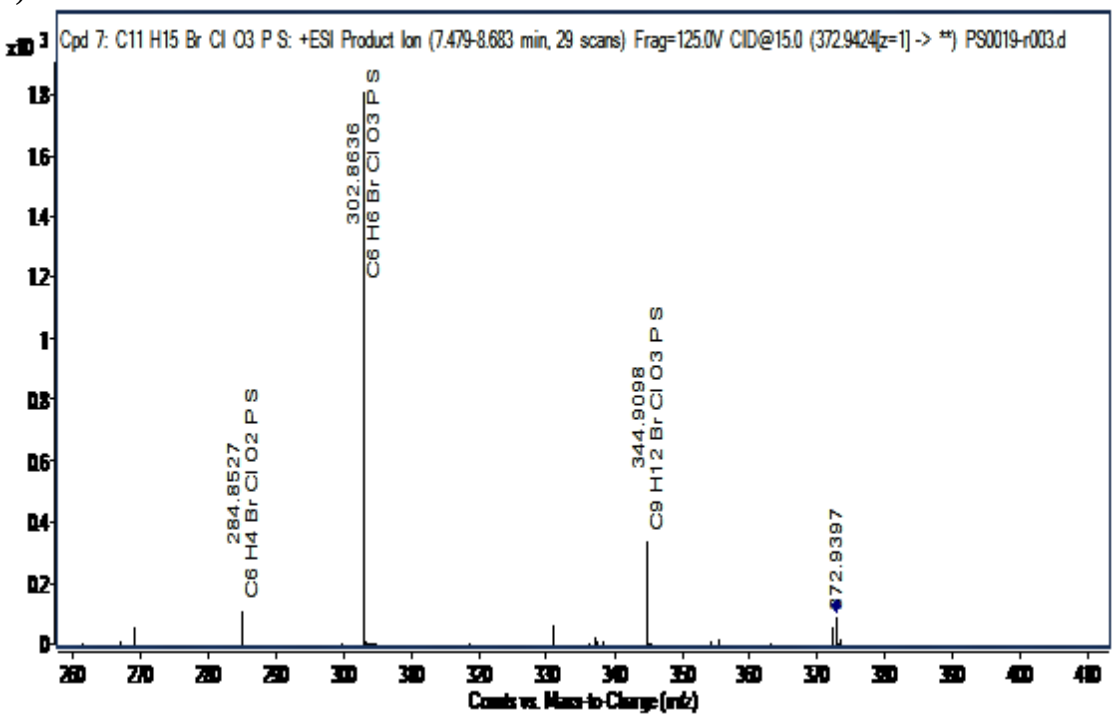
Extracted Ion Chromatogram (EIC) of Profenofos

(ii)



Mass Spectrum of Profenofos with the molecular formula, $[M+H]^+$

(iii)



Mass spectrum of Profenofos with the molecular formula of product ion, $[M+H]^+$

Figure 5(a)-(f): The Extracted Ion Chromatograms (EIC) and mass spectrums of pesticide used to identify the molecular formula of the pesticide compound and product ion as the confirmation of the compound.

 # The blue diamond shape in mass spectrum show the precursor ion of each pesticide.

It was found that a very clean extracted ion chromatogram and mass spectrum was obtained for every single pesticides compound showed that the samples obtained after extraction and clean-up process contained less co-extractive matrices.

3.4 Identification of Pesticides

Pesticides in vegetables sample were identified by the accurate mass measurement of molecular ion and confirmed by the accurate mass measurement of the characteristic fragments of the ions (product ions). **Table 4** shows the calculated molecular mass of pesticides compared to the molecular mass observed from the mass spectrum in the experiment. The differences of the mass were less than 7 ppm or less than 1.7 mDa which were in the acceptable range and profenofos only showed a very small mass error (0.12 ppm) compare to other.

Pesticide	Formula (M)	Mass (m/z)	Calculated Mass (m/z)	Diff. (ppm)	Diff. (mDa)
Acephate	$C_4 H_{10} N O_3 P S$	183.01292	183.0119	-5.56	-1.02
Carbaryl	$C_{12} H_{11} N O_2$	201.08044	201.07898	-7.24	-1.46
Dimethoate	$C_5 H_{12} N O_3 P S_2$	229.00098	228.99962	-5.91	-1.35
Quinalphos	$C_{12} H_{15} N_2 O_3 P S$	298.05532	298.0541	-4.1	-1.22
Diazinon	$C_{12} H_{21} N_2 O_3 P S$	304.10276	304.10105	-5.62	-1.71
Profenofos	$C_{11} H_{15} Br Cl O_3 P S$	371.93519	371.93514	-0.12	-0.04

Table 4: The difference between the observed and calculated mass in parts per million (ppm) and milliDaltons (mDa) for the pesticides used in this study.

The observed abundance as a percentage of the total abundance of the isotopes cluster were also compared to the predicted abundance as a percentage of the total abundance of the isotopes cluster and it was found that the differences were less than 1 %. The details of the molecular formula and relative abundance were shown in Appendix A.

The accurate mass measurement of product ions for each pesticide also has been used as confirmatory to identify the empirical formula of pesticides. The mass spectrums of characteristic fragments ion of each pesticides compound were shown in the **Figure 5** above. The predicted molecular formula of the product ions were in **Table 5** below:

5(a) Acephate.

Peak	m/z	Abund%	Formula	Calc. m/z	Diff (ppm)	Diff (mDa)
1	124.98302	36.32	$C_2 H_6 O_2 P S$	124.98206	-7.64	-0.95
2	142.99333	100	$C_2 H_8 O_3 P S$	142.99263	-4.94	-0.71

5(b) Carbaryl

Peak	m/z	Abund%	Formula	Calc m/z	Diff (ppm)	Diff (mDa)
1	145.06488	100	$C_{10} H_9 O$	145.06479	-0.62	-0.09

5(c) Dimethoate

Peak	m/z	Abund%	Formula	Calc m/z	Diff (ppm)	Diff (mDa)
1	124.98226	100	$C_2 H_6 O_2 P S$	124.98206	-1.59	-0.2
2	170.9699	78.58	$C_3 H_8 O_2 P S_2$	170.96978	-0.67	-0.11

5(d) Quinalphos

Peak	m/z	Abund%	Formula	Calc m/z	Diff (ppm)	Diff (mDa)
1	124.98209	41.01	$C_2 H_6 O_2 P S$	124.98206	-0.25	-0.03
2	163.03271	100	$C_8 H_7 N_2 S$	163.03245	-1.61	-0.26
3	242.99912	98.84	$C_8 H_8 N_2 O_3 P S$	242.99878	-1.41	-0.34

5(e) Diazinon

Peak	m/z	Abund%	Formula	Calc m/z	Diff (ppm)	Diff (mDa)
1	153.10246	77.84	$C_8 H_{13} N_2 O$	153.10224	-1.42	-0.22
2	169.0798	100	$C_8 H_{13} N_2 S$	169.0794	-2.38	-0.4

5(f) Profenofos

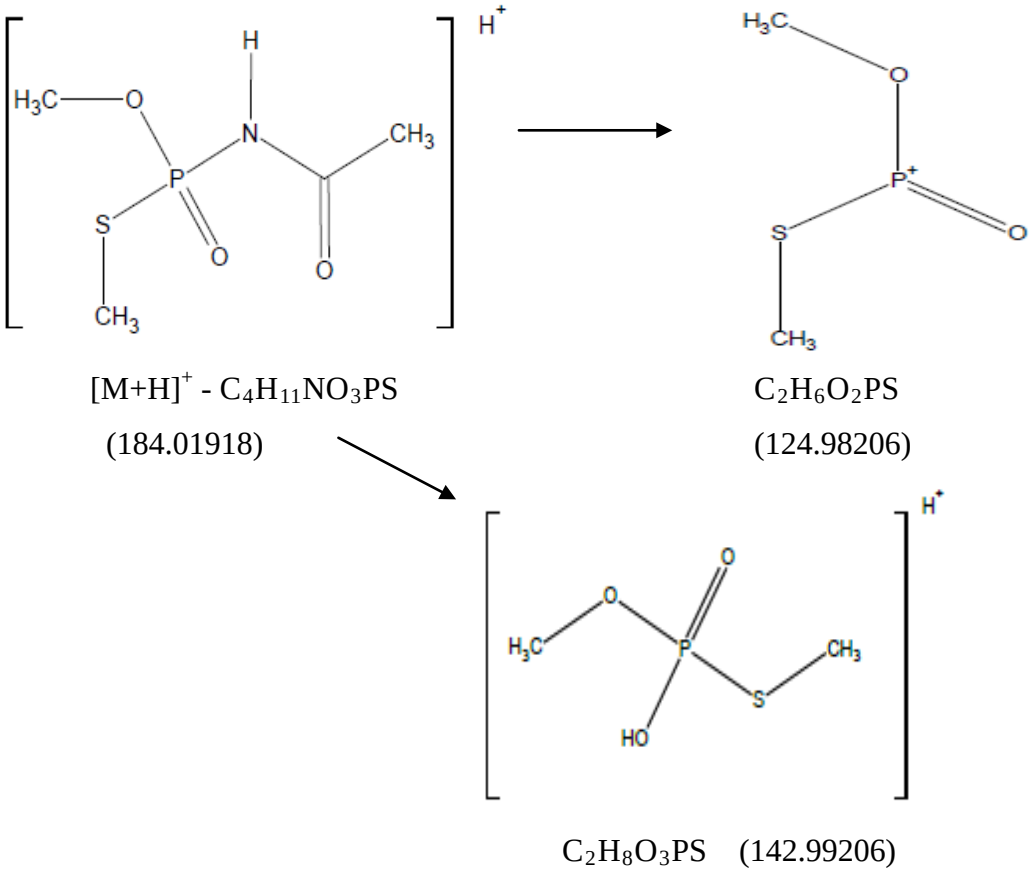
Peak	m/z	Abund%	Formula	Calc m/z	Diff (ppm)	Diff (mDa)
1	302.86358	100	C_6 H_6 Br Cl O_3 P	302.86417	1.94	0.59

Table 5 (a)–(f) The differences between the observed and calculated mass in parts per million (ppm) and milliDaltons (mDa) for the product ions of pesticides used in this study

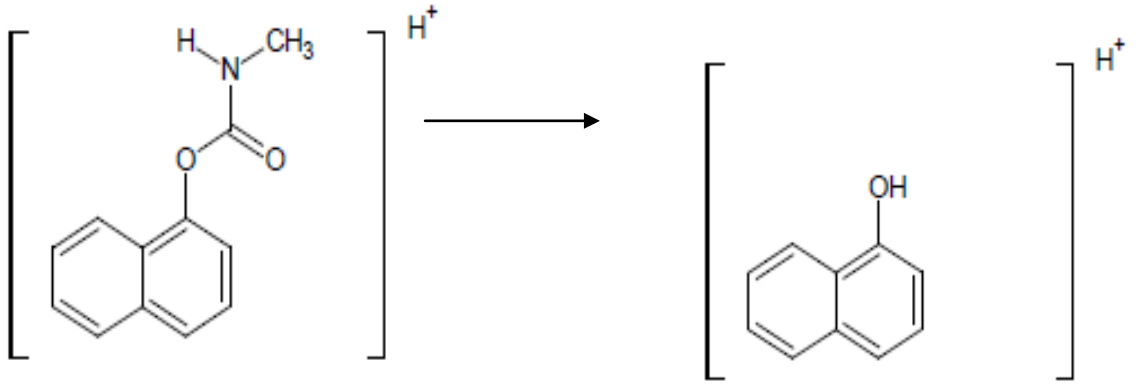
The product ions with the relative abundance 35 to 100% were chosen as identification of the pesticides. The mass error for the product ion used as identification for all pesticide investigated in this study were less than 3 ppm except acephate show a higher mass error, that was 5 ppm. The details of the all the fragments ion (product ions) for all the six types of pesticides were shown in Appendix B.

The suggested structural formula of the pesticide compounds and their characteristic fragments ion were shown as in **Figure 6** below.

6(a) Acephate



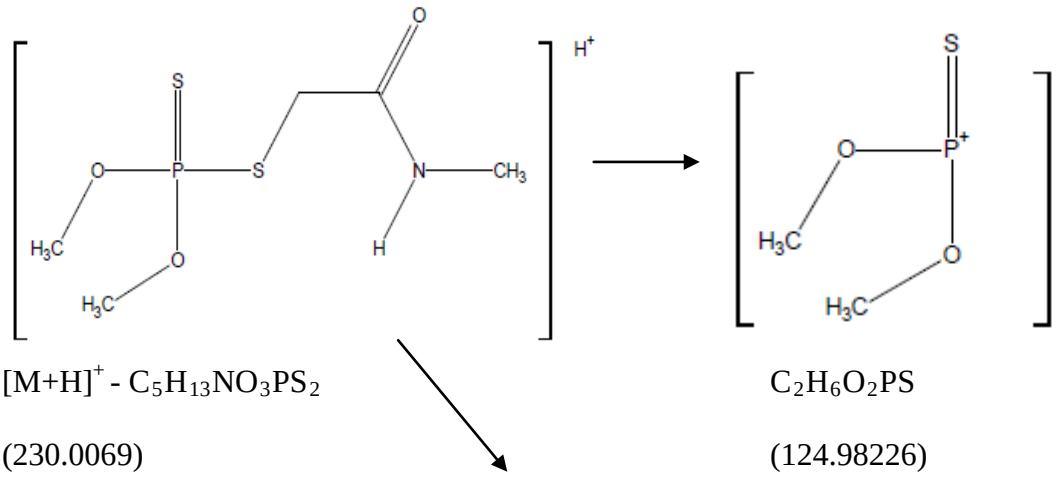
6(b) Carbaryl



$[M+H]^+ - C_{12}H_{12}NO_2$ (202.08626)

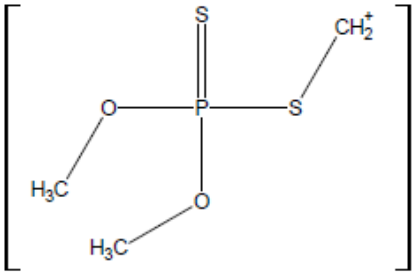
$C_{10}H_9O$ (145.06479)

6(c) Dimethoate



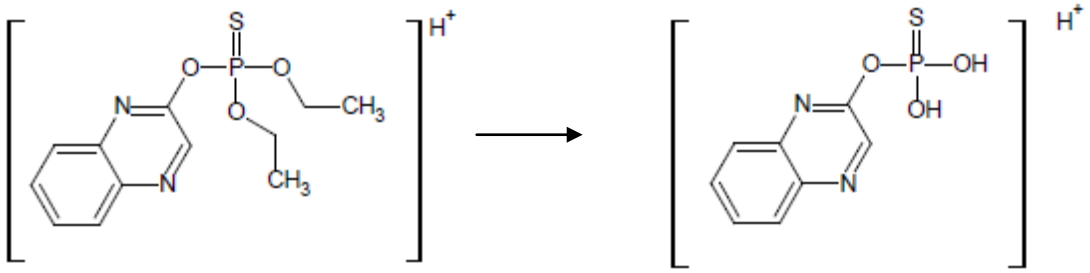
$[M+H]^+ - C_5H_{13}NO_3PS_2$ (230.0069)

$C_2H_6O_2PS$ (124.98226)



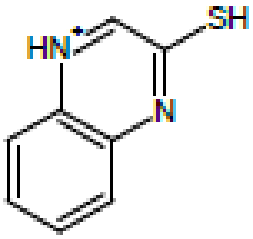
$C_3H_8O_2PS_2$ (170.96978)

6 (d) Quinalphos

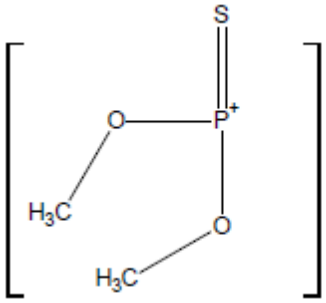


$[M+H]^+$ - $C_{12}H_{16}N_2O_3PS$ (299.06138)

$C_8H_8N_2O_3PS$ (242.99878)

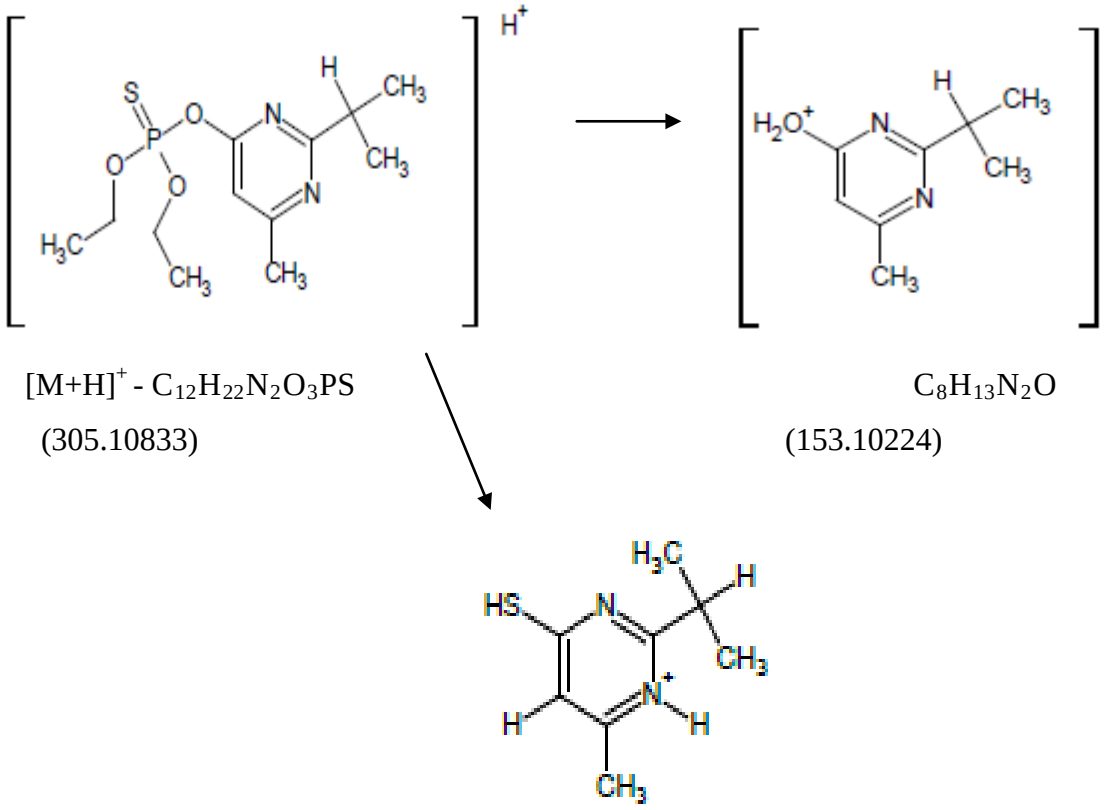


$C_8H_7N_2S$ (163.03245)



$C_2H_6O_2PS$ (124.98206)

6 (e) Diazinon



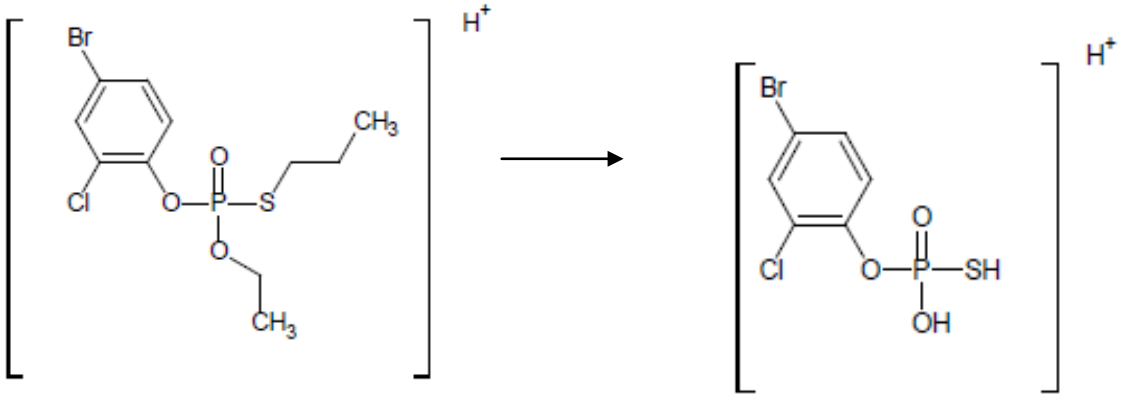
$[M+H]^+$ - $C_{12}H_{22}N_2O_3PS$
(305.10833)

$C_8H_{13}N_2O$
(153.10224)

$C_8H_{13}N_2S$
(169.0794)

[29]

6 (f) Profenofos



$C_{11}H_{16}BrClO_3PS$
(372.94242)

$C_6H_6BrClO_3PS$
(302.86417)

Figure 6(a)-(f): Suggested structural formula for protonated molecular formula of six types of pesticide and their relative fragments ion.

3.5 Linearity of Calibration Curves

Calibration curves for each pesticide were plotted using the response (peak area) of extracted ion chromatogram of quantification ions of each pesticide against concentration of pesticide in standard mixture of pesticides. The known concentration of standard mixture of pesticide was prepared by appropriate serial dilution of the mixed standard stock solution.

The software used for data processing to construct calibration curves was Agilent MassHunter Workstation Sofware –Quatitative Analysis (QTOF) version B.01.04. The characteristic fragment ions and the $[M+H]^+$ were used as quantification and qualification ion. The concentration of pesticide in vegetables sample was then calculated from the linear equation of the calibration graph. The recoveries and linearity of the method was examined. This multiresidue method was demonstrated on 4 types of vegetables samples - *Sawi hijau (leafy vegetables), Kailan (Kale), Yau Mak (Romaine Lettuce) and Tai Pak Choy (Chinese Cabbage).*

External standard method was used to determine the concentration of pesticide in the sample. The interferences from sample matrix effects were omitted in this study. This was due to most of the studies showed that it is not necessary to have further clean-up process after sample extraction.[7] This is because only a small amount of sample will be injected into liquid chromatography column and the specificity of LC-MS/MS (ability of to distinguish the analytes being measured from other substances).[5] Besides that, in most of the cases, no significant differences response of pesticides in sample matrix compared to the pesticides standard in pure solvent calibration,[7,24] therefore, solvent based standards could be used for accurate quantitation of real sample.[14]

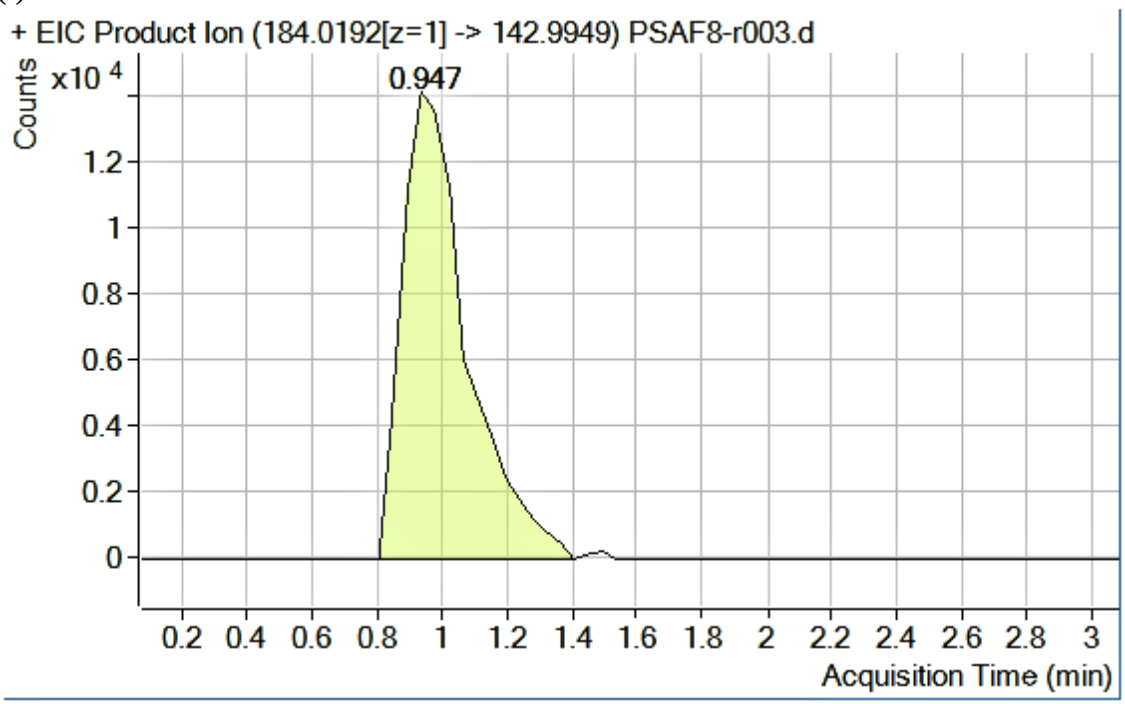
In this study, only 2µL of samples was injected into the analytical instrument and the samples had been clean-up by using Florisil which can eliminate most of the

interfering matrices. Matrices effect should delay or shortened the retention time of pesticides in the liquid chromatography column. The figures in the Appendix C showed the extracted ion chromatogram for different pesticide in different concentration and samples, and the retention time was shown on the top of each chromatogram peak. By comparing the retention time, it was found that the retention time in both methanol and pesticides are almost the same with the corresponded intensity/response From the extracted ion chromatogram, the retention time of each pesticide in the methanol and in samples were not much differences and all in the range, so, it can be proved that the chromatogram were not much interfered by matrices effects in this study, therefore, pesticide in methanol were used as calibration graph and the concentration of pesticides are determine by interpolating or extrapolating of the calibration curves.

