

# Development of Simultaneous Dimethoate, Carbaryl and Fenvalerate Extraction from Tangerine (*Citrus reticulata* Blanco) Peel by Using SPE-HPLC

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**Abstract**— Simultaneous extraction of three residual pesticides from different classes: dimethoate, carbaryl and fenvalerate, as representatives of organophosphate, carbamate and pyrethroid, from Tangerine (*Citrus reticulata* Blanco) peel was developed. The methodology was carried out by means of solvent extraction in ultrasonic bath. After that the extract was evaporated to near dryness on a vacuum rotary evaporator (40±1°C). The residue was then redissolved and followed by C18 - solid phase extraction (SPE) clean-up step. Typically, the parameters influencing the extraction such as extracting solvent, sonication time and the clean-up step condition were investigated. The determination was performed by high performance liquid chromatography (HPLC) equipped with ultraviolet detector (UV) and liquid chromatography/mass spectrometry (LC/MS). Average percentage recoveries of dimethoate, carbaryl and fenvalerate were found to be 91%, 80% and 97%, respectively. The precision with relative standard deviation (R.S.D) ranged from 0.63 - 4.19% for intra-day (n = 8) and from 0.57 - 4.90 % for inter-day (n = 8, 6 days).

**Keywords**—carbaryl, dimethoate, fenvalerate, liquid chromatography, solid phase extraction

## I. Introduction

Pesticides are widespread used to control pests in fruits and vegetables for increasing agriculture production. They impart a vital role for the enhancement of agricultural commodities according to the requirement of world population. They are extensively sprayed on fruits, vegetables, rice and cotton to protect the crops from different infestations, pests, insects, fungi, bacteria, weeds, nematodes, rodents and other pests. Many different classes of pesticides are used for a wide variety of pest control.

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In the last two decades organochlorine insecticides (e.g. DDT, aldrin and lindane) have been progressively replaced by carbamate (e.g. carbaryl, methomyl and aldicarb), organophosphate (e.g. parathion, malathion and dimethoate) and pyrethroid (e.g. fenvalerate and permethrin) (Fig. 1) due to their lower environmental persistence than organochlorine.

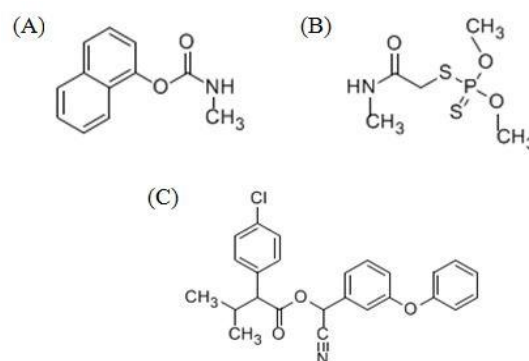


Figure 1 The structures of (A) carbaryl (B) dimethoate (C) Fenvalerate.

In the North of Thailand, a large amount of pesticides has been used to control the Tangerine (*Citrus reticulata* Blanco) pests. The presence of residues in fruits results in serious food safety problems and human health [1-2]. The toxicity of pesticide can produce adverse effects in human's health by skin contact, inhalation and ingestion. Furthermore, they are also an inhibitor of the acetylcholinesterase (AChE) [3-4] in nerve-impulse transmission [5-6]. The levels of pesticide residues are controlled by Maximum Residue Limits (MRLs), which are established by each country and/or organization [7]. The MRLs of dimethoate, carbaryl and fenvalerate in fruits and vegetables which permitted by FAO/WHO food standard are 5, 15 and 2 mg/L, respectively [8]. According to high toxicity of these pesticides, sensitive and selective methods are required to detect their presence in fruits. Generally, dimethoate and fenvalerate have always been investigated by GC-NPD and GC-ECD, respectively, which are selective detector, but HPLC does offer some advantages [9]. Due to physical and chemical properties such as thermal instability and low volatility, carbaryl does not perform well in GC technique without the time-consuming process of derivatization [5]. Thus the use of HPLC is preferable [10]. Various organic solvent used to extract pesticide residues are acetone [11], ethyl acetate [3, 12-13], acetonitrile [5], dichloromethane [14], n-hexane [15], light petroleum ether

[16] and a mixture of organic solvents are also used like dichloromethane-cyclohexane [17], dichloromethane-acetone [18] because they can extract a wide range of polarity compounds [19]. The sample extraction in fruits is often complicated by the presence of co-extract or matrix compounds so the clean-up steps are necessary prior to analysis [20]. Among the clean-up step methods, solid phase extraction (SPE) is gaining consideration owing to less used in solvent consumption [21]. SPE has been developed involving procedures using cartridges of C18 [22], florisil [23], activated carbon etc. Several methods established the simultaneous determination of carbamate and organophosphate pesticides in fruits and vegetables [5, 16] but the analysis did not cover pesticide residues in pyrethroid group. The purpose of this study was to develop of simultaneously carbaryl, dimethoate and fenvalerate residual extraction from Tangerine (*Citrus reticulata* Blanco) by using SPE-HPLC.