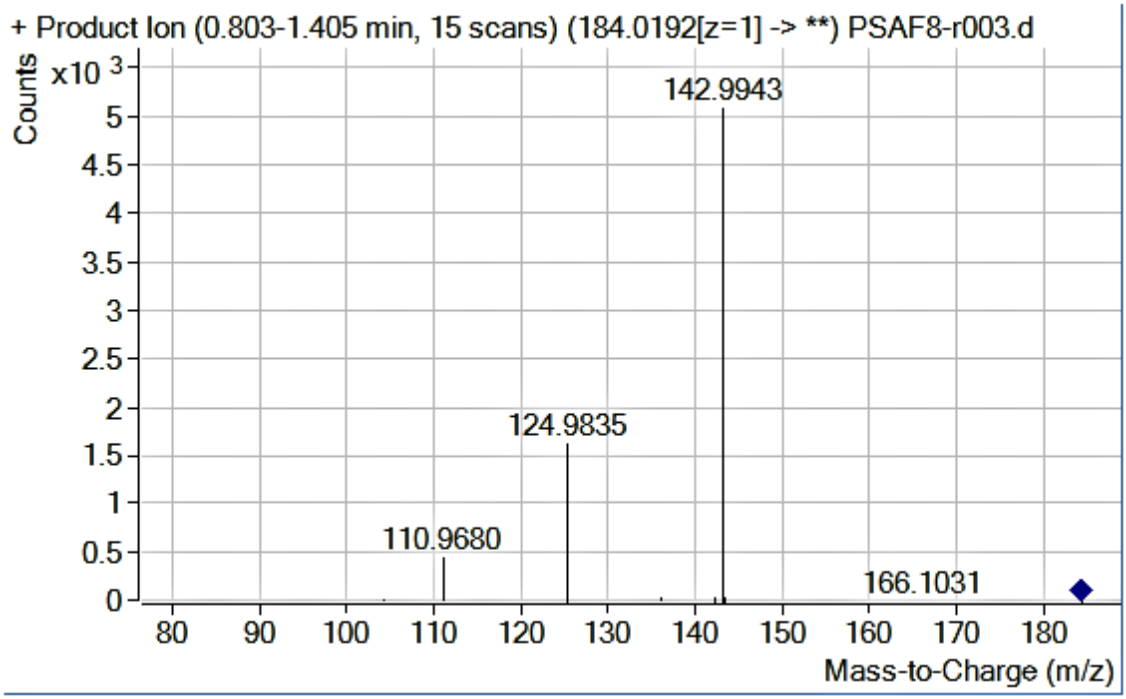
The examples of extracted ion chromatogram of quantifier (characteristic fragment ions) and mass spectrum of the pesticides used to construct calibration graph were shown in **Figure 7** below:

7 (a) Acephate

(i)



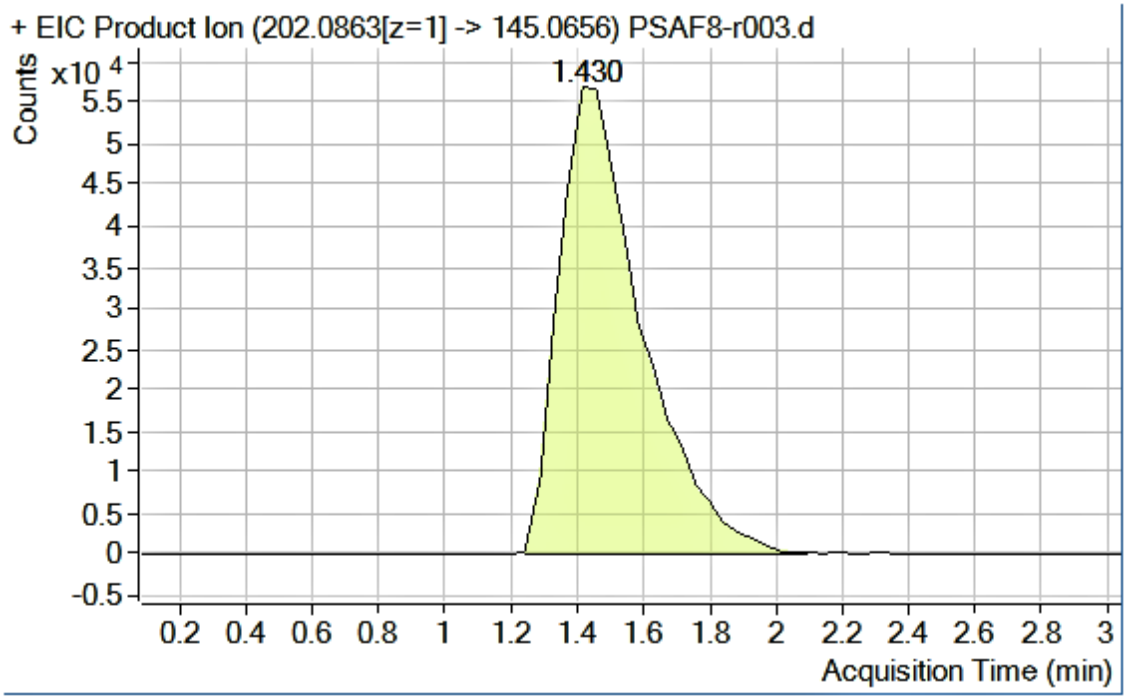
(ii)



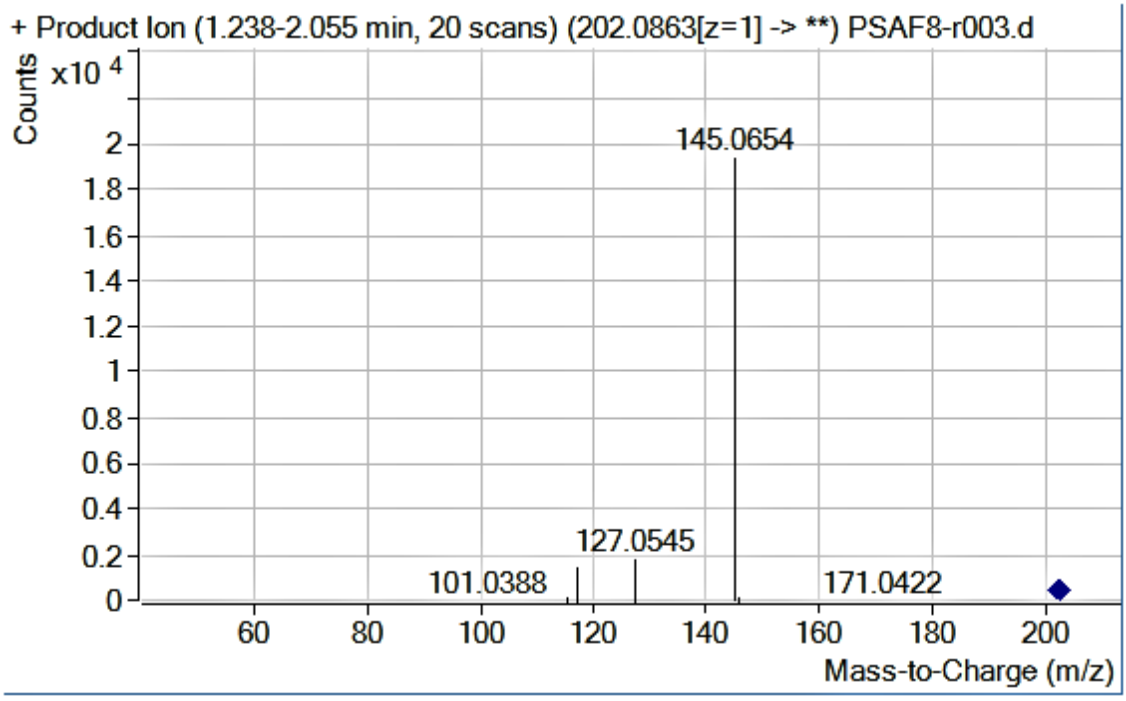
#The blue dot in the spectrum indicated the precursor ion, [M+H]$^+$ of the pesticide

7 (b) Carbaryl

(i)

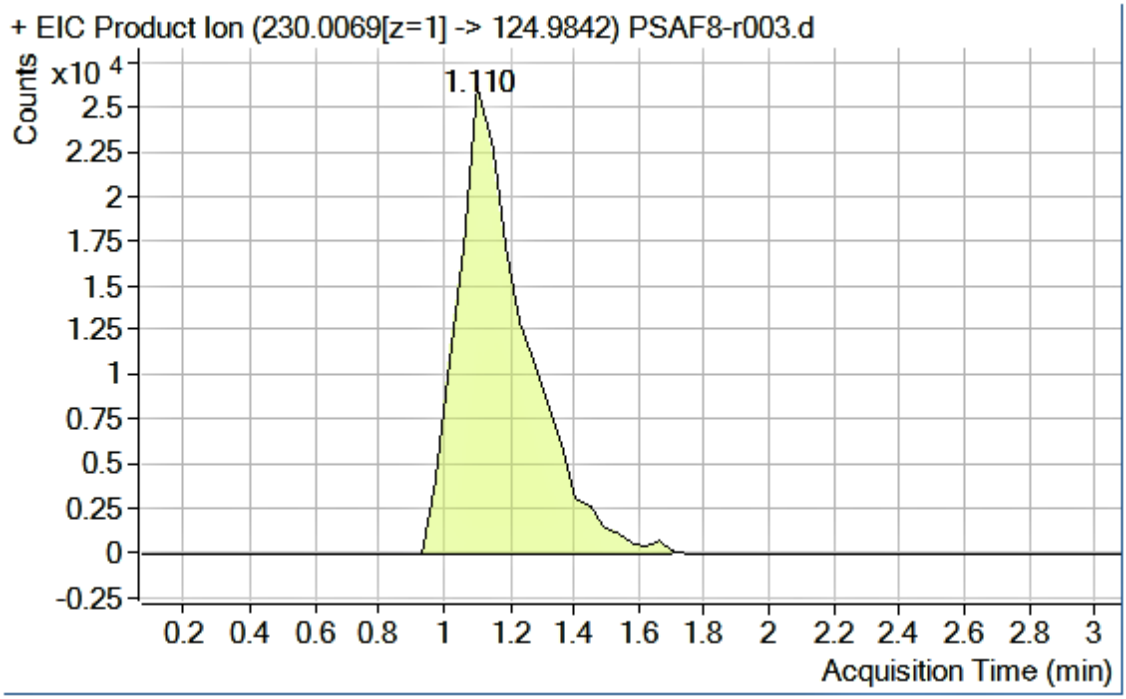


(ii)

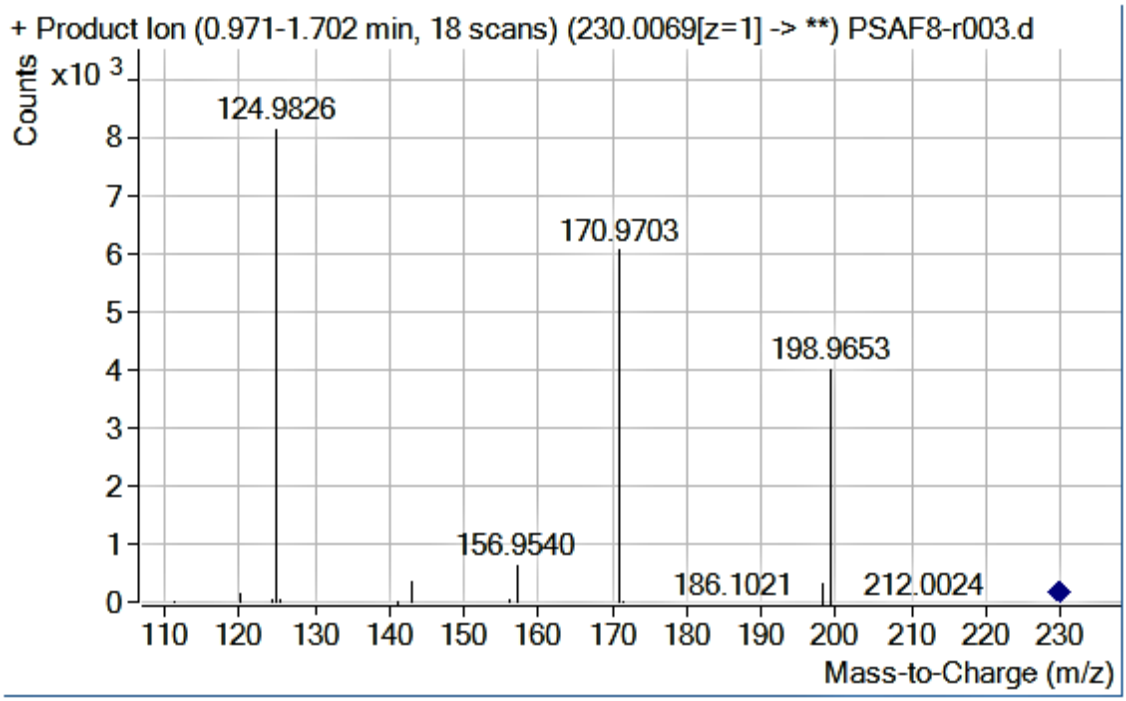


7(c) Dimethoate

(i)

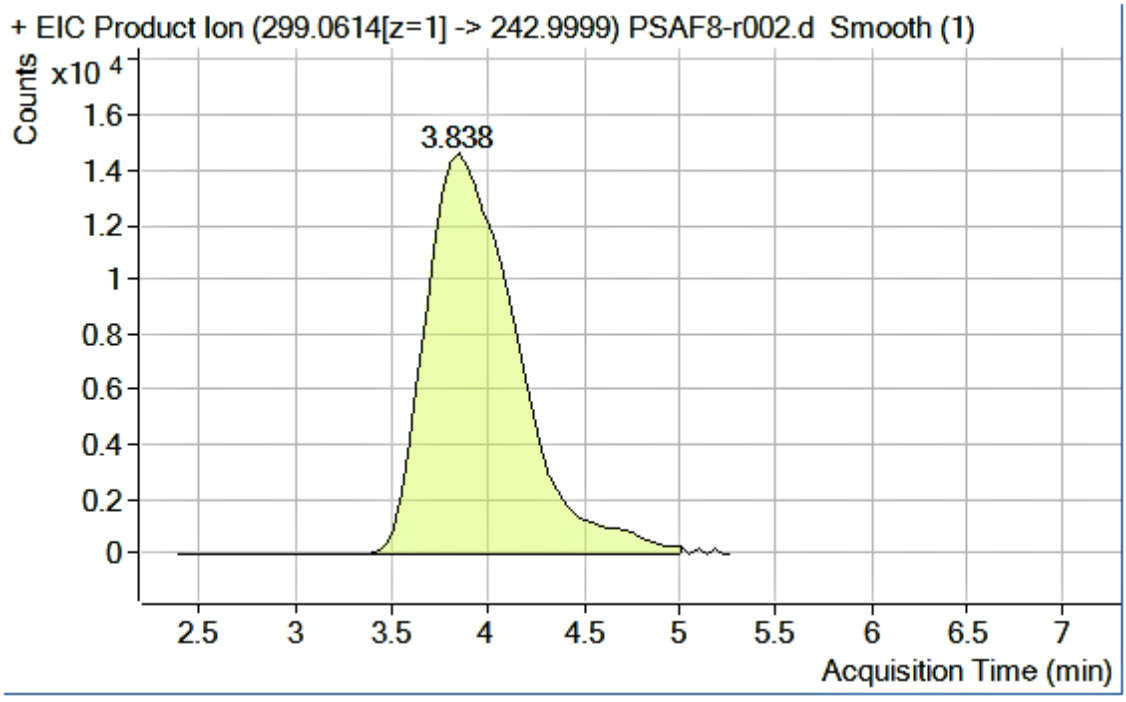


(ii)

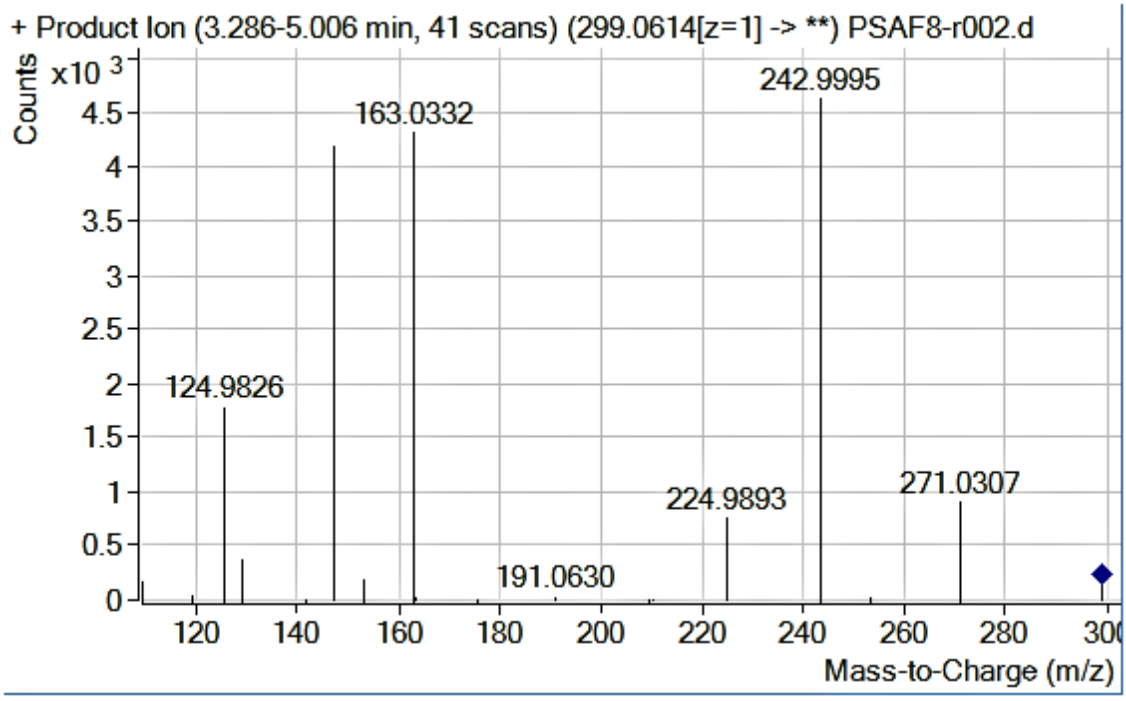


7(d) Quinalphos

(i)

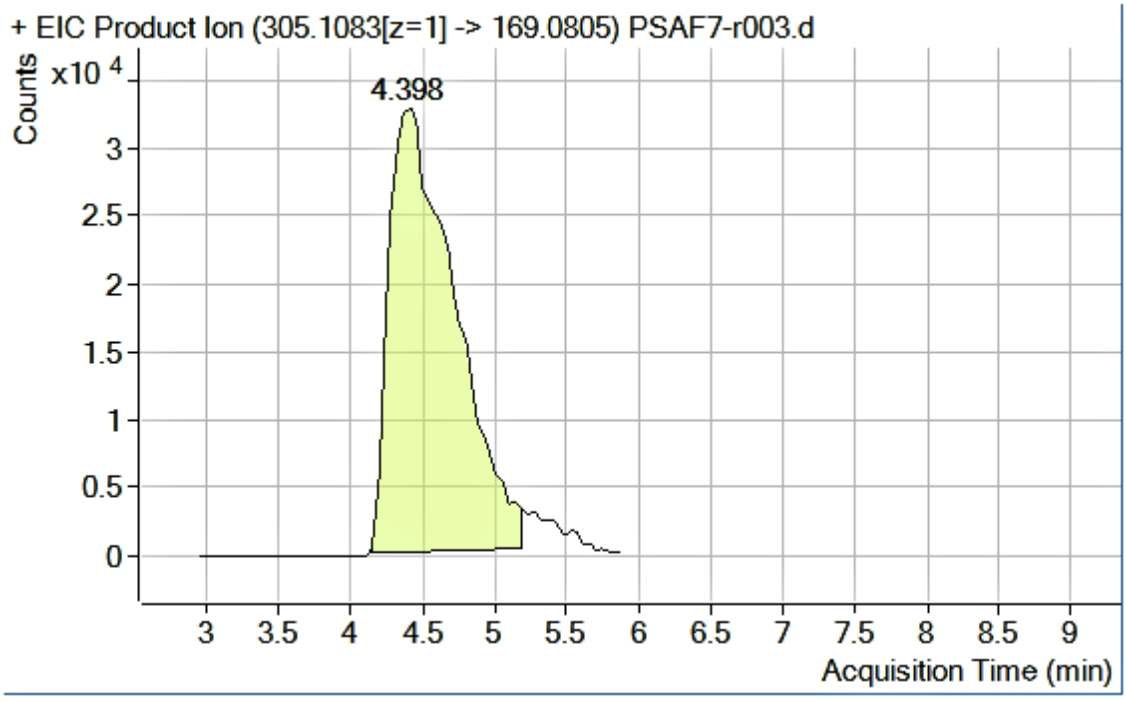


(ii)

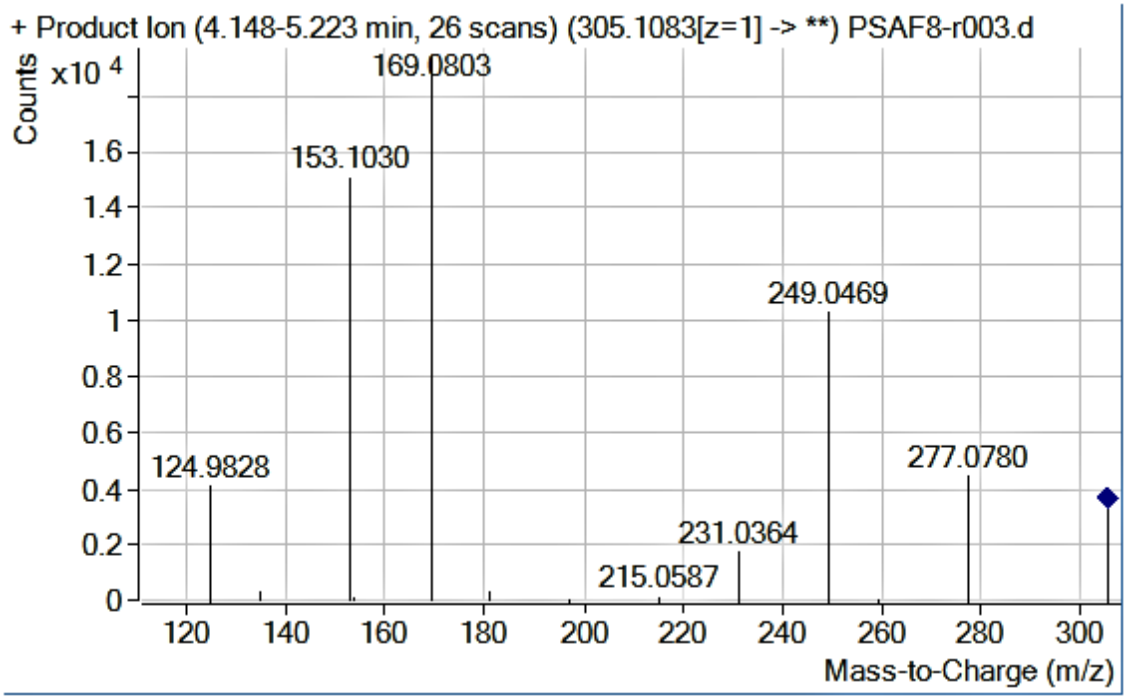


7(e) Diazinon

(i)

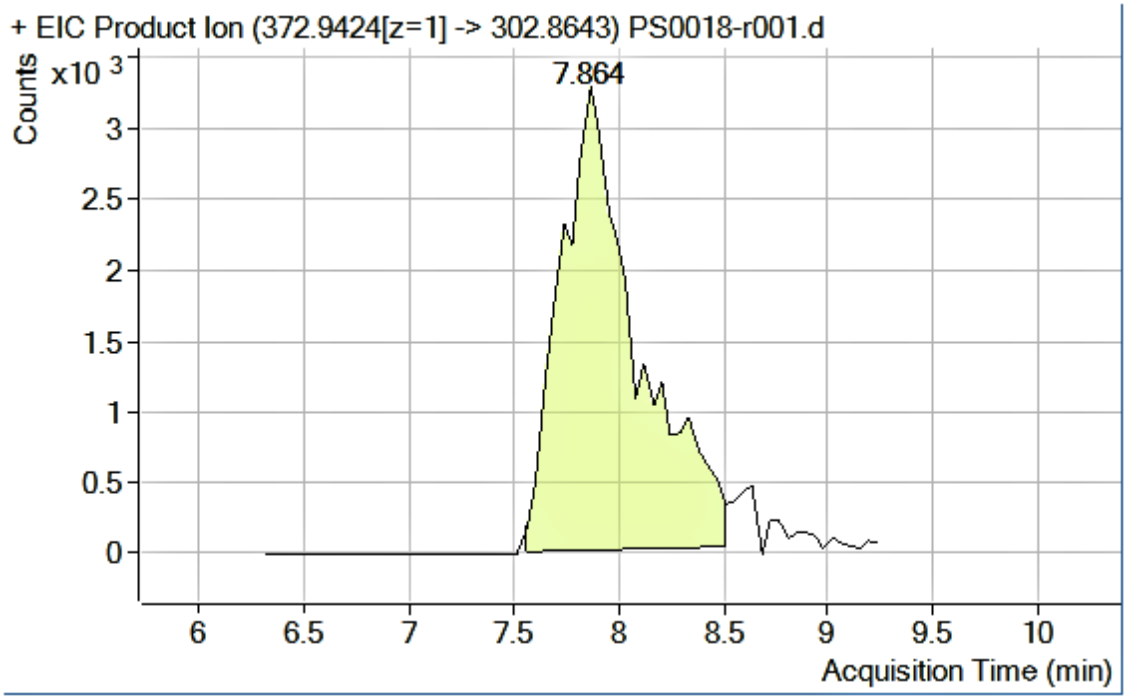


(ii)



7(f) Profenofos

(i)



(ii)

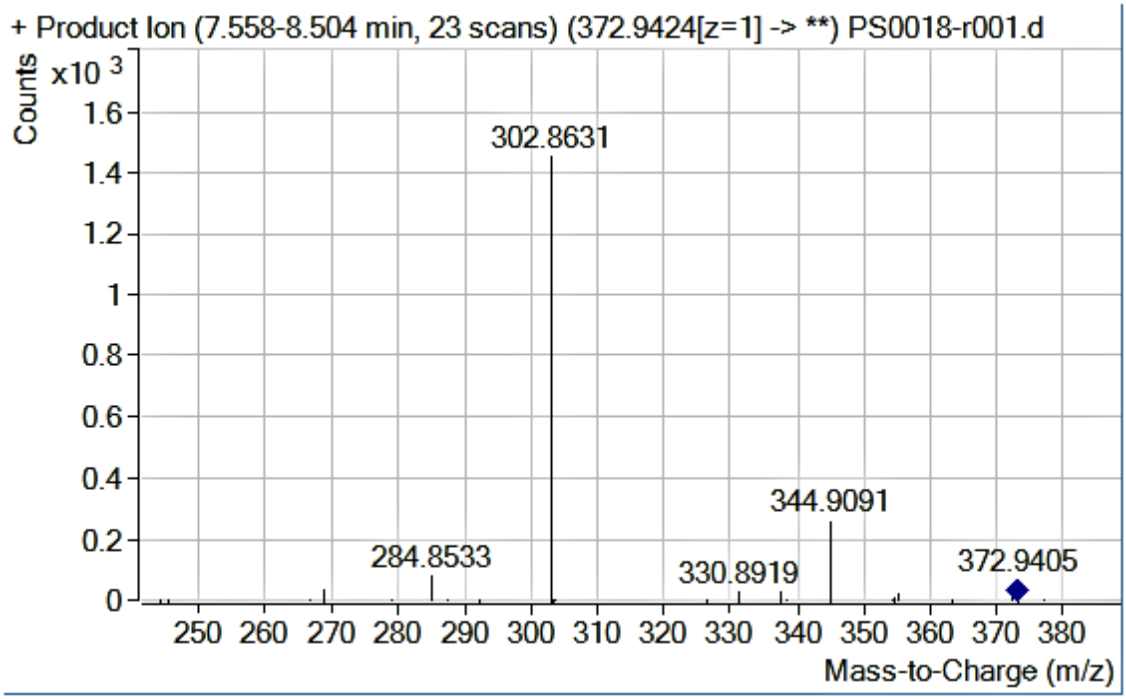


Figure 7 (a)-(f) are the examples of (i) chromatograms and (ii) mass spectrums in targeted MS/MS mode of pesticides used to construct calibration curves.

The accurate mass measurement of the protonated precursor ions and product ions were used for both identification and quantification purposes.[14] The quantifiers used for calibration graph were as in **Table 6** below:

Pesticide	Precursor ion (m/z)	Product ion 1(m/z)	Product ion 2 (m/z)
Acephate	184.0192	142.9943	124.9835
Carbaryl	202.0863	145.0654	-
Dimethoate	230.0069	124.9826	170.9703
Quinalphos	299.0614	163.0332	242.9995
Diazinon	305.1083	169.0803	153.1030
Profenofos	372.9424	302.8631	-

Table 6: Precursor ions and products ions used to construct calibration graph for six types of pesticides in this study.

The tables in Appendix D show the relative responses and concentration of individual pesticides used to construct the calibration curves and the calibrations curves

were shown in Appendix E for each pesticide. The linearity of the chromatographic determination of all the 6 pesticides was examined for concentration ranges as shown in **Table** 7.

Pesticide	Linearity Coefficient, R^2
Acephate	0.999
Carbaryl	0.999
Dimethoate	0.997
Quinalphos	0.998
Diazinon	0.996
Profenofos	0.999

Table7: Linearity coefficient, R^2 of pesticides compound.

The results of all the pesticide calibration curves were within the acceptable values for the linear correlation coefficient ($r^2 \geq 0.99$)

3.6 Recoveries of Pesticides

The recoveries of the six types of pesticides in vegetables were examined at 1 fortification level. Duplicate samples of approximately 5 g each of homogenized, chopped and blended vegetables were each spike with 1 ml of mixed standard 0.2 mg/L stock solution respectively. The spikes samples were extracted with the procedures as shown in **Figure 1** in page 14. The recoveries of the pesticides were in the range of 50% to 130% except for carbaryl which was below 35%.

The concentration of pesticide in the vegetables samples was determined by external standard method. The response (peak area) in the extracted ion chromatogram of quantifier was used to determine the concentration of pesticide by applying the linear regression equation as in the calibration graphs. The method used to calculate the concentration of pesticide from graph, concentration of pesticide in sample and the recovery were shown in Appendix **F. Table 8** below shows the mass of vegetables

samples, concentration of pesticide from graph (mg/L), concentration of pesticide in samples (mg/kg) and the % of recovery in the vegetables samples in this study.

8(a) Acephate

Sample		Mass (g)	Concentration from calibration graph (mg/L)	Concentration (mg/kg)	% of Recovery
Kailan	unspiked	5.1484	n.d	-	84.1
	spiked	5.0193	0.0336	0.0335	
Yau Mak	unspiked	5.0136	n.d	-	97.2
	spike	5.0002	0.0389	0.0389	
Sawi hijau	unspiked	4.9807	n.d	-	87.7
	spiked	5.1788	0.0351	0.0339	
Tai Pak Choy	unspiked	4.8910	n.d	-	93.8
	spiked	5.3141	0.0375	0.0353	

- n.d. – not detected

8 (b) Carbaryl

Sample		Mass (g)	Concentration from calibration graph (mg/L)	Concentration (mg/kg)	% of Recovery
Kailan	unspiked	5.1484	n.d.	-	25.8
	spiked	5.0193	0.0103	0.0103	
Yau Mak	unspiked	5.0136	n.d.	-	35.5
	spike	5.0002	0.0142	0.0142	
Sawi hijau	unspiked	4.9807	n.d.	-	21.3
	spiked	5.1788	0.0085	0.0082	
Tai Pak Choy	unspiked	4.8910	n.d.	-	33.3
	spiked	5.3141	0.0133	0.0125	

8(c) Dimethoate

Sample		Mass (g)	Concentration from calibration graph (mg/L)	Concentration (mg/kg)	% of Recovery
Kailan	unspiked	5.1484	0.0392	0.0380	54.9
	spiked	5.0193	0.0601	0.0599	
Yau Mak	unspiked	5.0136	n.d	-	133.2
	spike	5.0002	0.0533	0.0533	
Sawi hijau	unspiked	4.9807	n.d	-	73.6
	spiked	5.1788	0.0294	0.0284	
Tai Pak Choy	unspiked	4.8910	n.d	-	78.6
	spiked	5.3141	0.0314	0.0296	

8 (d) Quinalphos

Sample		Mass (g)	Concentration from calibration graph (mg/L)	Concentration (mg/kg)	% of Recovery
Kailan	unspiked	5.1484	0.1096	0.1064	56.9
	spiked	5.0193	0.1296	0.1291	
Yau Mak	unspiked	5.0136	0.0964	0.0961	75.3
	spike	5.0002	0.1262	0.1262	
Sawi hijau	unspiked	4.9807	0.0885	0.0889	117.0
	spiked	5.1788	0.1388	0.1340	
Tai Pak Choy	unspiked	4.8910	0.0956	0.0978	99.9
	spiked	5.3141	0.1439	0.1354	

8 (e) Diazinon

Sample		Mass (g)	Concentration from calibration graph (mg/L)	Concentration (mg/kg)	% of Recovery
Kailan	unspiked	5.1484	0.1298	0.1260	53.4
	spiked	5.0193	0.1479	0.1473	
Yau Mak	unspiked	5.0136	0.1160	0.1156	49.7
	spike	5.0002	0.1355	0.1355	
Sawi hijau	unspiked	4.9807	0.0983	0.0987	136.9
	spiked	5.1788	0.1570	0.1516	
Tai Pak Choy	unspiked	4.8910	0.0996	0.1018	112.8
	spiked	5.3141	0.1533	0.1443	

8 (f) Profenofos

Sample		Mass (g)	Concentration from calibration graph (mg/L)	Concentration (mg/kg)	% of Recovery
Kailan	unspiked	5.1484	0.3899	0.3786	99.7
	spiked	5.0193	0.4200	0.4184	
Yau Mak	unspiked	5.0136	0.1052	0.1050	94.4
	spike	5.0002	0.1427	0.1427	
Sawi hijau	unspiked	4.9807	0.1151	0.1156	89.7
	spiked	5.1788	0.1556	0.1502	
Tai Pak Choy	unspiked	4.8910	0.1100	0.1125	118.7
	spiked	5.3141	0.1670	0.1571	

Table 8: The mass of vegetables samples, concentration of pesticide from graph (mg/L), concentration of pesticide in samples (mg/kg) and the % of recovery in the vegetables samples in this study.

The concentration of pesticide in the unspiked vegetables samples were summarized as in **Table 9**.

Pesticide	Concentration of pesticide detected (mg/kg)			
	Kailan (Kale)	Yau Mak (Romaine lettuce)	Sawi hijau (Leafy vegetables)	Tai Pak Choy (Chinese Cabbage)
Acephate	n.d.	n.d.	n.d.	n.d.
Carbaryl	n.d.	n.d.	n.d.	n.d.
Dimethoate	0.0380	n.d.	n.d.	n.d.
Quinalphos	0.1064	0.0961	0.0889	0.0978
Diazinon	0.1260	0.1156	0.0987	0.1018
Profenofos	0.3786	0.1050	0.1156	0.1125

Table 9: Concentration of pesticide in unspike vegetables samples (mg/kg)

Table 10 below shows the maximum residue level (MRL) of pesticides in vegetables as in Malaysia Food Act, 1983 (Act 281) & Regulations:[30]

Pesticide	Vegetable Samples	MRLs (mg/kg)
Acephate	Kale	5
	Lettuce	5
	Cabbage	2
Carbaryl	Chinese Cabbage	5
Dimethoate	Kale	0.5
	Lettuce	2
	Leafy vegetables	2
Quinalphos	Cabbage	0.1
Diazinon	Kale	0.5
	Chinese Cabbage	0.5
Profenofos	Kale	2
	Cabbage	1

Table 10: MRLs of pesticides in vegetables in this study as in Malaysia Food Act,1983

"Not prescribed" means the Maximum Residue Limits are not required.

By comparing the MRLs stated in the Malaysia Food Act, 1983 in Table 10 with the concentration of pesticides detected in Table 9. None of the vegetable samples investigated exceed the MRLs.

Table 11 below shows the percentage of recovery in the vegetables samples for each type of pesticide.

Sample / Pesticide	Percentage of the recovery (%)			
	Kailan (Kale)	Yau Mak (Romaine lettuce)	Sawi hijau (Leafy vegetables)	Tai Pak Choy (Chinese Cabbage)
Acephate	84.1	97.2	87.7	93.8
Carbaryl	25.8	35.5	21.3	31.3
Dimethoate	54.9	133.2	73.6	78.6
Quinalphos	56.9	75.3	117.0	99.9
Diazinon	53.4	49.7	136.9	112.8
Profenofos	99.7	94.4	89.7	118.7

Table 11: The percentage of recovery of the pesticides in vegetables sample in this study.

It was found that the percentage of the recovery was satisfied in all the pesticide except for Carbaryl. Florisil column is not suitable for samples contain polar pesticide because it will remove most of the organophosphate and carbamate pesticides. However, by using aceetonitrile to eluate the pesticide in the Florisil clean-up, all the organophosphate pesticides were successfully eluated as an acceptable percentage of recovery was obtained but not for carbamate pesticides. Percentage of recovery for both acephate and profenofos in all the vegetables investigated were among the best which were in the range of 80-120% while dimethoate, quinalphos and diazinon also showed an acceptable range in 50-130%. However, Carbaryl (carbamates pesticide) only showed

very low recovery, less than 35% which was out of range. Other sample clean-up method should be used to increase the recovery of carbaryl (carbamates) in this study.

3.7 Detection Limit

The Limit of Detection (LOD) is defined as the lowest concentration that can be determined to be statistically different from a blank. This concentration is recommended to be three standard deviations above the measured average difference between the sample and blank signals, which corresponds to the 99% confidence level.[30]

A preliminary estimation of the LOD was calculated as shown in Appendix G. [31] **Table 12** showed the limits of detection (LOD) of the 6 pesticides with the linearity coefficient, $R^2 > 0.99$.

Pesticide	LOD (mg/kg))	Linearity coefficient,
Acephate	0.0171	0.999
Carbaryl	0.0099	0.999
Dimethoate	0.0262	0.997
Quinalphos	0.0183	0.998
Diazinon	0.0307	0.996
Profenofos	0.0133	0.999

Table 12: LOD of 6 types of pesticides used in the study

The LOD was further estimated from the chromatogram where the S/N ratio =3, it was found the LOD was lower than what had estimated from the preliminary estimation. The chromatograms of each of the pesticides with the concentration of 0.01 mg/L (Appendix H) was used with the calculated average S/N ratio=3. The steps and the average S/N ratio were shown in Appendix I. It was found that the LOD for each of the pesticide were at 0.01 mg/kg (10ppb).

Chapter 4

Conclusion

Ultrasonic solvent extraction method had been used in this study to extract the pesticides from the vegetables matrices because it is more efficient compare to homogenous method. Ethyl acetate was chosen as it is immiscible with water and less co-extracted matrices were extracted together with the interested pesticides. Anhydrous sodium sulphate was used to force all the polar pesticides to the ethyl acetate layer and absorbed some of the water content in the vegetables. Florisil column had been used for sample clean up even though it is not suitable for moderate polar to polar pesticides but it is suitable to used with vegetables matrices as compare to alumina and silica gel to produce a clean eluant. Acetonitrile which is suitable for eluating moderate polar and polar pesticides was used to overcome the drawbacks of the Florisil column. The recovery for all the moderate polar to polar pesticide such as acephate, dimethoate, diazinon, quinalphos and profenofos (all the organophosphates pesticides) were in the range showed that acetonitrile was suitable to overcome the drawback of the Florisil column but not for carbaryl which still showed a low recovery. Carbaryl showed a low recovery may be due to the matrix effect and lost in the clean up steps by using Florisil colum. Other clean-up method should be use to obtain good recovery of carbamate pesticides in vegetables, for example, the gel permeation chromatography method. Matrix matched method should be used to avoid matrix effects for quantitative analysis so that a better recovery results able to be obtained for carbaryl. Even though there was no significant change in the retention time of carbaryl in sample matrices compare to carbaryl in pure methanol solvent but the signal suppression or enhancement due to the matrix effects may cause the low recoveries in vegetables.

A clean, less background noise extracted ion chromatogram and mass spectrum was obtained showed the high selectivity of LC-Q-TOF-MS technique. High mass accuracy measurement for all the pesticide showed that the LC-Q-TOF MS is a powerful tool for identification of pesticides. The mass errors were less than 7 ppm or 1.7 mDa which were in the acceptable range, and profenofos showed only a very small mass error (0.12 ppm) compare to other pesticides. The accurate mass measurement has narrow down the possibility of the false identification of empirical formula of the pesticides, furthermore, the accurate mass measurement of characteristic fragment ions give a double confirmation of the identity of the pesticides compound. The limit of detection (LOD) for all the pesticides investigated in this study was 0.01 ppm. The retention times for all the pesticide were less than 10 minutes. Only a very small volume of sample (2µL) was required to provide sufficient chromatography and mass spectrometry peaks.

For the quantitative analysis of pesticides, a good linear coefficient regression, > 0.99 for all the pesticides was obtained for the concentration range of 0.02ppm to 0.4 ppm with. The calibration curves were obtained from the pesticides in pure methanol solvent by assuming no significant effects from the matrix effects. The assumption was made as the recoveries of the pesticides were in the acceptable range except for carbaryl and the retention time for each individual pesticides were compared in different types of vegetables sample. The similar retention time showed that no significant matrix effect because retention time should be shifted if there is significant matrix effect.

As a conclusion, ultrasonic solvent extraction method by using ethyl-acetate as extraction solvent followed by Florisil clean-up was able to extract organophophate pesticides from vegetables with a good recovery and the LC-Q-TOF-MS analytical technique provides simple, rapid and accurate technique for identify and quantify pesticides residue in vegetables.

In future, the sample extraction and the sample clean-up method can be manipulated to increase the percentage of recoveries of pesticides especially the carbamates pesticides. The conditions in the LC-Q-TOF analytical instruments can also be manipulated to further reduce the mass error to less than 7 ppm as obtained in this styudy for more accurate identification purpose. The second transitions /product ions should be obtained for carbaryl and profenofos by manipulated the collision energy in the Targeted MS/MS mode in order to fulfill the requirement as in the European Union (EU) Council Directive regarding analytical method and interpretation of results where three identification points are needed for correct LC-MS/MS confirmation. **[15]**

References

1. B. Kmellár, P. F.-U.-A. (2008). Validation and uncertainty study of a comprehensive list of 160 pesticide residues in multi-class vegetables by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A, 1215* , 37–50.

2. Didier Ortelli, P. E. (2004). Multiresidue analysis of 74 pesticides in fruits and vegetables by liquid chromatography–electrospray–tandem mass spectrometry. *Analytica Chimica Acta 520* , 33–45.

3. Yolanda Pic´o, M. l. (2007). Identification of unknown pesticides in fruits using ultra-performance liquid chromatography–quadrupole time-of-flight mass spectrometry Imazalil as a case study of quantification. *Journal of Chromatography A, 1176* , 123–134.

4. Zorka Knezˇevic´, M. S. (2009). Screening of fresh fruit and vegetables for pesticide residues on Croatian market. *Food Control 20* , 419–422.

5. Carla Soler, Y. P. (2007). Recent trends in liquid chromatography-tandem mass spectrometry to determine pesticides and their metabolites in food. *Trends in Analytical Chemistry, Vol. 26, No. 2, ,* 103-115.

6. Nasreddine, L. &.-M. (2002). Food contamination by metals and pesticides in the European Union. Should we worry? *Toxicology Letters, 127* , 29–41.

7. Anna Sannino, L. B. (2004). Application of liquid chromatography with electrospray tandem mass spectrometry to the determination of a new generation of pesticides in processed fruits and vegetables. *Journal of Chromatography A, 1036* , 161–169.

8. Sanchez-Brunete, B. A. (2005). Determination of pesticide residues by GC-MS using analyte protectants to counteract the matrix effect. *Analytical Sciences, Vol. 21* , 1291-1296.

9. Ondrej Lacina, J. U. (2010). Identification/quantification of multiple pesticide residues in food plants by ultra-high-performance liquid chromatography-time-of-flight mass spectrometry. *Journal of Chromatography A, 1217* , 648-659.

10. A. Garrido Frenich, J. M. (2004). Monitoring multi-class pesticide residues in fresh fruits and vegetables by liquid chromatography with tandem mass spectrometry. *Journal of Chromatography A, 1048* , 199–206.

11. Kit Granby, J. H. (2004). Analysis of pesticides in fruit, vegetables and cereals using methanolic extraction and detection by liquid chromatography–tandem mass spectrometry. *Analytica Chimica Acta 520* , 165–176.

12. Maurice Hiemstra, A. d. (2007). Comprehensive multi-residue method for the target analysis of pesticides in crops using liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A, 1154* , 3–25.

13. Oscar Nunez, E. M. (2005). LC-MS/MS analysis of organic toxics in food. *Trends in Analytical Chemistry, Vol.24, No.7 , 24,7*, 683-703.

14. Imma Ferrer, J. F.-R.-A. (2005). Multi-residue pesticide analysis in fruits and vegetables by liquid chromatography–time-of-flight mass spectrometry. *Journal of Chromatography A* .

15. COMMISSION DECISION of 12 August 2002,implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. (2002). *Official Journal of the European Communities* , L 221/8-L 221/36.

16. Jan F. Van Bocxlaer, S. R. (2005). Confirmation of the identity of residues using quadrupole time-of flight mass spectrometry. *Analytica Chimica Acta 529* , 65-73.

17. Damia Barcelo, M. P. (2007). Challenges and achievements of LC-MS in environmental analysis: 25 years on. *Trends in Analytical Chemistry, Vol. 26, No. 1,* , 2-11.

18. Fjeldsted, J. (2003). Time-of-flight mass spectrometry, technical overview,Agilent application note,5989-0373EN, Agilent, USA,.

19. Imma Ferrer, E. M. (2007). Multi-residue method for the analysis of 101 pesticides and their degradates in food and water samples by liquid chromatography/time-of-flight mass spectrometry. *Journal of Chromatography A, 1175* , 24–37.

20. Jian Pan (2008). Analysis of pesticide multi-residues in leafy vegetables by ultrasonic solvent extraction and liquid chromatography-tandem mass spectrometry. *Ultrasonics Sonochemistry 15* , 25–32.

21. Tuija Pihlström, G. B.-G. (2007). Analysis of pesticide residues in fruit and vegetables with ethyl acetate extraction using gas and liquid chromatography with tandem mass spectrometric detection. *Anal Bioanal Chem 389* , 1773–1789.

22. (n.d.). Retrieved 18 April, 2010, from http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3600c.pdf

23. Tomomi Goto, Y. I. (2006). The high throughput analysis of N-methyl carbamate pesticides in fruits and vegetables by liquid chromatography electrospray ionization tandem mass spectrometry using a short column. *Analytica Chimica Acta 555* , 225–232.

24. Ana Agüera, S. L.-A. (2004). One-year routine application of a new method based on liquid chromatography–tandem mass spectrometry to the analysis of 16 multiclass pesticides in vegetable samples. *Journal of Chromatography A, 1045* , 125–135.

25. Lam, D.C. (n.d.). Retrieved 18 April, 2010, from http://www.govtlab.gov.hk/g/texchange/pesticide2.pdf

26. Kuet, M. A. *Pesticide Residues in Local Vegetables in Sarawak is under Control Trend analysis from 2000 to 2006.*

27. Meloan, C. E. (1996). *Pesticides laboratory training manual* . Gaithersburg, Md. : AOAC International.

28. (n.d.). Retrieved 18 April, 2010, from http://www.epa.gov/waterscience/methods/method/files/632.pdf

29. Vasilis N. Kouloumbos, D.F. (August 2003). Identification of photocatalytic degradation products of diazinon in TiO2 aqueous suspensions using GC/MS/MS and LC/MS with quadrupole time-of-flight mass spectrometry. *Journal of the American Society for Mass Spectrometry, Volume 14, Issue 8*, 803-817.

30. *Food Act 1983 (Act 281) & Regulations (As at 5th December 2008).* (2008), International Law Book Servives.

31. (n.d.). Retrieved 18 April, 2010, from http://www.dnr.state.wi.us/org/es/science/lc/OUTREACH/Publications/LOD%20Guidance%20Document.pdf

32. (n.d.). Retrieved 18 April, 2010, from http://www.hc-sc.gc.ca/cps-spc/alt_formats/pacrb-dgapcr/pdf/pubs/pest/pol-guide/spn/spn2003-02-eng.pdf

Appendix A

(a) Acephate

m/z	Ion	Formula	Abundance
184.02019	$(M+H)^+$	$C_4 H_{11} N O_3 P S$	65911.4

Formula (M)	Ion Formula	Mass	Calc Mass	m/z	Calc m/z	Diff (ppm)	Diff (mDa)	DBE
$C_4 H_{10} N O_3 P S$	$C_4 H_{11} N O_3 P S$	183.01292	183.0119	184.02019	184.01918	-5.56	-1.02	1

Isotope	Abund Sum%	Calc Abund Sum%	m/z	Calc m/z	Diff (ppm)
1	91.14	90.13	184.02019	184.01918	-5.53
2	4.25	5.16	185.02736	185.02166	-30.78
3	4.6	4.71	186.01797	186.01621	-9.49

(b) Carbaryl

m/z	Ion	Formula	Abundance
202.08771	(M+H)+	C_{12} H_{12} N O_2	81744.3

Formula (M)	Ion Formula	Mass	Calc Mass	m/z	Calc m/z	Diff (ppm)	Diff (mDa)	DBE
C_{12} H_{11} N O_2	C_{12} H_{12} N O_2	201.08044	201.07898	202.08771	202.08626	-7.24	-1.46	8

Isotope	Abund Sum%	Calc Abund Sum%	m/z	Calc m/z	Diff (ppm)
1	87.23	87.09	202.08771	202.08626	-7.22
2	11.65	11.81	203.09011	203.08947	-3.11
3	1.12	1.1	204.09512	204.09197	-15.42

(c) Dimethoate

m/z	Ion	Formula	Abundance
230.00825	(M+H)+	$C_5 H_{13} N O_3 P S_2$	159820.7

Formula (M)	Ion Formula	Mass	Calc Mass	m/z	Calc m/z	Diff (ppm)	Diff (mDa)	DBE
$C_5 H_{12} N O_3 P S_2$	$C_5 H_{13} N O_3 P S_2$	229.00098	228.99962	230.00825	230.0069	-5.91	-1.35	1

Isotope	Abund Sum%	Calc Abund Sum%	m/z	Calc m/z	Diff
1	85.76	84.68	230.00825	230.0069	-5.89
2	6.2	6.45	231.01027	231.0092	-4.66
3	7.82	8.31	232.00457	232.00345	-4.81
4	0.21	0.56	233.00614	233.00596	-0.77

(d) Quinalphos

m/z	Ion	Formula	Abundance
299.0626	(M+H)+	C_{12} H_{16} N_2 O_3 P S	168718.8

Formula (M)	Ion Formula	Mass	Calc Mass	m/z	Calc m/z	Diff (ppm)	Diff (mDa)	DBE
C_{12} H_{15} N_2 O_3 P S	C_{12} H_{16} N_2 O_3 P S	298.05532	298.0541	299.0626	299.06138	-4.1	-1.22	7

Isotope	Abund Sum%	Calc Abund Sum%	m/z	Calc m/z	Diff (ppm)
1	83.1	82.19	299.0626	299.06138	-4.09
2	12.02	12.16	300.06518	300.06425	-3.09
3	4.37	5.02	301.0612	301.05968	-5.05
4	0.52	0.63	302.06343	302.06178	-5.46

(e) Diazinon

m/z	Ion	Formula	Abundance
305.11004	(M+H)+	C_{12} H_{22} N_2 O_3 P S	253591.8

Formula (M)	Ion Formula	Mass	Calc Mass	m/z	Calc m/z	Diff (ppm)	Diff (mDa)	DBE
C_{12} H_{21} N_2 O_3 P S	C_{12} H_{22} N_2 O_3 P S	304.10276	304.10105	305.11004	305.10833	-5.62	-1.71	4

Isotope	Abund Sum%	Calc Abund Sum%	m/z	Calc m/z	Diff
1	82.66	82.13	305.11004	305.10833	-5.61
2	12.19	12.21	306.11234	306.11122	-3.66
3	4.64	5.03	307.10839	307.10665	-5.67
4	0.51	0.63	308.11175	308.10875	-9.73

(f) Profenofos

m/z	Ion	Formula	Abundance
372.94239	(M+H)+	C_{11} H_{16} Br Cl O_3 P	33784.2

Formula (M)	Ion Formula	Mass	Calc Mass	m/z	Calc m/z	Diff (ppm)	Diff (mDa)	DBE
C_{11} H_{15} Br Cl O_3 P	C_{11} H_{16} Br Cl O_3 P S	371.93519	371.93514	372.94239	372.94242	-0.12	-0.04	4

Isotope	Abund Sum%	Calc Abund Sum%	m/z	Calc m/z	Diff (ppm)
1	31.75	32.12	372.94239	372.94242	0.08
2	4.09	4.17	373.94544	373.94558	0.37
3	42.91	43.4	374.94025	374.94016	-0.22
4	5.43	5.6	375.94292	375.9433	1.03
5	11.88	12.46	376.93728	376.93759	0.84
6	2.04	1.57	377.94289	377.94063	-5.98
7	1.75	0.62	378.95051	378.9358	-38.82
8	0.16	0.07	379.94068	379.93827	-6.35

Appendix B

(a) Acephate

Peak	m/z	Abund%	Formula	Calc m/z	Diff (ppm)	Diff (mDa)
1	110.96657	12.81	$C H_4 O_2 P S$	110.96641	-1.4	-0.16
2	124.98302	36.32	$C_2 H_6 O_2 P S$	124.98206	-7.64	-0.95
3	142.99333	100	$C_2 H_8 O_3 P S$	142.99263	-4.94	-0.71

(b) Carbaryl

Peak	m/z	Abund%	Formula	Calc m/z	Diff (ppm)	Diff (mDa)
1	117.06993	7.07	$C_9 H_9$	117.06988	-0.42	-0.05
2	127.05424	10.44	$C_{10} H_7$	127.05423	-0.07	-0.01
3	145.06488	100	$C_{10} H_9 O$	145.06479	-0.62	-0.09

(c) Dimethoate

Peak	m/z	Abund%	Formula	Calc m/z	Diff (ppm)	Diff (mDa)
1	124.98226	100	$C_2 H_6 O_2 P S$	124.98206	-1.59	-0.2
2	142.99305	6.67	$C_2 H_8 O_3 P S$	142.99263	-2.93	-0.42
3	156.95341	9.15	$C_2 H_6 O_2 P S_2$	156.95413	4.59	0.72
4	170.9699	78.58	$C_3 H_8 O_2 P S_2$	170.96978	-0.67	-0.11
5	198.96506	45.44	$C_4 H_8 O_3 P S_2$	198.9647	-1.81	-0.36

(d) Quinalphos

Peak	m/z	Abund%	Formula	Calc m/z	Diff (ppm)	Diff (mDa)
1	124.98209	41.01	$C_2 H_6 O_2 P S$	124.98206	-0.25	-0.03
2	129.04475	8.99	$C_8 H_5 N_2$	129.04472	-0.19	-0.03
3	147.05526	95.03	$C_8 H_7 N_2 O$	147.05529	0.21	0.03
4	153.01302	5.4	$C_4 H_{10} O_2 P S$	153.01336	2.26	0.35
5	163.03271	100	$C_8 H_7 N_2 S$	163.03245	-1.61	-0.26
6	224.98836	17.29	$C_8 H_6 N_2 O_2 P S$	224.98821	-0.66	-0.15
7	242.99912	98.84	$C_8 H_8 N_2 O_3 P S$	242.99878	-1.41	-0.34
8	271.02959	19.89	$C_{10} H_{12} N_2 O_3 P S$	271.03008	1.77	0.48

(e) Diazinon

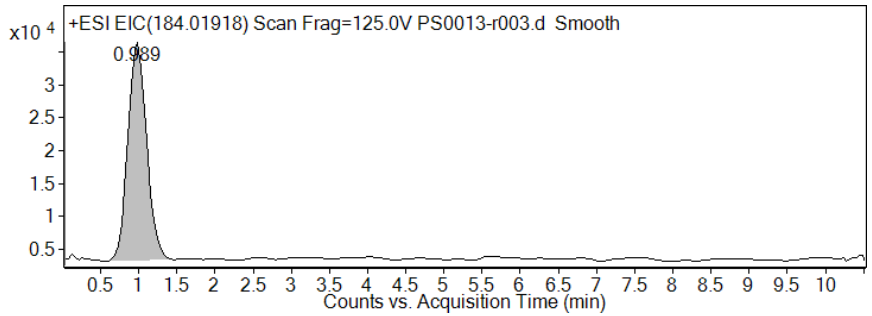
Peak	m/z	Abund%	Formula	Calc m/z	Diff (ppm)	Diff (mDa)
1	124.98241	22.5	$C H_5 N_2 O S_2$	124.98378	10.96	1.37
2	153.10246	77.84	$C_8 H_{13} N_2 O$	153.10224	-1.42	-0.22
3	169.0798	100	$C_8 H_{13} N_2 S$	169.0794	-2.38	-0.4
4	231.03506	10	$C_7 H_1 1 N_4 O S_2$	231.03688	7.88	1.82
5	249.04611	57.81	$C_7 H_{13} N_4 O_2 S_2$	249.04744	5.34	1.33
6	277.07652	24.28	$C_9 H_{17} N4 O_2 S_2$	277.07874	8.02	2.22

(f) Profenofos

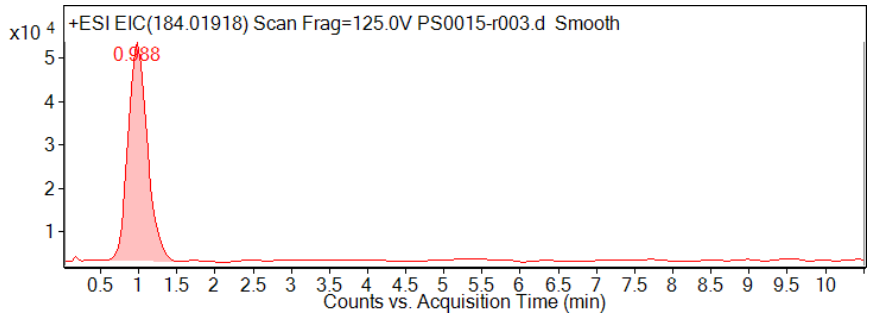
Peak	m/z	Abund%	Formula	Calc m/z	Diff (ppm)	Diff (mDa)
1	284.85274	6.21	$C_6 H_4 Br Cl O_2 P S$	284.8536	3.02	0.86
2	302.86358	100	$C_6 H_6 Br Cl O_3 P S$	302.86417	1.94	0.59
3	344.90977	18.83	$C9 H_{12} Br Cl O_3 P S$	344.91112	3.9	1.34

APPENDIX C

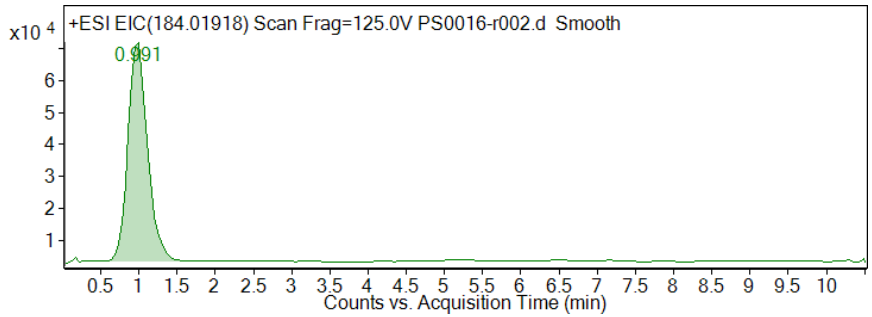
8.(a) 0.02 ppm



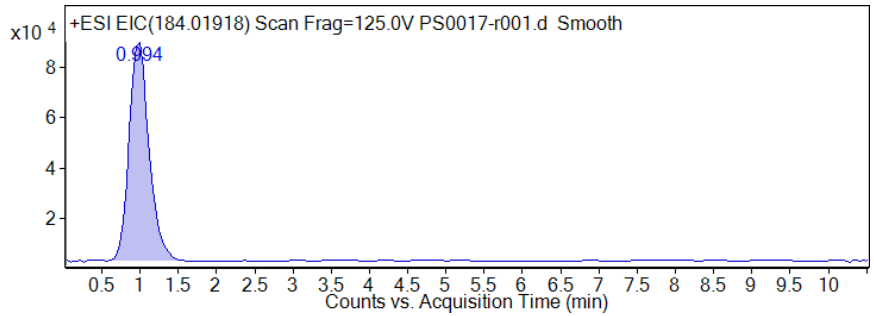
8(b) 0.04 ppm



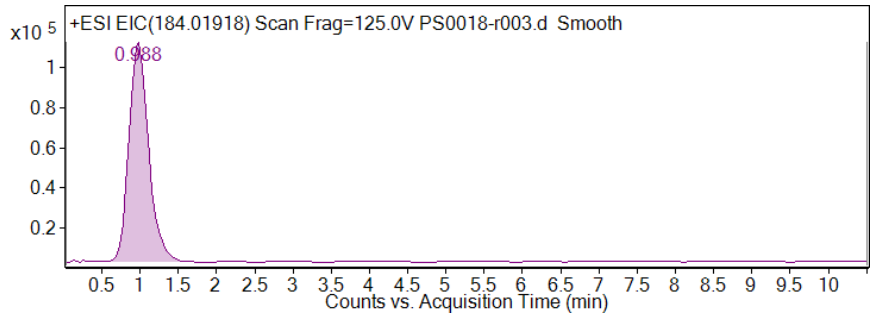
8(c) 0.05 ppm



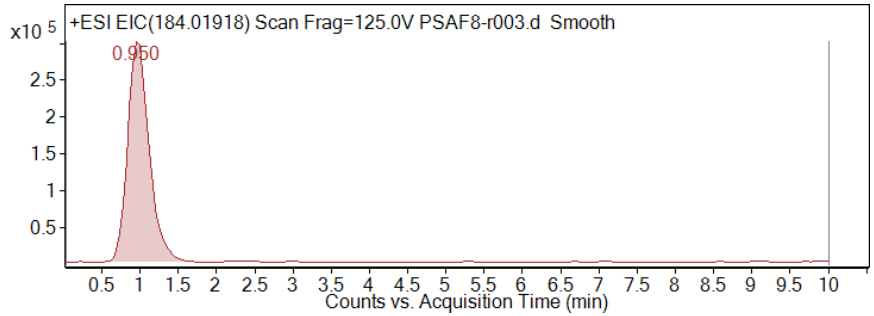
8(d) 0.08 ppm



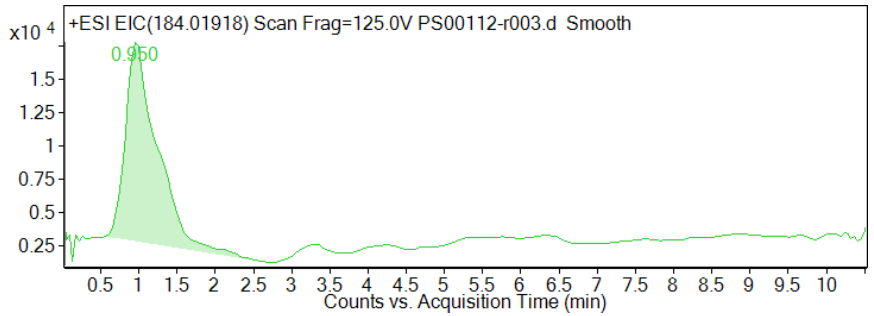
8(e) 0.10 ppm



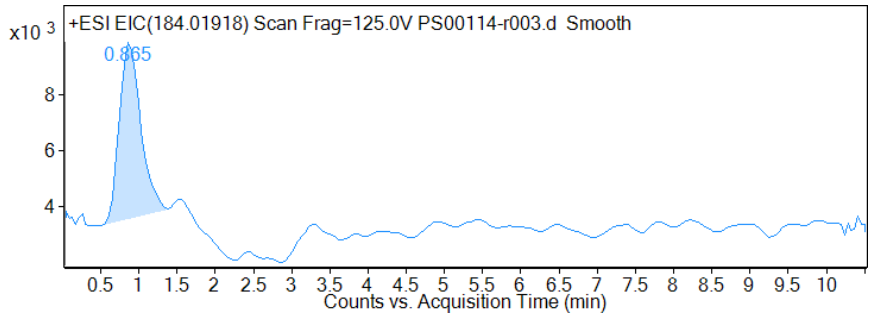
8(f) 0.40 ppm



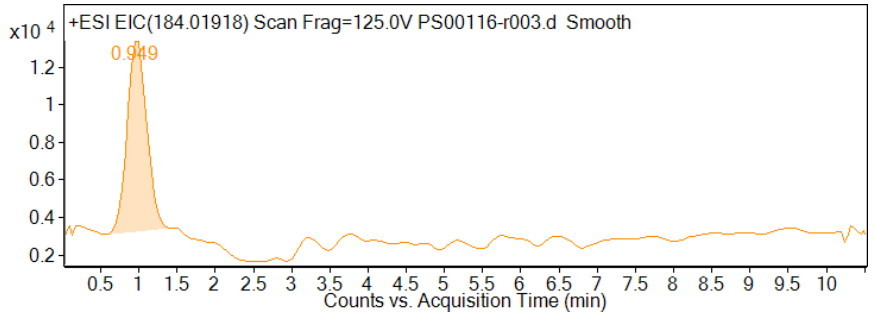
8(g) Kai Lan



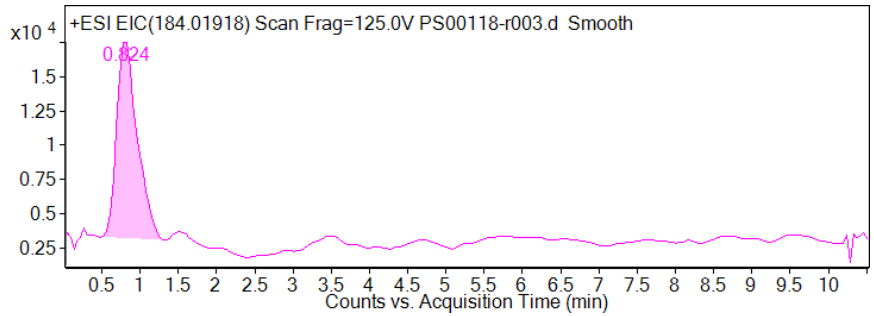
8(h) Yau Mak



8(i) Sawi hijau



8(j) Tai Pak Choy



8(k)

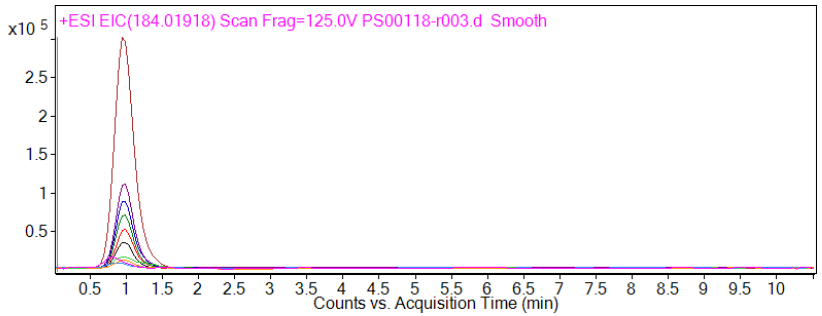
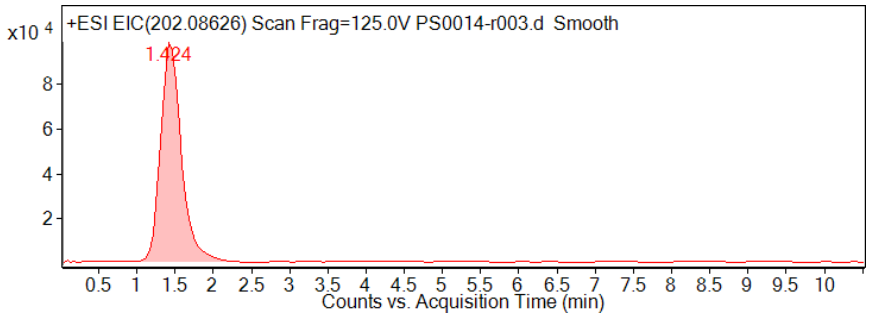
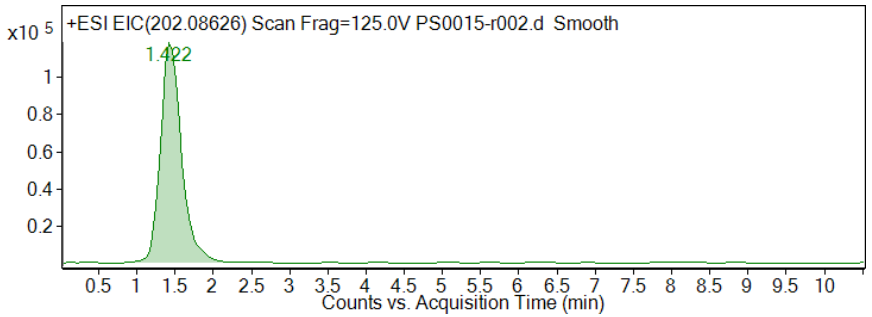


Figure8: (a) – (f) are the extracted ion chromatograms (EIC) of Acephate in different concentration ranges from 0.02-0.4 ppm , (g)-(j) are EIC from vegetables samples and (k) is the chromatogram of (a) to (j) in the overlaid mode.

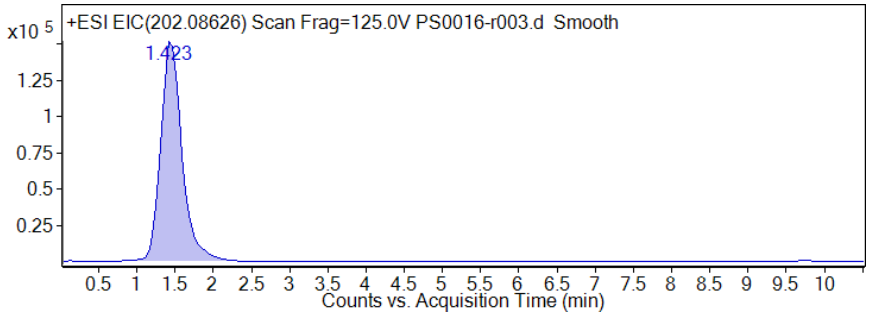
9.(a) 0.03 ppm



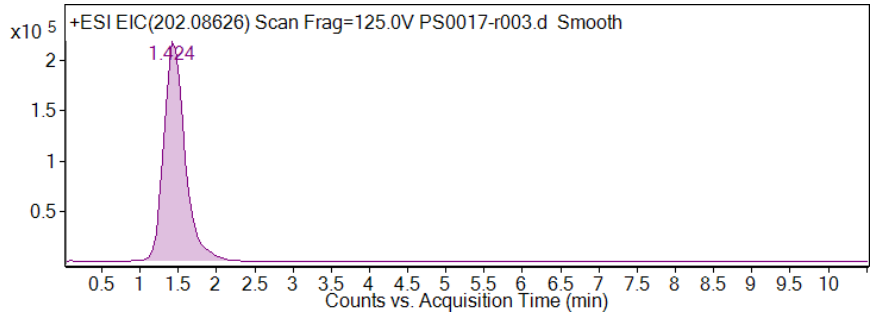
9(b) 0.04 ppm



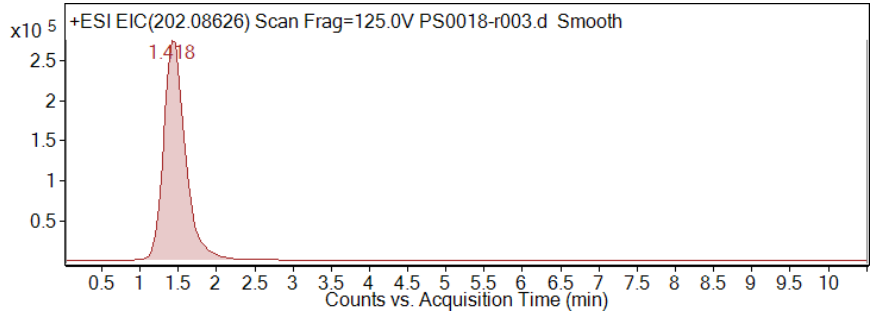
9(c) 0.05 ppm



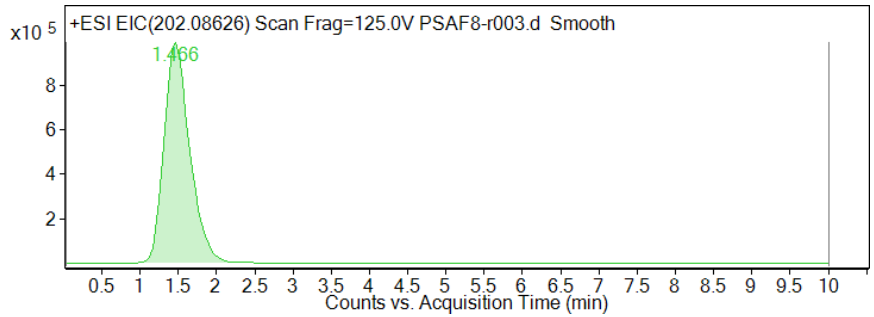
9(d) 0.08 ppm



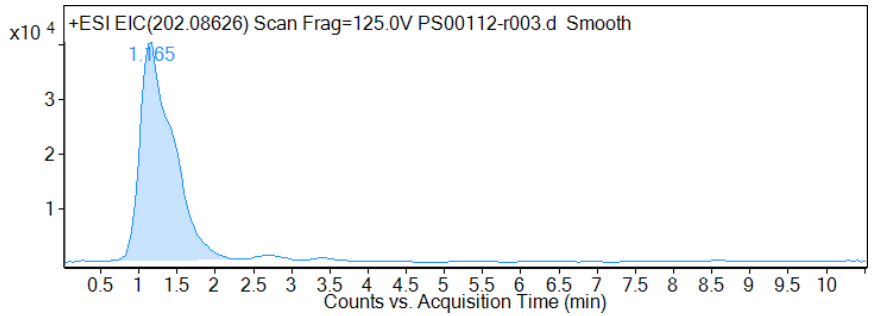
9(e) 0.10 ppm



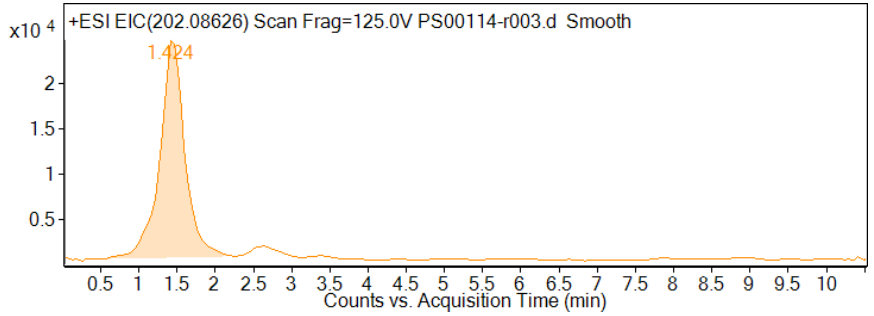
9(f) 0.40 ppm



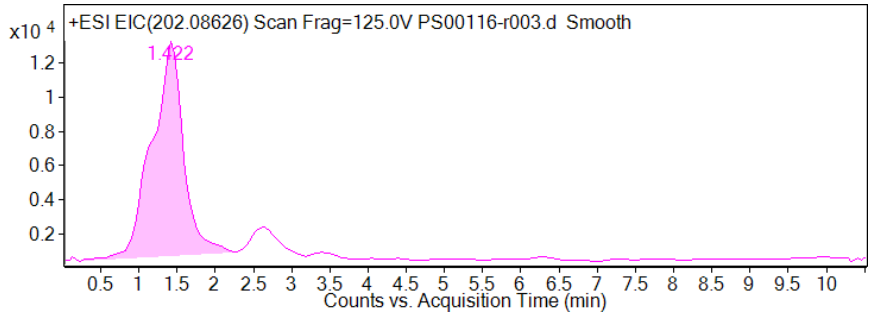
9(g) Kai Lan



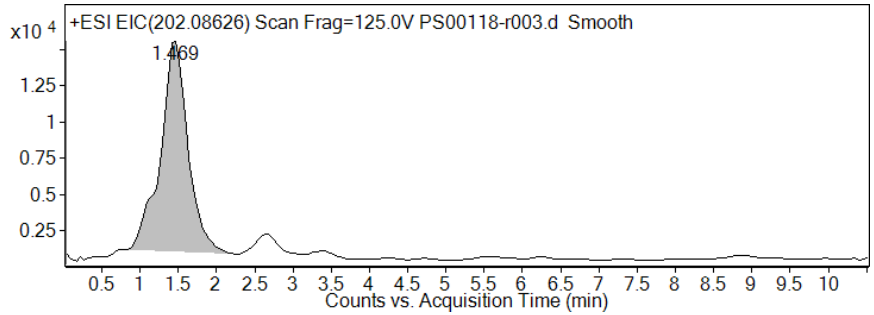
9(h) Yau Mak



9(i) Sawi hijau



9(j) Tai Pak Choy



9(k)

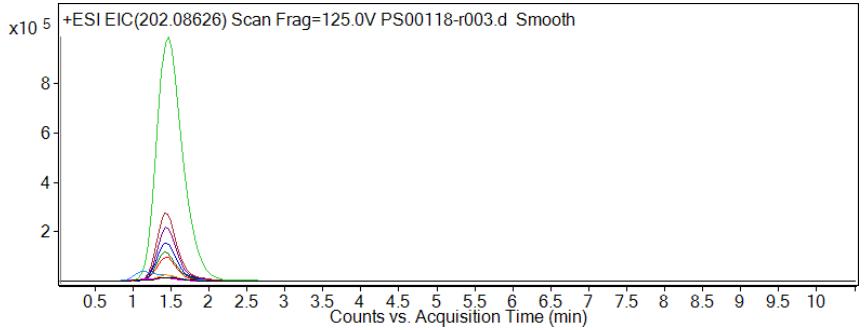
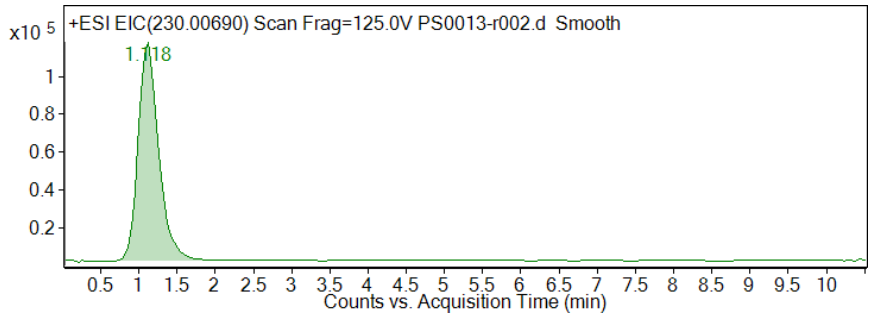
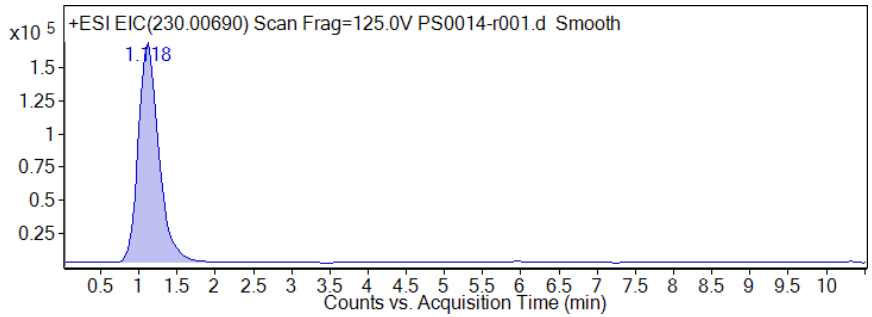


Figure9: (a) – (f) are the extracted ion chromatogram (EIC) of Carbaryl in different concentration ranges from 0.03-0.4 ppm, (g)-(j) are EIC of vegetables samples and (k) is the chromatogram of (a) to (j) in the overlaid mode

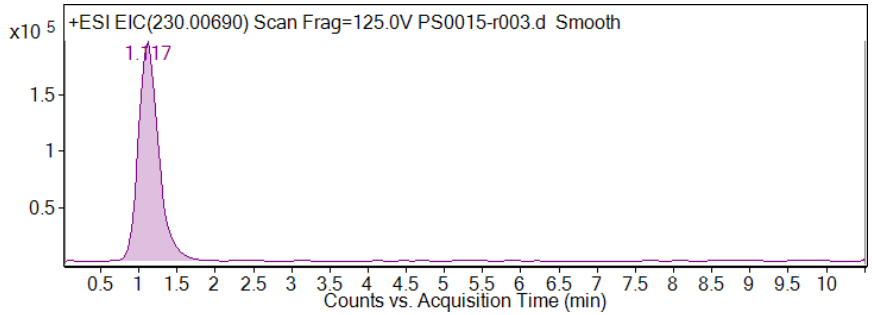
10(a) 0.02 ppm



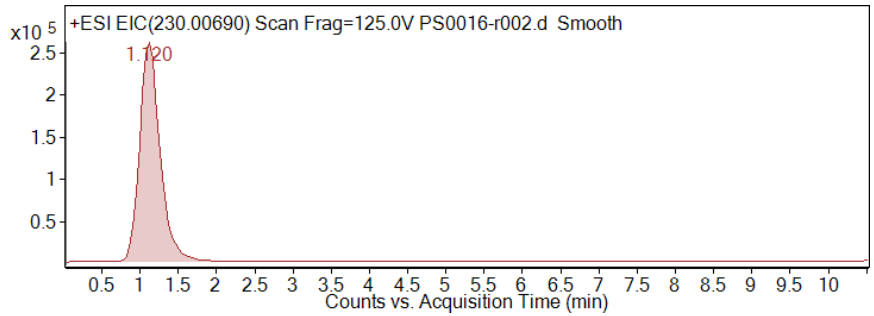
10(b) 0.03 ppm



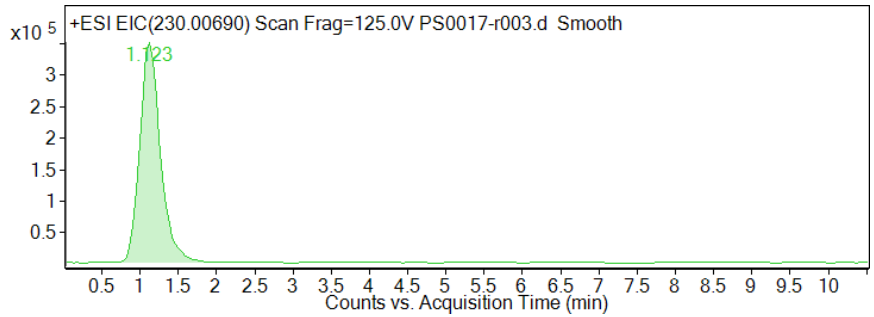
10(c) 0.04 ppm



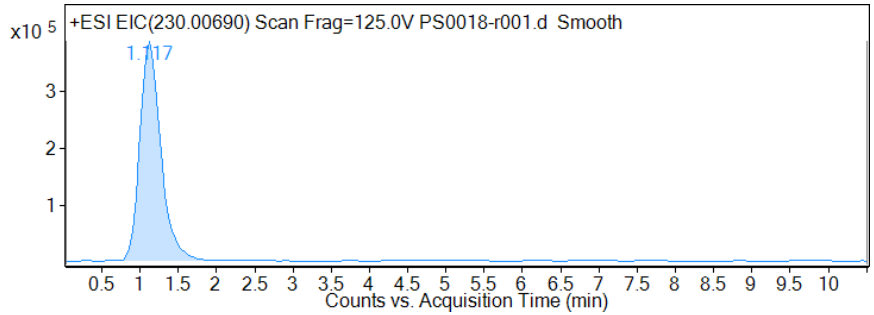
10(d) 0.05 ppm



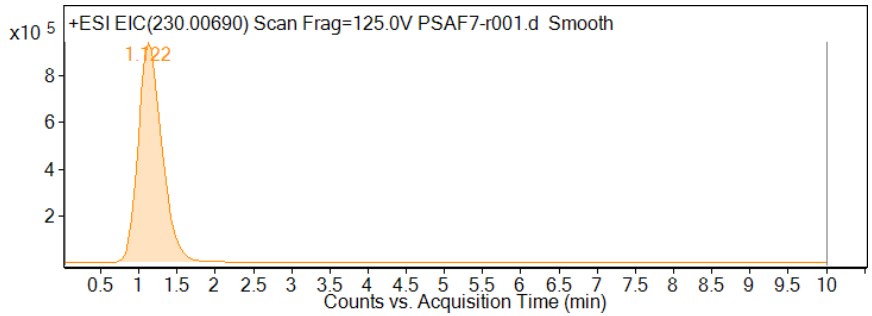
10(e) 0.08 ppm



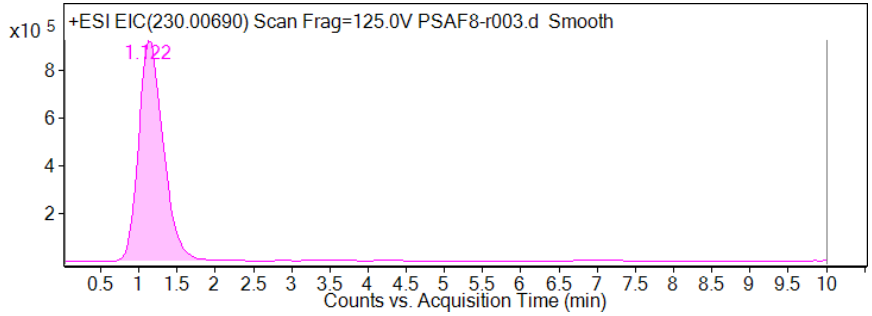
10(f) 0.10 ppm



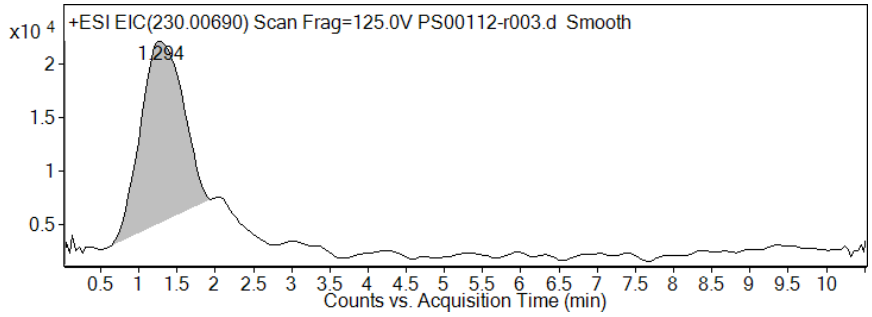
10(g) 0.30 ppm



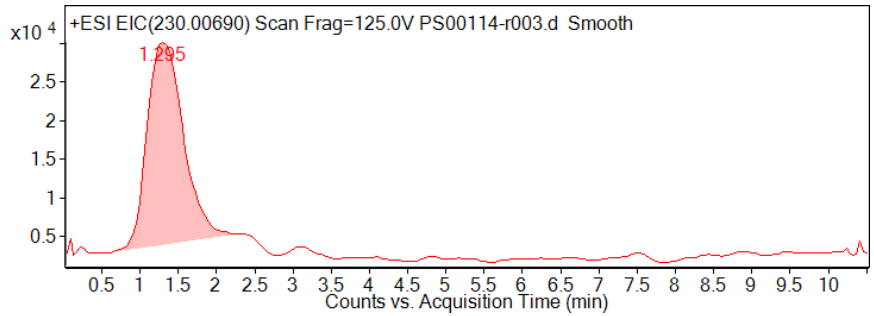
10(h) 0.40 ppm



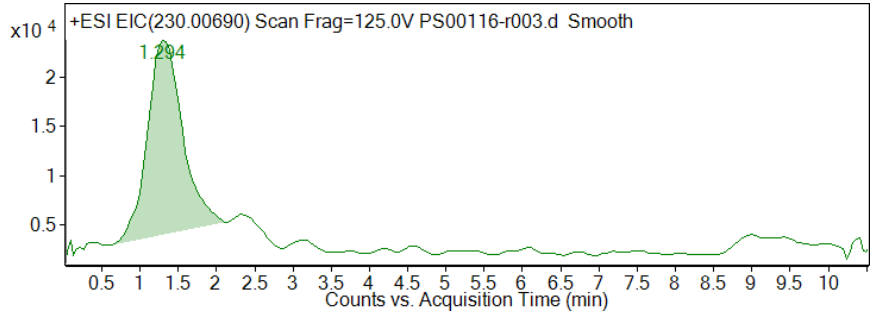
10(i) Kai Lan



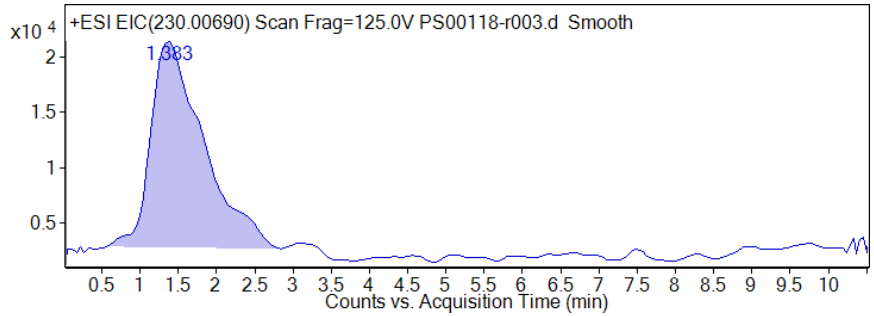
10(j) Yau Mak



10(k) Sawi hijau



10(l) Tai Pak Choy



10(m)

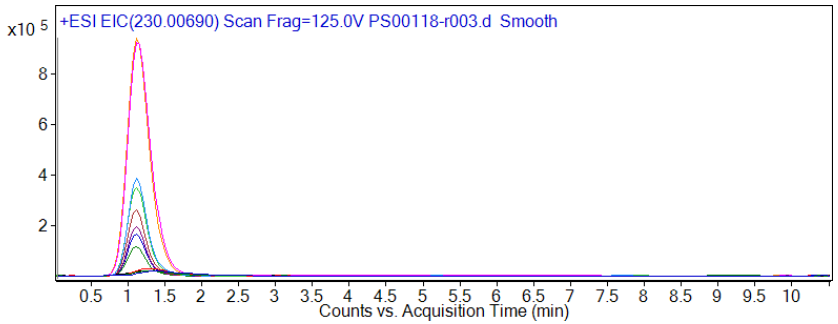
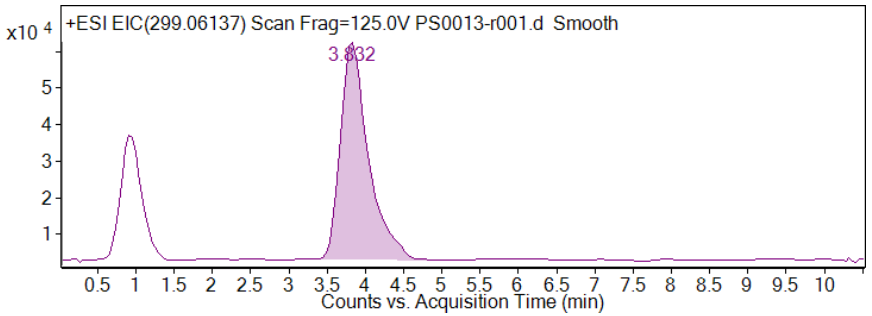
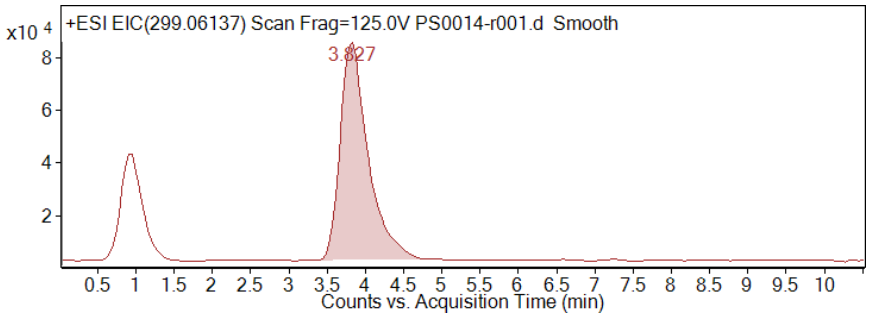


Figure10: (a) – (h) are the extracted ion chromatogram (EIC) of Dimethoate in different concentration ranges from 0.02-0.4 ppm, (i)-(l) are EIC of vegetables samples and (m) is the chromatogram of (a) to (l) in the overlaid mode.

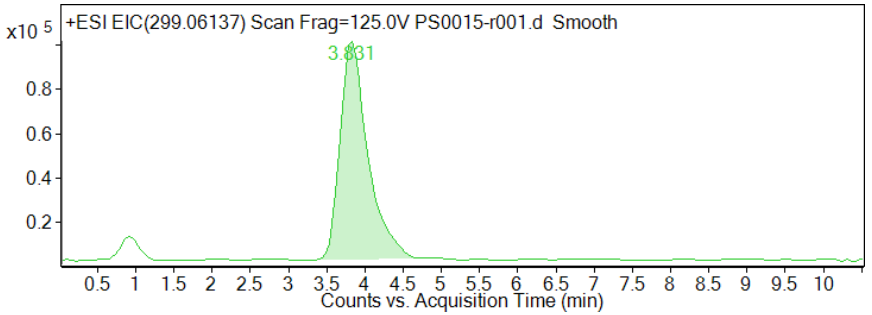
11(a) 0.02 ppm



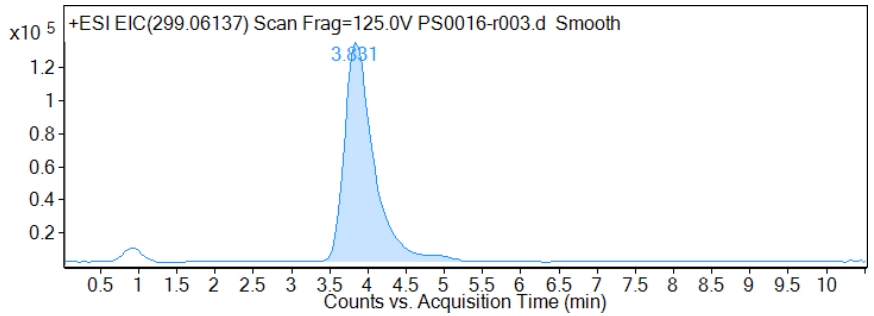
11(b) 0.03 ppm



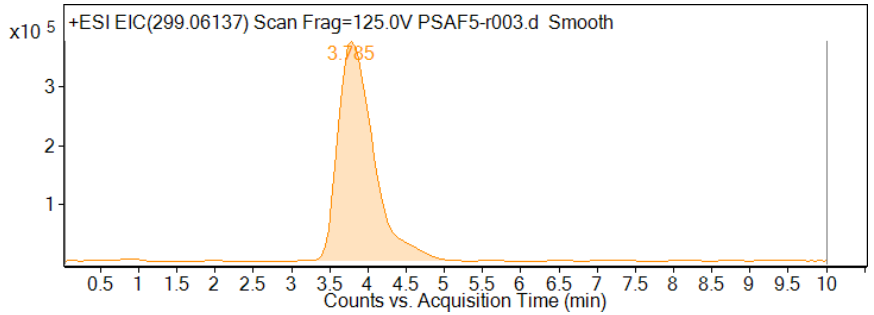
11(c) 0.04 ppm



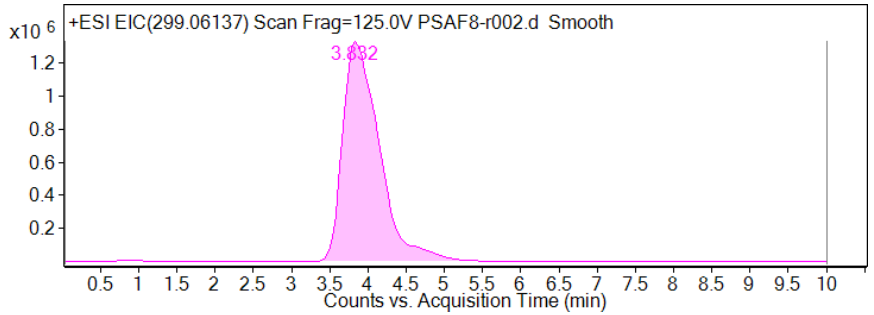
11(d) 0.05 ppm



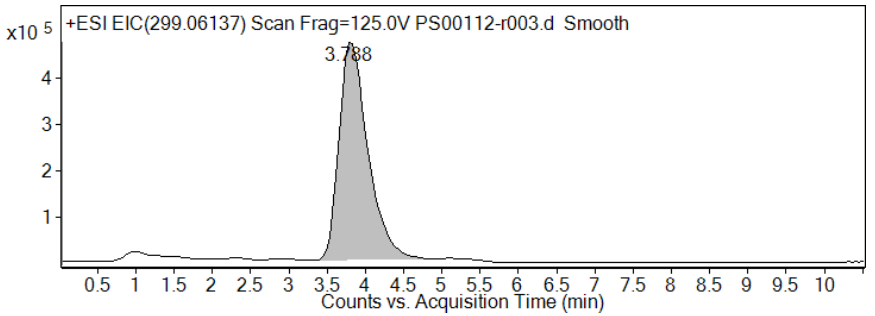
11(e) 0.10 ppm



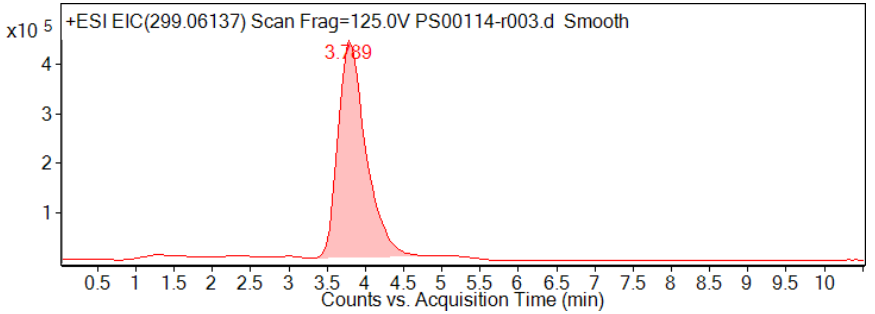
11(f) 0.40 ppm



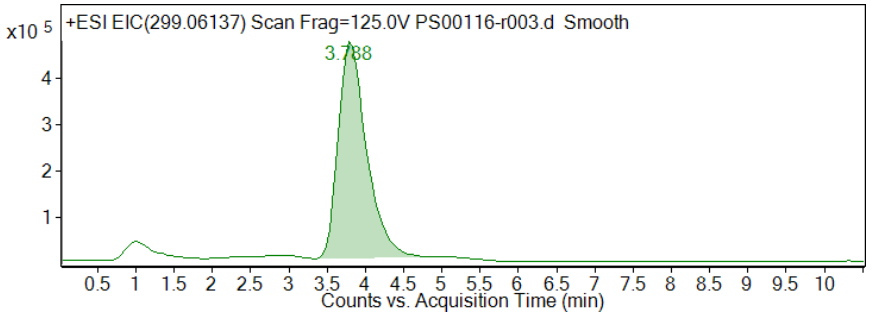
11(g) Kai Lan



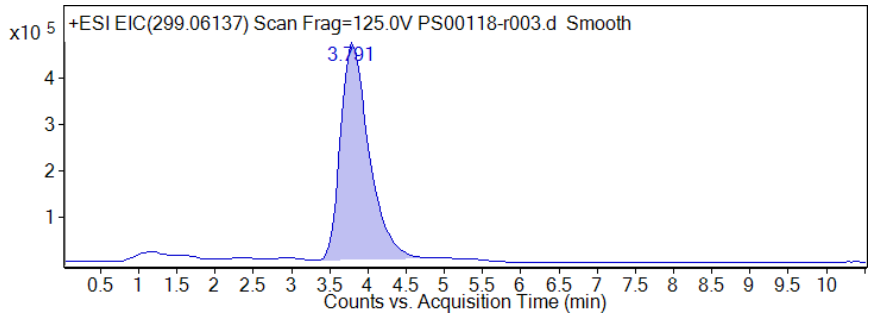
11(h) Yau Mak



11(i) Sawi hijau



11(j) Tai Pak Choy



11(k)

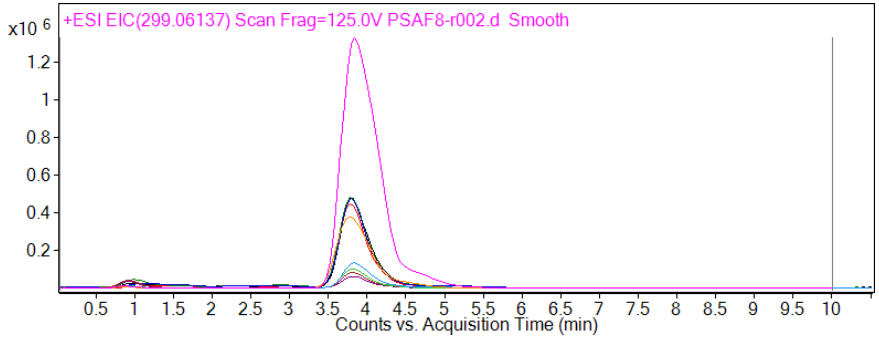
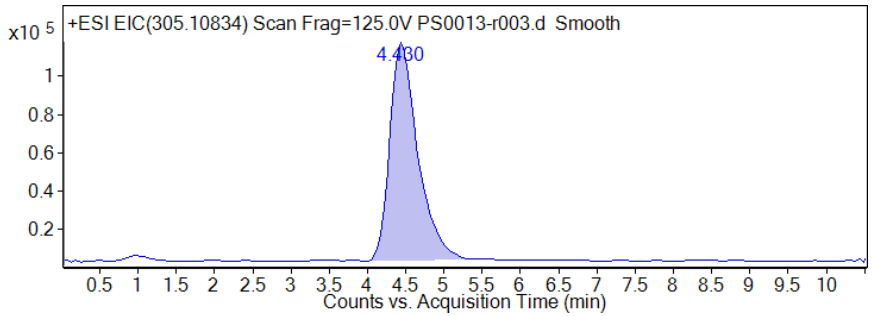
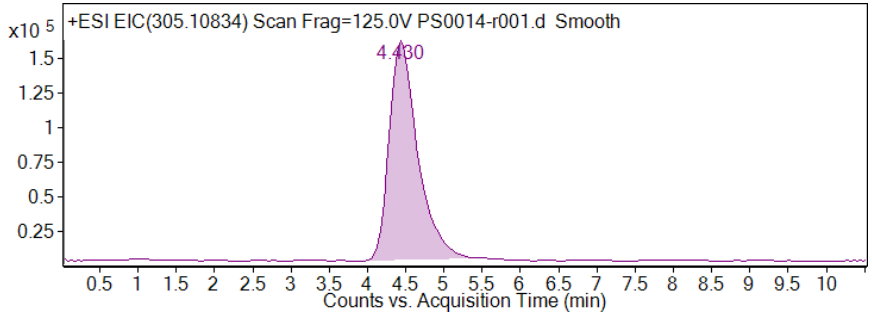


Figure11: (a) – (f) are the extracted ion chromatogram (EIC) of Quinalphos in different concentration ranges from 0.02-0.4 ppm ,(g)-(j) are EIC of vegetables samples and (k) is the chromatogram of (a) to (j) in the overlaid mode.

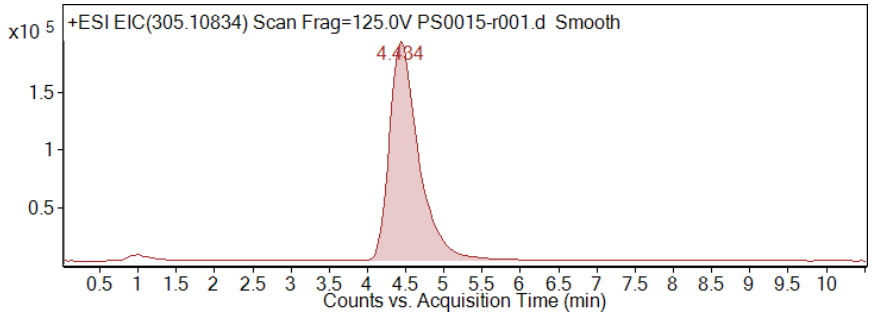
12(a) 0.02 ppm



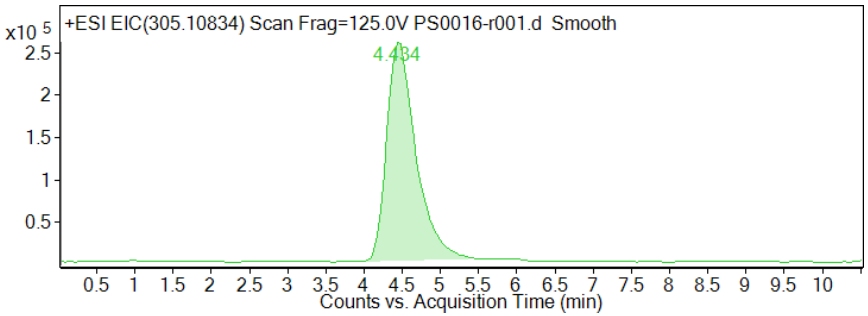
12(b) 0.03 ppm



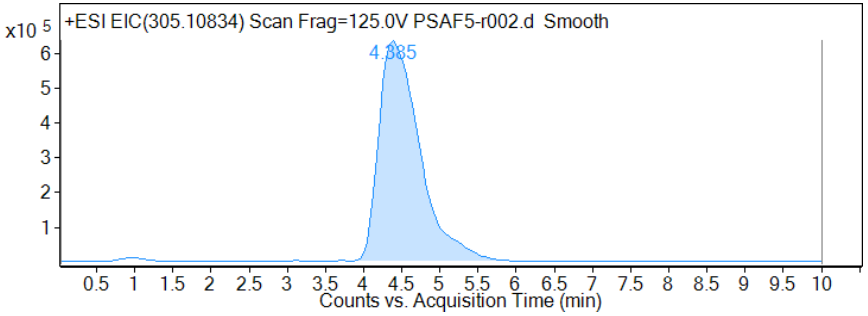
12(c) 0.04 ppm



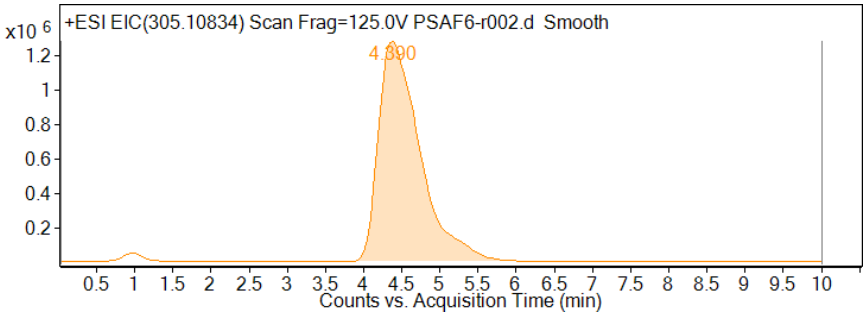
12(d) 0.05 ppm



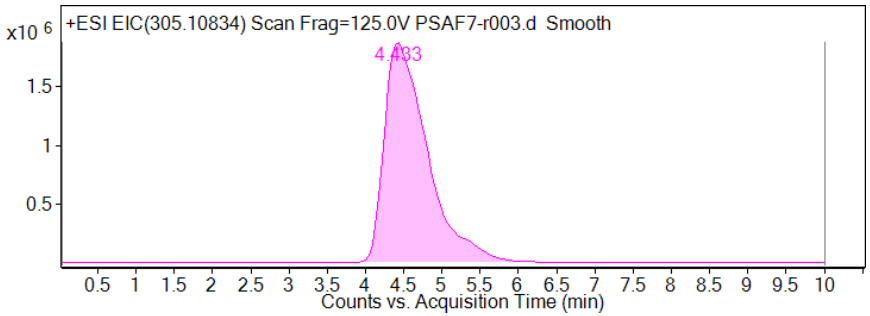
12(e) 0.10 ppm



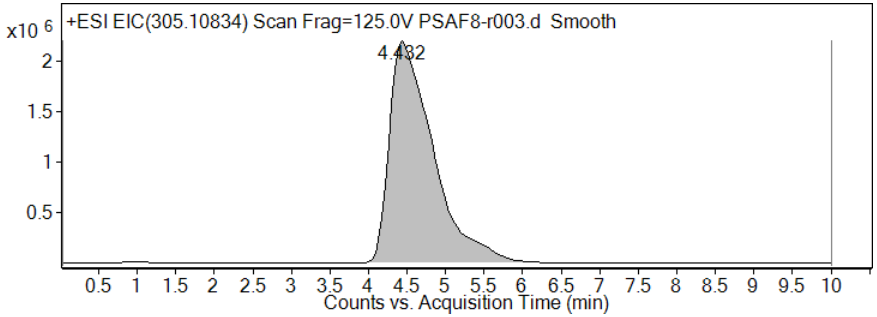
12(f) 0.20ppm



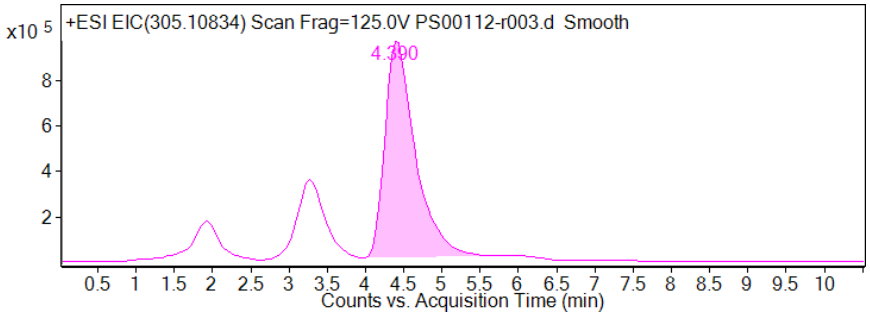
12(g) 0.30 ppm



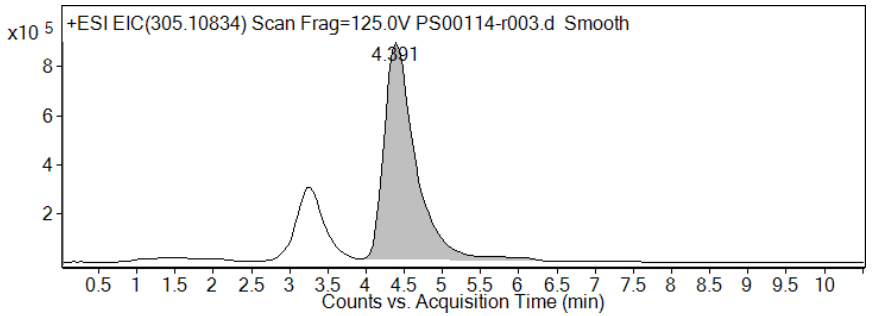
12(h) 0.40 ppm



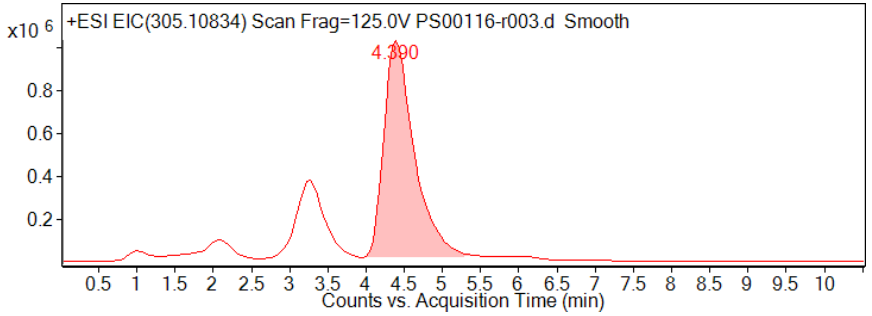
12(i) Kai Lan



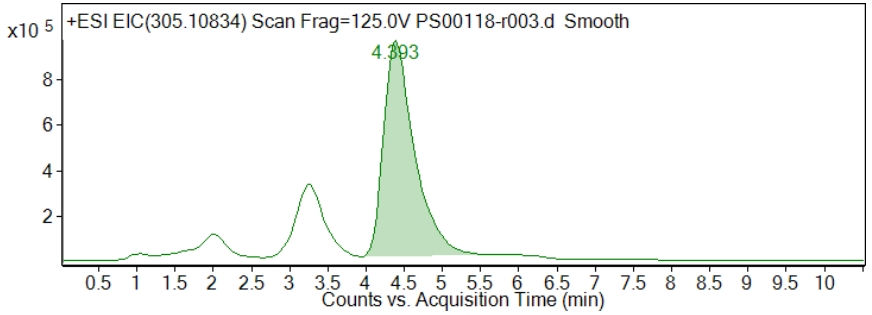
12(j) Yau Mak



12(k) Sawi hijau



12(l) Tai Pak Choy



12(m)

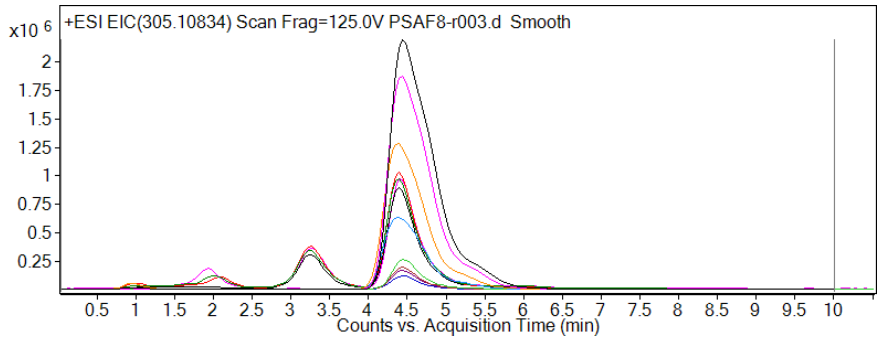
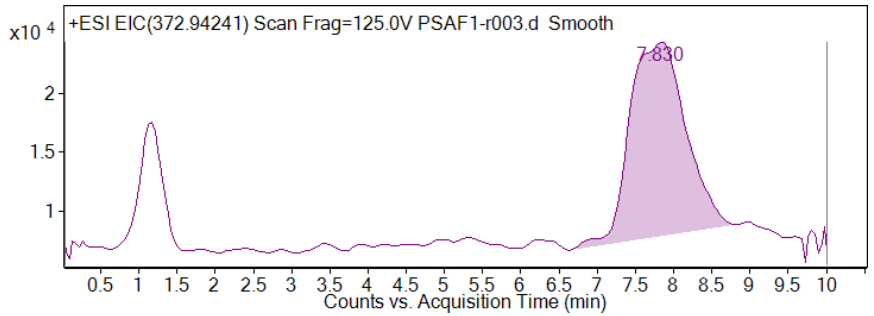
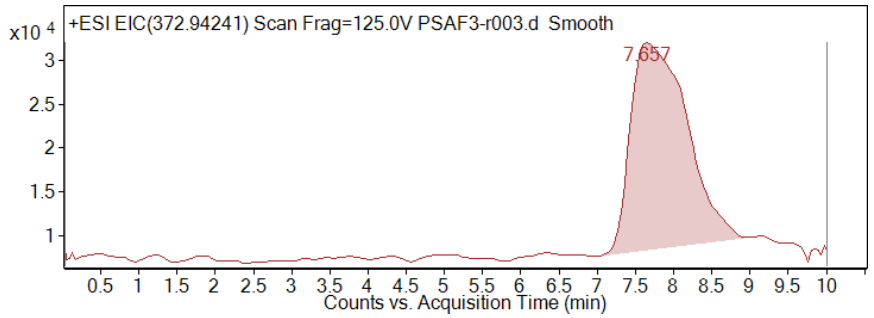


Figure 12: (a) – (h) are the extracted ion chromatogram (EIC) of Diazinon in different concentration ranges from 0.02-0.4 ppm, (i)-(l) are EIC vegetables samples and (m) is the chromatogram of (a) to (l) in the overlaid mode.

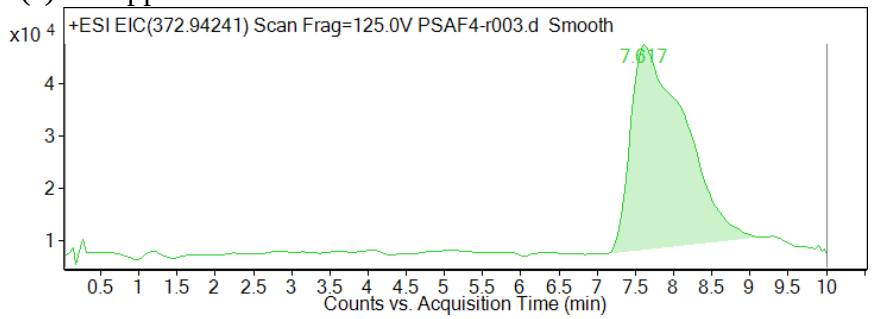
13(a) 0.02 ppm



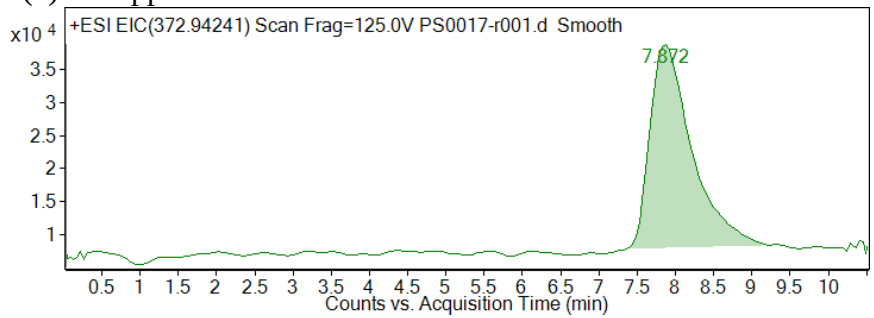
13(b) 0.03 ppm



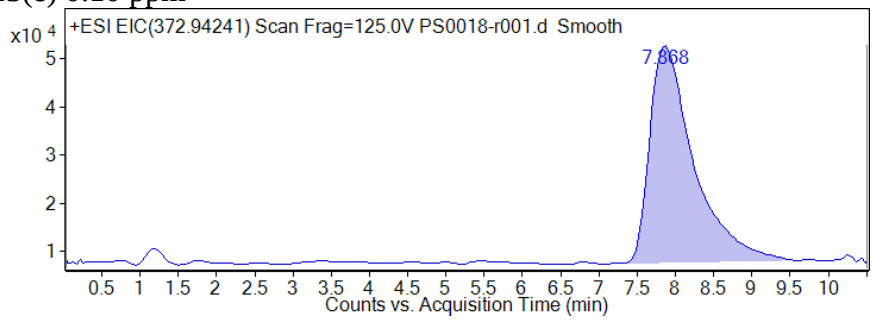
13(c) 0.05 ppm



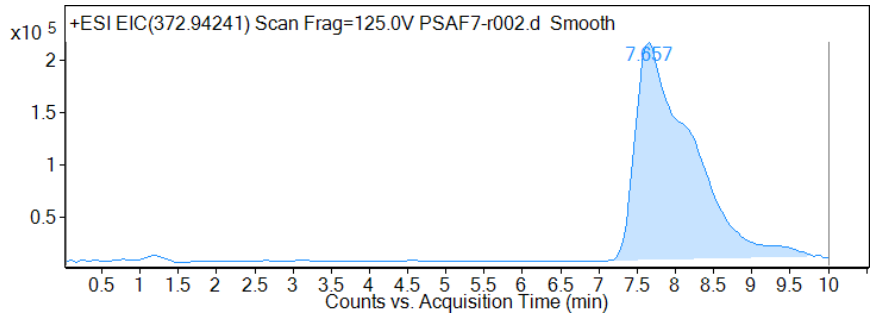
13(d) 0.08 ppm



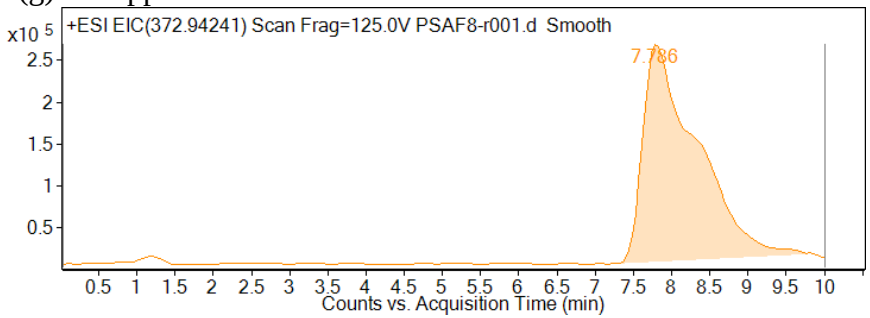
13(e) 0.10 ppm



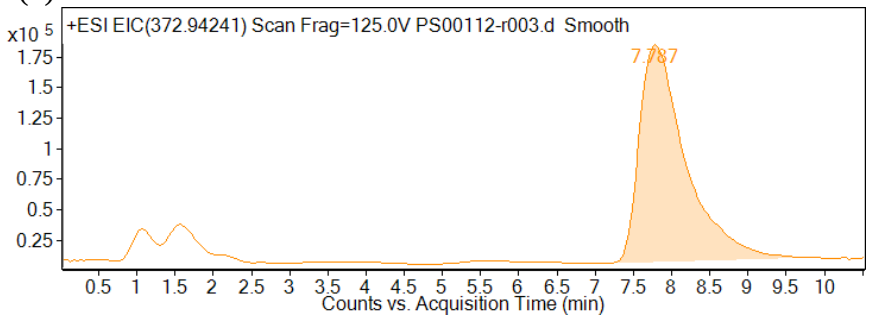
13(f) 0.30 ppm



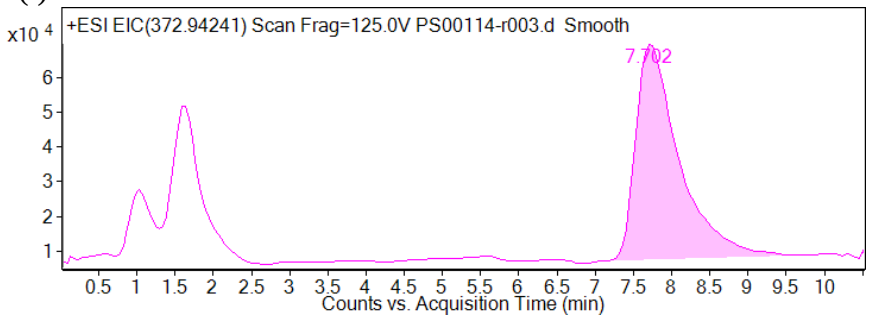
13(g) 0.40 ppm



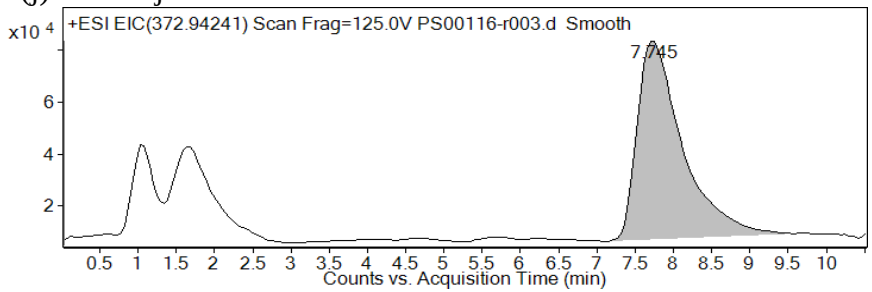
13(h) Kai Lan



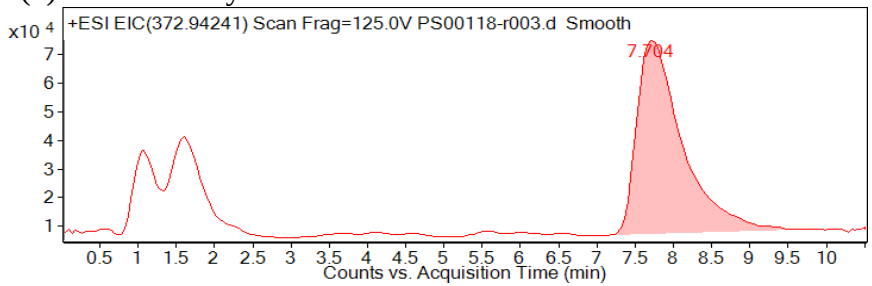
13(i) Yau Mak



13(j) Sawi hijau



13(k) Tai Pak Choy



13(l)

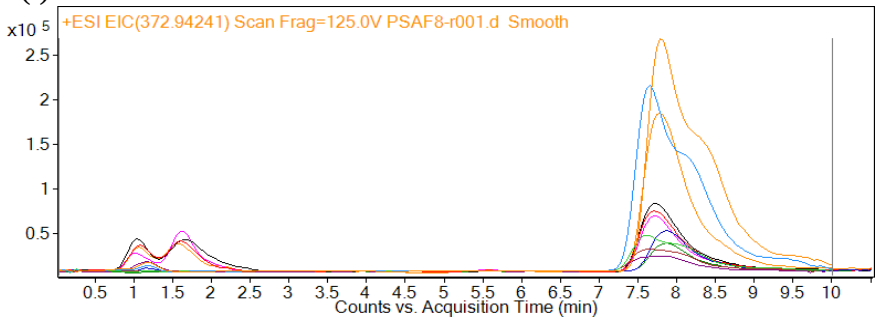


Figure 13: (a) – (g) are the extracted ion chromatogram (EIC) of Profenofos in different concentration ranges from 0.02-0.4 ppm, (h)-(k) are EIC ofvegetables samples and (l) is the chromatogram of (a) to (k) in the overlaid mode.

Appendix D

(a)

Acephate Method	Acephate Results	
Concentration (mg/L)	Retention Time	Response (Peak Area)
0.020	0.969	17023
0.040	0.972	28344
0.050	0.970	36986
0.080	0.955	47358
0.100	0.964	60288
0.400	0.947	196871

(b)

Carbaryl Method	Carbaryl Results	
Concentration (mg/L)	Retention Time	Response (Peak Area)
0.020	1.411	57033
0.030	1.419	76611
0.040	1.42	100453
0.050	1.413	129694
0.080	1.412	189437
0.100	1.411	237919

(c)

Dimethoate Method	Dimethoate Results	
Concentration (mg/L)	Retention Time	Response (Peak Area)
0.020	1.093	37241
0.030	1.097	48149
0.040	1.098	58309
0.050	1.097	59159
0.080	1.097	103744
0.100	1.098	110325
0.200	1.093	308047
0.300	1.097	378671

(d)

Quinalphos Method	Quinalphos Results	
Concentration (mg/L)	Retention Time	Response (Peak Area)
0.020	3.821	15708
0.030	3.82	22903
0.040	3.737	28599
0.050	3.917	38631
0.100	3.727	114809
0.400	3.834	434498

(e)

Diazinon Method	Diazinon Results	
Concentration (mg/L)	Retention Time	Response (Peak Area)
0.020	4.384	49512
0.030	4.402	71801
0.040	4.437	81062
0.050	4.437	113329
0.100	4.312	321815
0.200	4.328	699548
0.300	4.398	1050338
0.400	4.392	1303390

(f)

Profenofos Method	Profenofos Results	
Concentration (mg/L)	Retention Time	Response (Peak Area)
0.010	7.565	18698
0.030	7.528	33208
0.050	7.57	50374
0.080	7.942	66738
0.100	7.864	83164
0.300	7.603	218710
0.400	7.748	297627

Table (a) – (f) show the response from the chromatograms of pesticides used to plot calibration curves as in Appendix E.

Appendix E

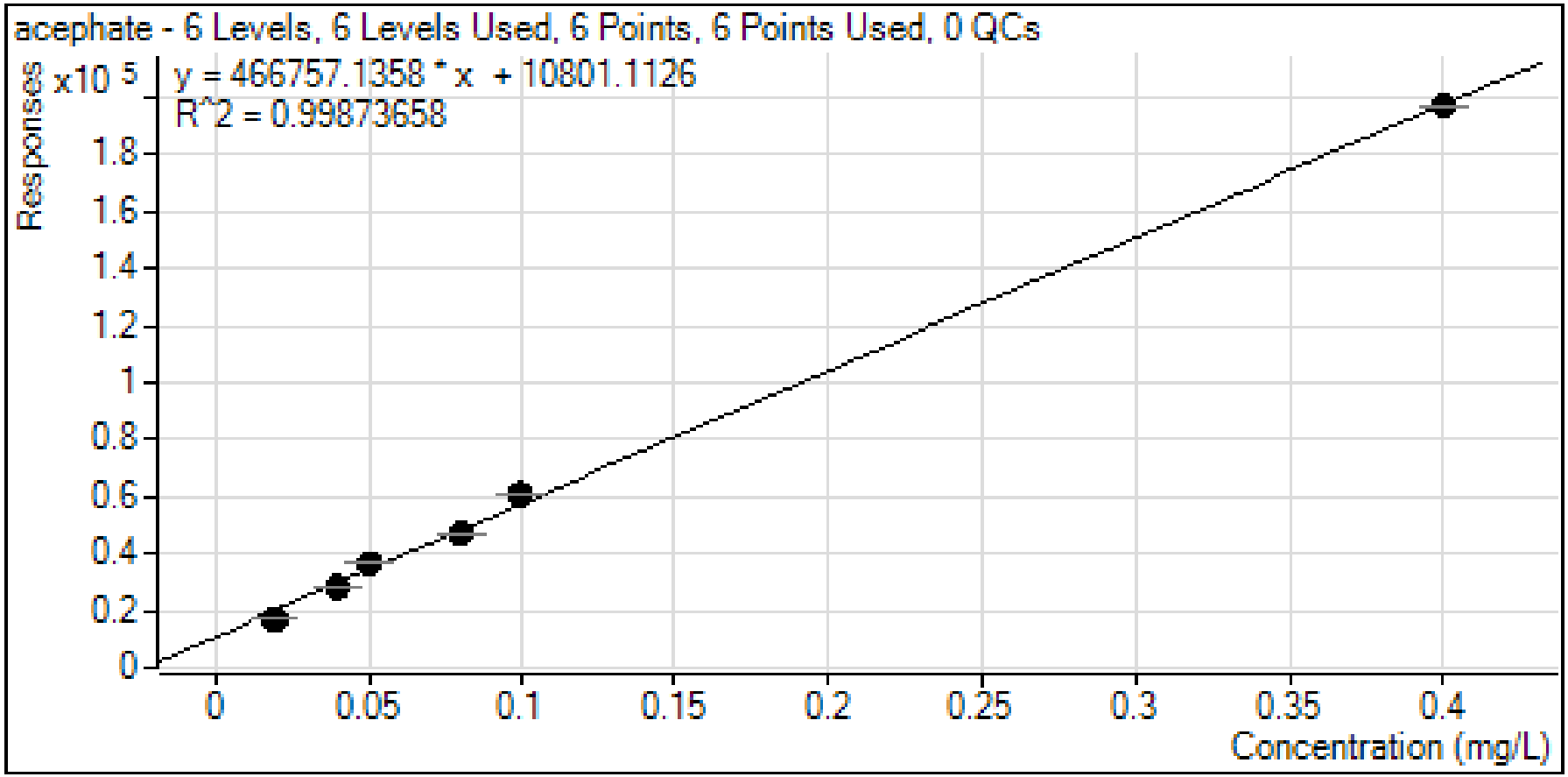


Figure 14(a): Calibration Curve for Acephate (0.02 -0.4 ppm)

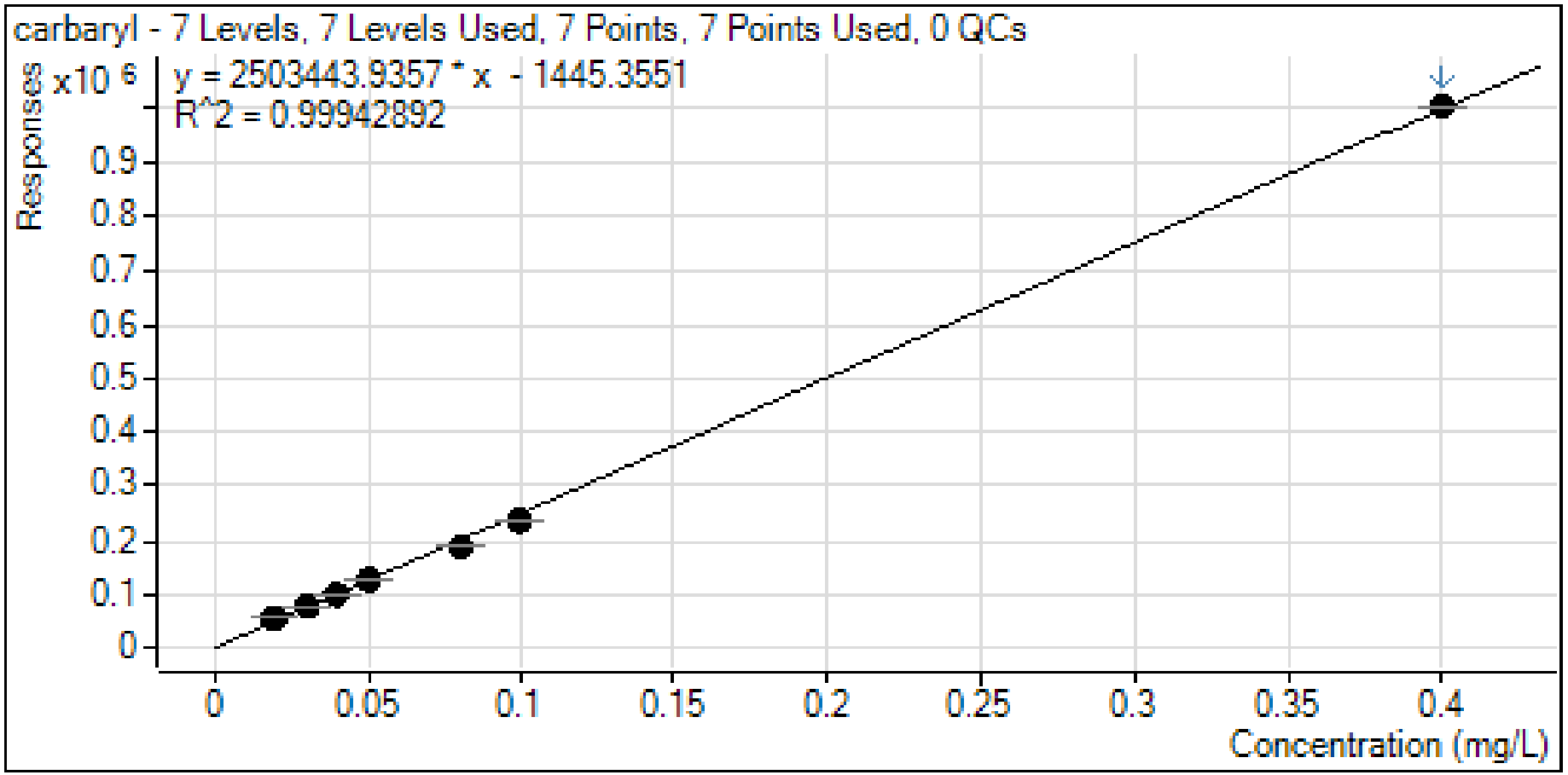


Figure 14(b): Calibration Curve for Carbaryl (0.02 -0.10 ppm)

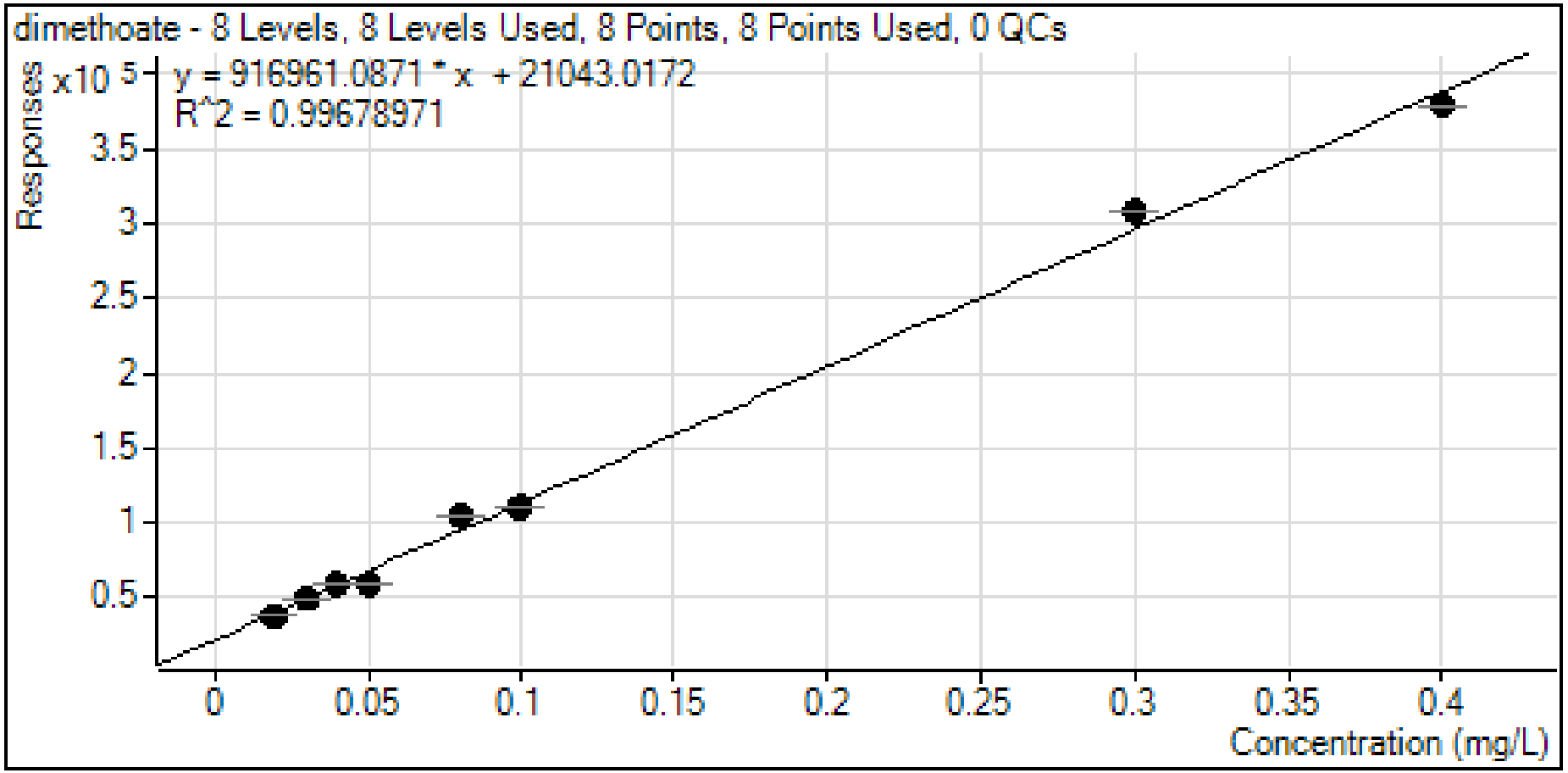


Figure 14(c): Calibration Curve for Dimethoate (0.02 -0.30 ppm)

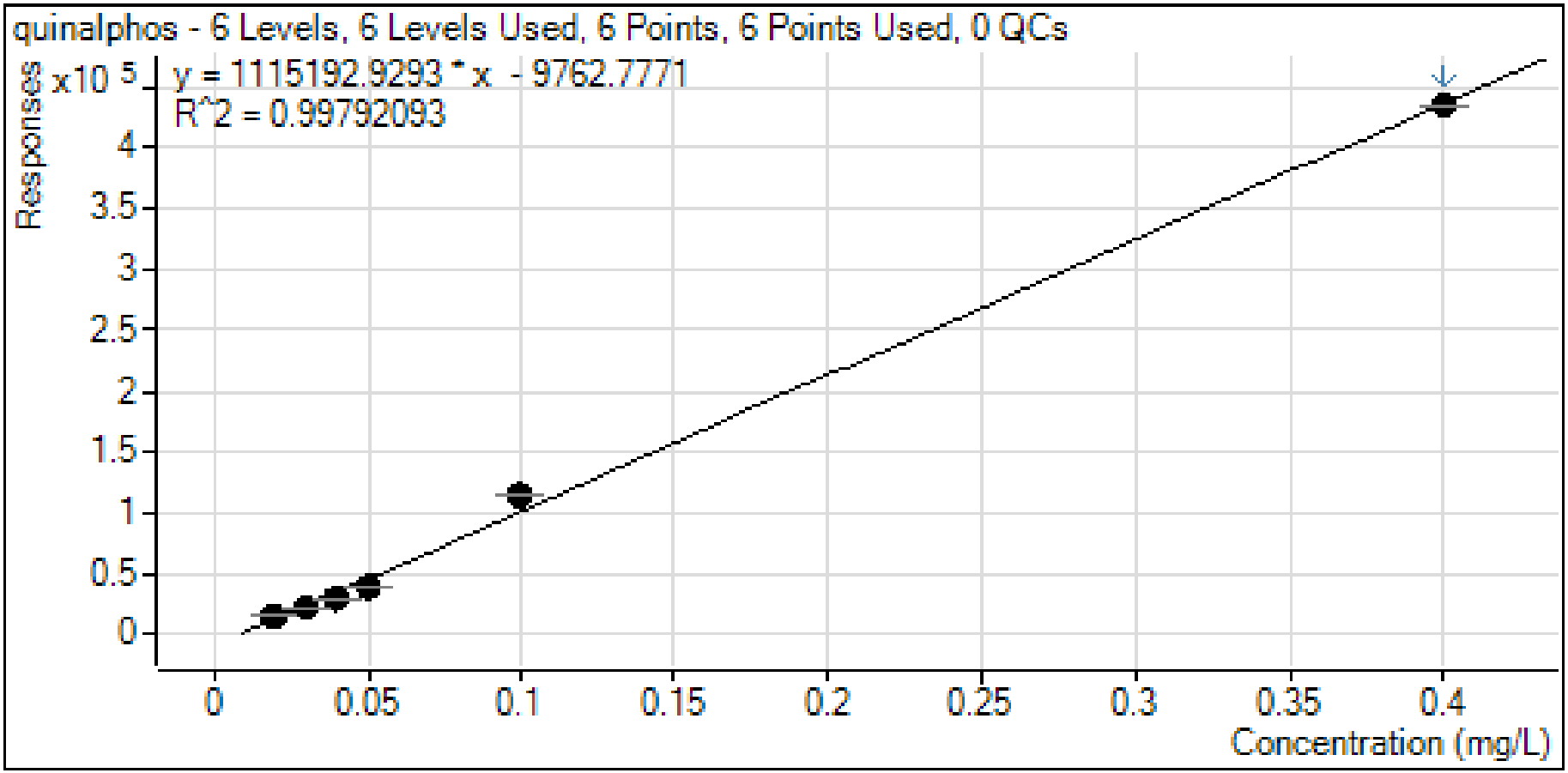


Figure 14(d): Calibration Curve for Quinalphos (0.02 -0.40 ppm)

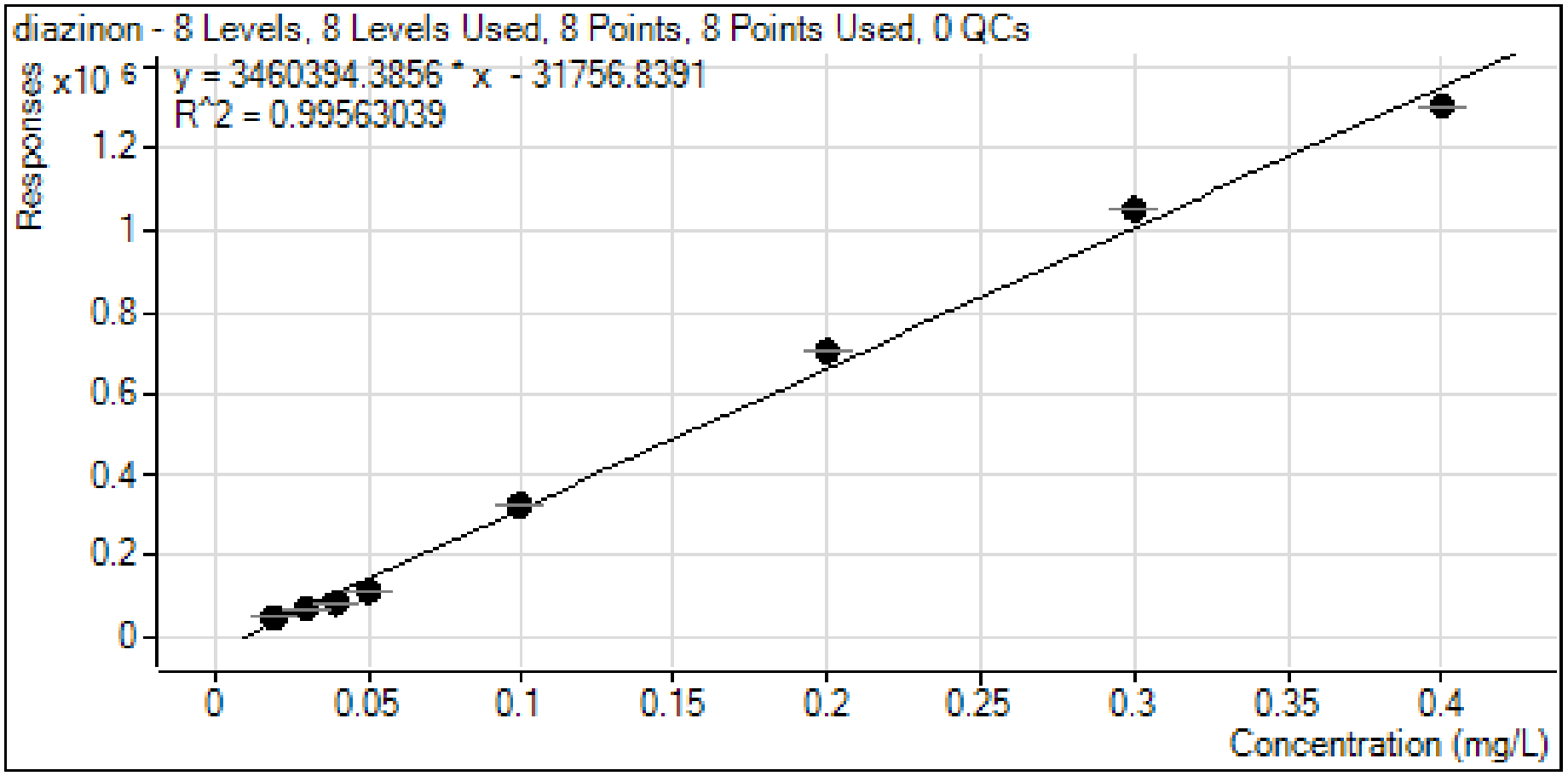


Figure 14(e): Calibration Curve for Diazinon (0.02 -0.40 ppm)

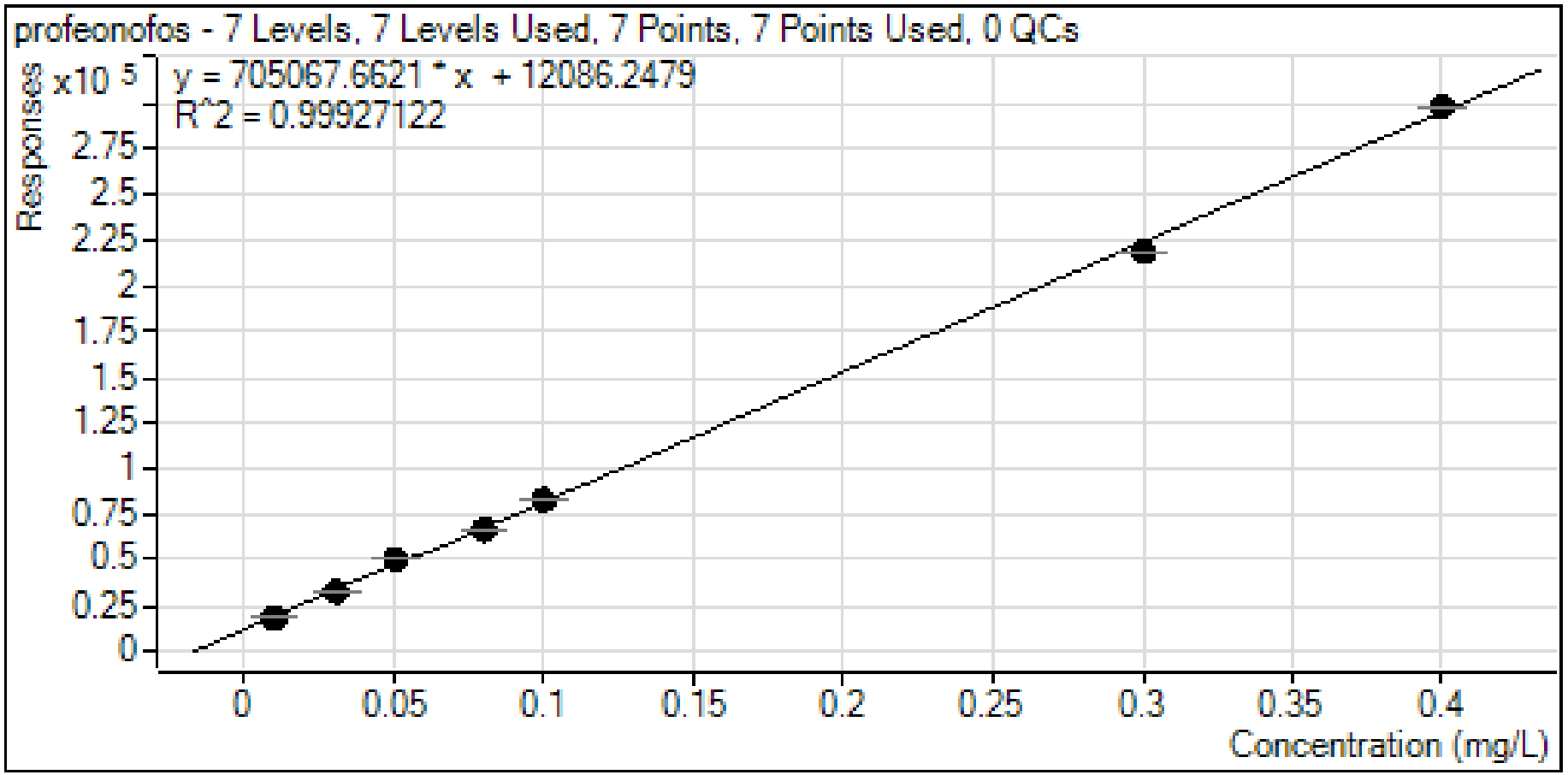


Figure 14(f): Calibration Curve for Profenofos (0.01 -0.40 ppm)

Appendix F

Concentration of pesticides was calculated by the following equation:

$$Concentration\ of\ pesticide\ in\ sample(mg/L) = \frac{concentration\ from\ calibration\ curve\ (mg/L)\ \times 5\ ml}{sample\ weight\ (g)}$$

Example:

Calculation of concentration of Profenofos in unspiked sample, Kai Lan (Chinese Kale):

Weight of unspiked sample = 5.1484 g

Concentration of Profenofos from the calibration curve = 0.3899 mg/L

Concentration of Profenofos in unspiked sample = $\frac{0.3899\ mg \times 5\ ml}{5.1484\ g}$

= 0.3786 mg/kg

= 0.3786 ppm

Spiking recovery was calculated by the following equation:

$$\%\ \text{of Recovery} = \frac{Concentration\ \ in\ spiked\ \ sample\ (mg/kg) - concentration\ \ in\ unspiked\ \ sample\ (mg/kg)}{\frac{concentration\ \ of\ standard\ \ mixture\ \ spiking\ \ solution\ (=0.2mg/L)}{weig\ ht\ of\ spiked\ \ sample\ \ (g)}} \times 100\%$$

Calculation of the % of Recovery for Profenofos in unspiked sample :

Concentration of Profenofos in spiked sample = 0.4184 ppm

Concentration of Profenofos in unspiked sample = 0.3786 ppm

Concentration of standard spiking = 0.2 mg/L

Weight of spiked sample = 5.0193 g

Weight of unspiked sample = 5.1484 g

$$\%\ of\ Recovery = \frac{0.4184ppm - 0.3786\ ppm}{\frac{0.2\ mg/L}{5.0193\ g}} \times 100\%$$

= 99.7 %

Appendix G

Example to calculate the Limit of Detection (LOD) for Profenofos: [32]

The standard solutions in the concentration range of 0.01-0.40 ppm for Profenofos were prepared. The response (peak area) for each concentration was obtained from the chromatogram and recorded as below. The expected peak area was calculated from the equation shown in the calibration curve (Appendix E).

Concentration (ppm)	Response (peak area), y_i	Expected Response , y_p	$y_i - y_p$	$(y_i - y_p)^2$
0.010	18697.7483	19136.9245	-439.1762	192875.7159
0.030	33207.9602	33238.2778	-30.3175	919.1524
0.050	50373.9108	47339.6310	3034.2798	9206853.9321
0.080	66738.1768	68491.6609	-1753.4841	3074706.4749
0.100	83163.7464	82593.0141	570.7323	325735.3307
0.300	218710.4812	223606.5465	-4896.0653	23971455.5321
0.400	297627.3437	294113.3127	3514.0310	12348413.5717
				∑= 49120959.7097

y_i was the observed instrument response and y_p was the predicted instrument response calculated from the linear equation from the calibration curve as in Appendix D. Standard deviation (s.d.) was then calculated by the following formula:

$$\text{s.d.} = \sqrt{\frac{\sum(y_i - y_p)^2}{n-2}} = \sqrt{\frac{49120959.7097}{7-2}} = 3134.356703$$

The linear equation as shown in the calibration curve of Profenofos was

$$y = 705{,}067.6621x + 12{,}086.2479$$

where y = response (peak area) and x = concentration

The estimated LOD was calculated as below:

1.

The peak area at the LOD (y_{LOD}) was calculated at 3 times the standard deviation,

y_{LOD} = (3 x s.d). + 12,086.2479

y_{LOD} = (3 x 3134.356703) + 12,086.2479

= 21489.318

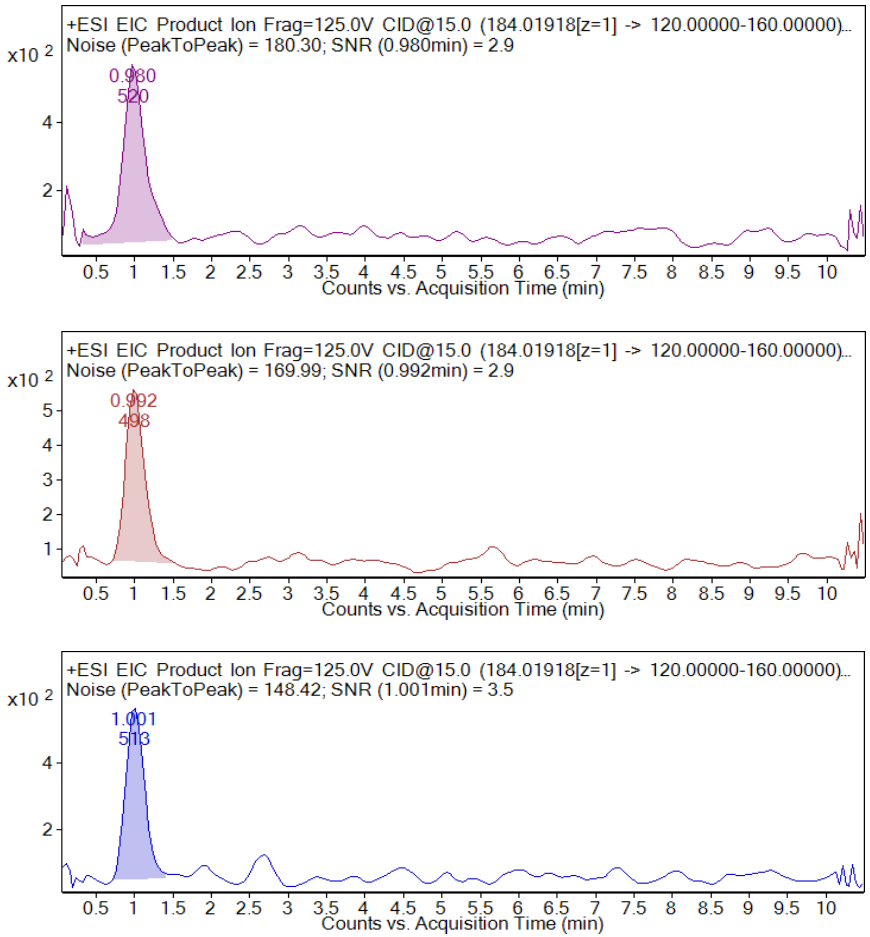
LOD of profenofos = $\frac{y_{LOD} - 12,086.2479}{705,067.6621}$

= $\frac{21,489.318 - 12,086.2479}{705,067.6621}$

= 0.0133 ppm

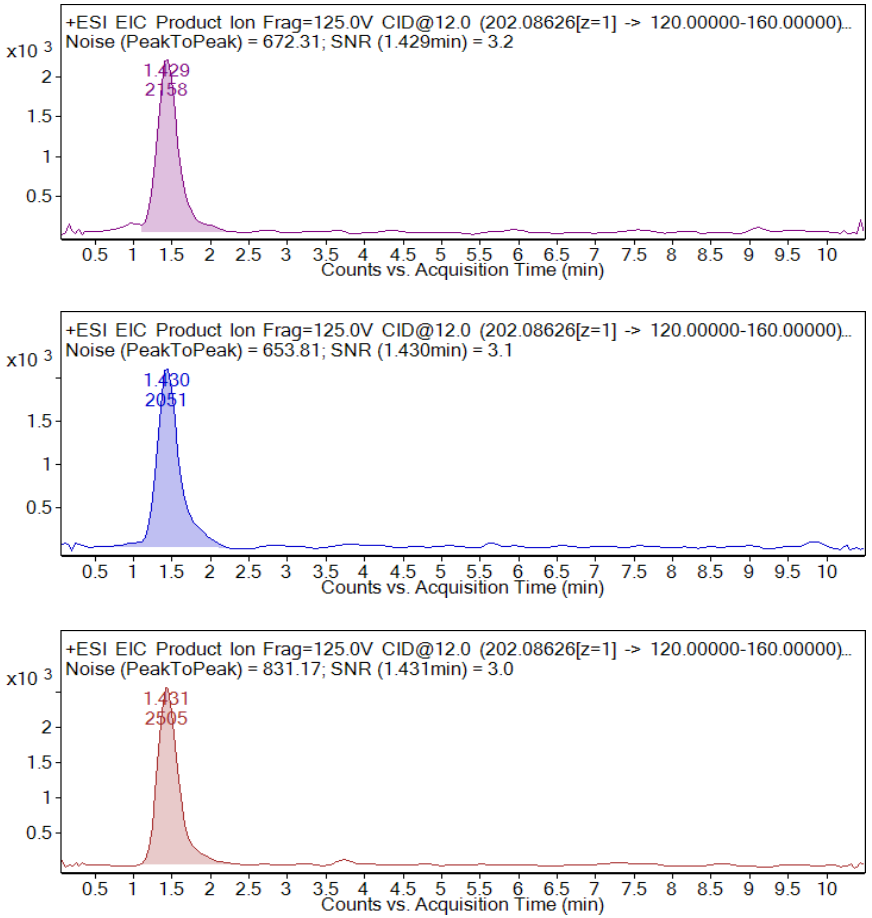
Appendix H

(a)



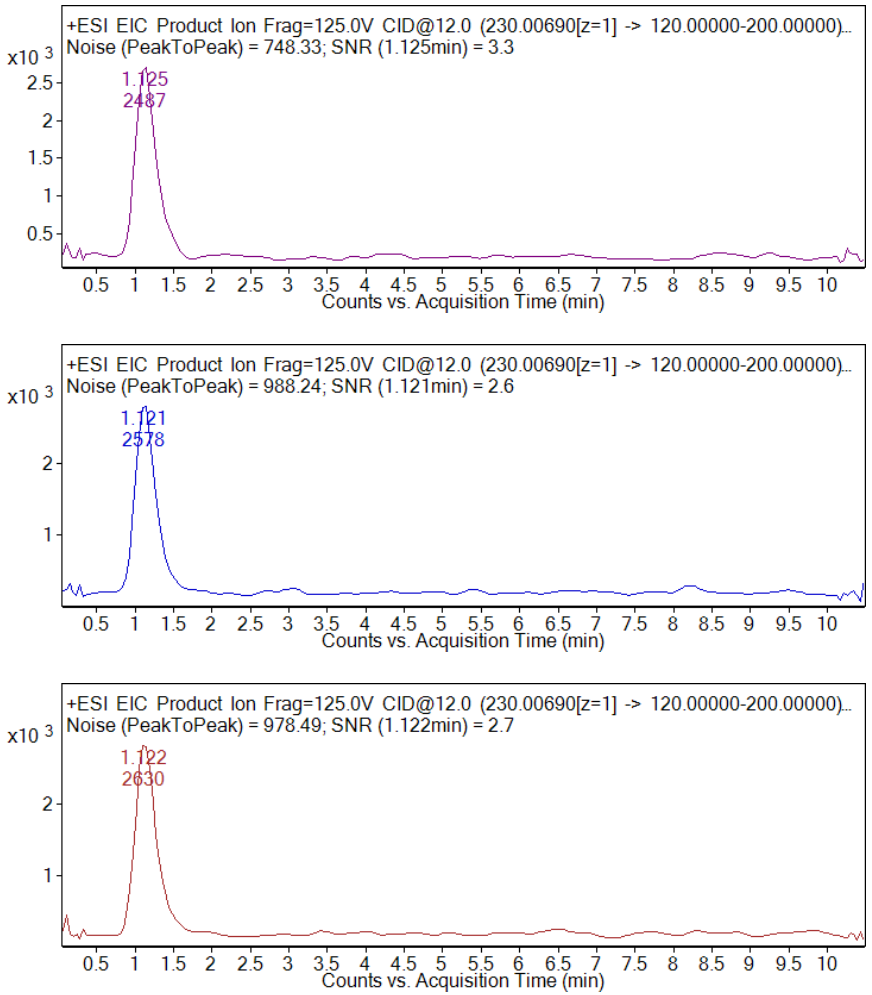
Examples of chromatograms used to calculate signal to noise(S/N) to estimate of Limit of Detection of Acephate.

(b)



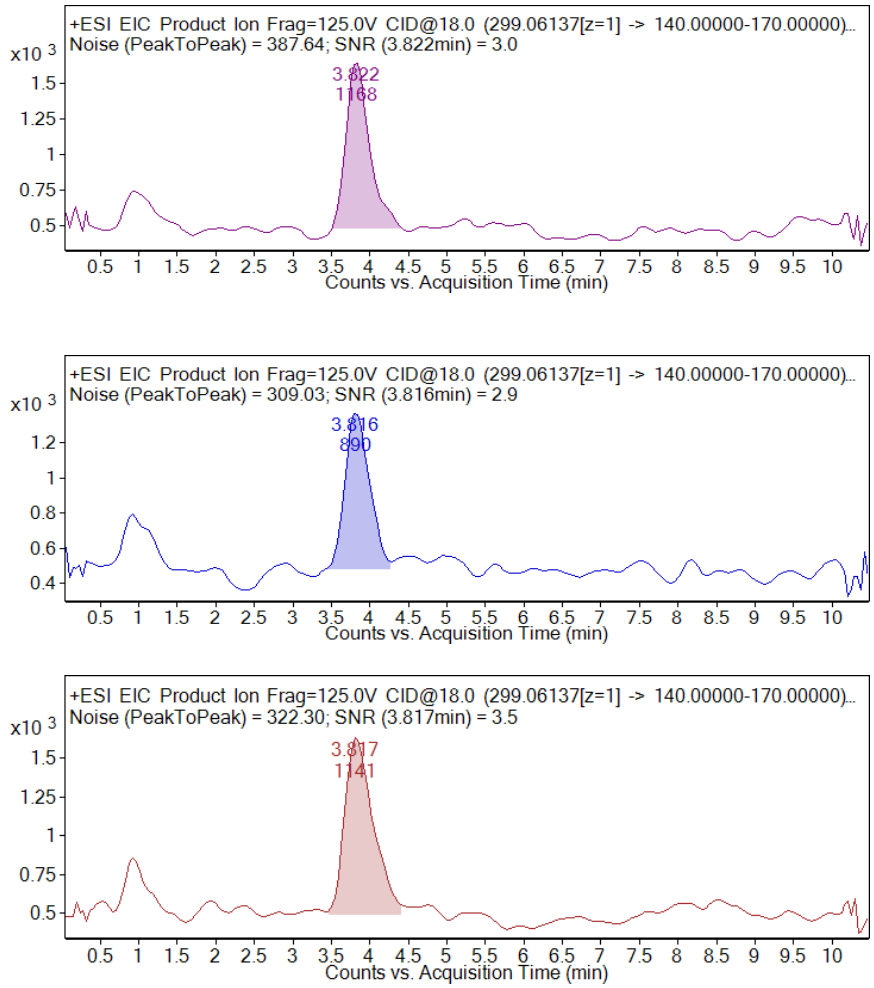
Examples of chromatograms used to calculate signal to noise(S/N) to estimate of Limit of Detection of Carbaryl.

(c)



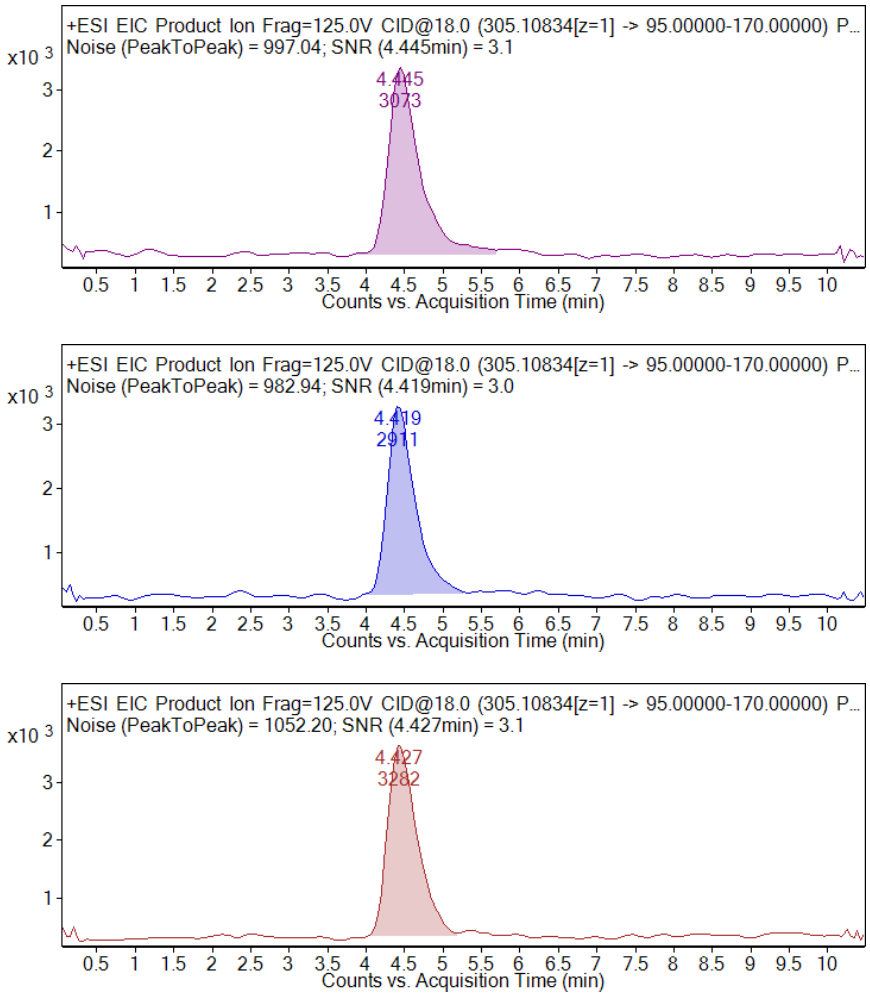
Examples of chromatogram used to calculate signal to noise(S/N) to estimate of Limit of Detection of Dimethoate

(d)



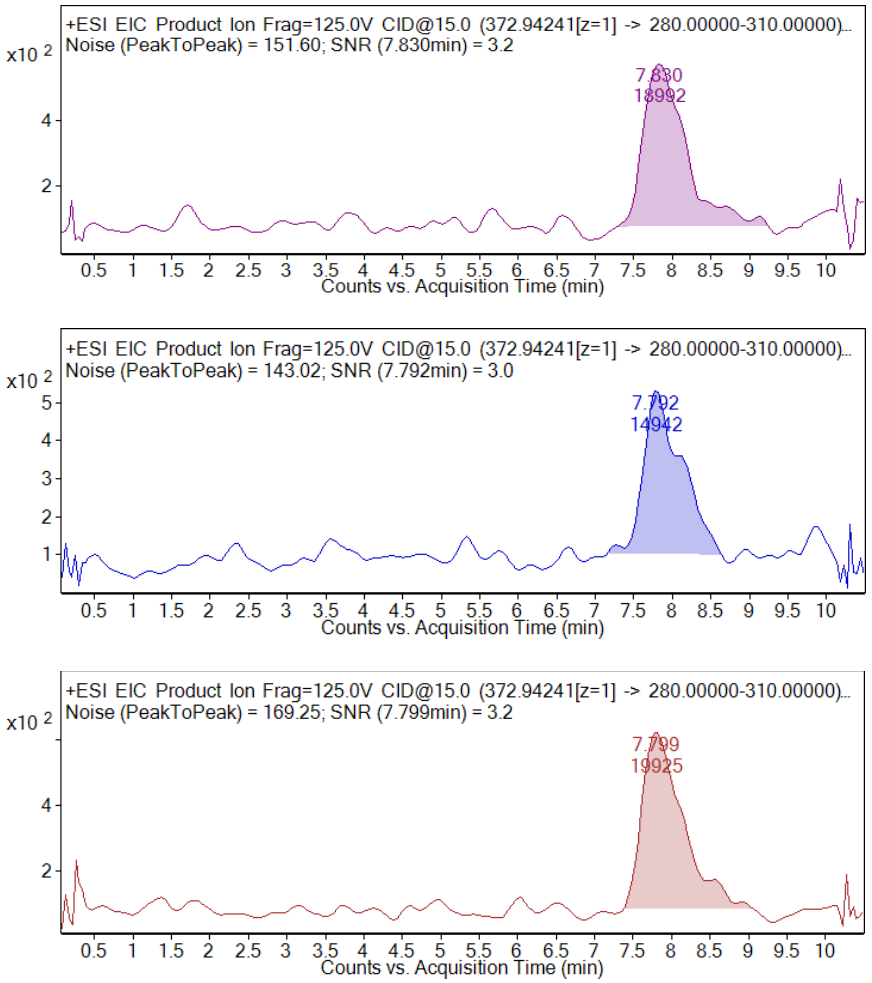
Examples of chromatogram used to calculate signal to noise(S/N) to estimate of Limit of Detection of Quinalphos.

(e)



Examples of chromatogram used to calculate signal to noise(S/N) to estimate of Limit of Detection of Diazinon.

(f)



Examples of chromatogram used to calculate signal to noise(S/N) to estimate of Limit of Detection of Profenofos

Appendix I

(a)

Signal to noise ratio (SNR) used to estimate LOD for Acephate at 0.01 ppm.

Chromatogram	RT	Area	Height	Width	SNR
1	1.001	8858	513	0.269	3.5
2	0.980	11071	520	0.289	2.9
3	0.992	8844	498	0.274	2.9

$$average\ SNR = \frac{3.5 + 2.9 + 2.9}{3}$$

$$= 3$$

(b)

Signal to noise ratio (SNR) used to estimate LOD for Carbaryl at 0.01 ppm.

Chromatogram	RT	Area	Height	Width	SNR
1	1.430	43388	2051	0.294	3.1
2	1.429	42662	2158	0.289	3.2
3	1.431	48708	2505	0.284	3.0

(c)

Signal to noise ratio (SNR) used to estimate LOD for Dimethoate at 0.01 ppm.

Chromatogram	RT	Area	Height	Width	SNR
1	1.121	49212	2578	0.286	2.6
2	1.125	47468	2487	0.287	2.6
3	1.122	52875	2630	0.299	2.7

(d)

Signal to noise ratio (SNR) used to estimate LOD for Quinalphos at 0.01 ppm.

Chromatogram	RT	Area	Height	Width	SNR
1	3.816	20105	890	0.356	2.9
2	3.822	25274	1168	0.323	3.0
3	3.817	29705	1141	0.397	3.5

(e)

Signal to noise ratio (SNR) used to estimate LOD for Diazinon at 0.01 ppm.

Chromatogram	RT	Area	Height	Width	SNR
1	4.419	74574	2911	0.374	3.0
2	4.445	89321	3073	0.399	3.1
3	4.427	88988	3282	0.413	3.1

(f)

Signal to noise ratio (SNR) used to estimate LOD for Profenofos at 0.01 ppm.

Chromatogram	RT	Area	Height	Width	SNR
1	7.792	14942	428	0.596	3.0
2	7.830	18992	491	0.589	3.2
3	7.799	19925	538	0.566	3.2

- RT – retention time
- Area – peak area
- Height – peak height
- Width – peak width
- SNR – signal to noise ratio
- LOD – Limit of detection