## II. Experimental

### A. Chemicals and reagents

All the reagents used were of analytical grade and the solvents of HPLC grade. The HPLC grade water obtained by purification of deionized water through a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the study. Organic solvents such as ethyl acetate, acetone and ethanol were acquired from LabScan (Bangkok, Thailand). HPLC-grade methanol were obtained from Merck (Darmstadt, Germany) and HPLC-grade acetonitrile obtained from BDH (Poole, UK). Pesticide analytical standards with purities of 98.5 - 99.0% were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Individual stock standard solution was prepared at concentration of 1000 mg/L in methanol. A mixed standard solution at various concentrations was prepared by dilution of the stock solutions in methanol. These solutions were stored in a refrigerator at 4°C. The mobile phases were filtered through a 0.45 µm nylon membrane filter (Supelco, Barcelona, Spain) and degassed under vacuum for 30 min prior to chromatographic separation. A 24-port vacuum manifold (JT Baker, Thailand) was employed for the filtration of extraction solvent. For SPE procedure, C18 cartridges (500 mg, 10 mL) obtained from Vertical Chromatography (Bangkok, Thailand) were used.

### B. Solvent Extraction Procedure

Orange samples were obtained from the local market. Ten grams of chopped orange peels were spiked with 1.00 mL of standard solution of dimethoate, carbaryl and fenvalerate at concentration level of 50, 15 and 20 mg L<sup>-1</sup>, respectively. After equilibration for 1 hour at room temperature to allow adsorption of pesticides, the sample was extracted with 50 mL of organic solvent in ultrasonic bath. Suitable extracting solvent such as ethyl acetate, acetone, ethanol and a mixture of ethyl acetate-acetone-ethanol (1:1:1, v/v), and sonication time were then investigated. The extract was filtered through a Whatman paper and collected in a 100 mL of round bottom flask. The extract was evaporated to near dryness in a vacuum

rotary evaporator (40±1°C) and the residue was then dissolved in 10 mL of deionized water.

### C. Solid Phase Extraction Procedure

2.00 mL of the extract solution was purified by passing through a C18 SPE cartridge vacuum manifold system which was previously conditioned with 3.00 mL of methanol following 3.00 mL of deionized water. The filtrate was collected immediately after passing the extractant through the cartridge (namely, unadsorbed solution). The eluting solvent was compared among a mixture of MeOH:H<sub>2</sub>O (7:3, v/v), ACN:H<sub>2</sub>O (7:3, v/v), acetone:H<sub>2</sub>O (7:3, v/v) and ACN, respectively. Finally each fraction was filtered through a 0.45 µm PTFE membrane filter and 20 µL of both the un-adsorbed solution and the eluent were injected in HPLC system under the optimum conditions. The purified and non-purified extracts from the same sample and both were processed in parallel. All procedures were carried out in triplicates.

### D. Instrumentation and chromatographic conditions

An Agilent Model 1100 Series LC system (Agilent Technologies, Palo Alto, CA, USA) which composed of a binary pump, a degasser and a diode array detector, with a 10 µL flow cell (10 mm path length), coupled with a computer was utilized. The acquisition and treatment data software are supplied by the manufacturer (HP Chemstation Software for LC). The HPLC system was connected to a mass spectrometer Agilent MSD (Agilent Technologies) equipped with an electrospray ionization (ESI) interface for confirmation of pesticides. The chromatographic separation was performed on a Phenomenex (Madrid, Spain) Envirosep-CM C18 column (175 mm x 3.2 mm i.d., 5 µm) preceded by a Phenomenex C18 guard column (4 mm x 4 mm i.d., 5 µm). The mobile phase consists of a binary gradient. It was a mixture of methanol-water at 1:9, v/v (mobile phase A) and 9:1, v/v (mobile phase B), both containing 5 mM ammonium acetate. The detector was set at λ<sub>max</sub>: 220 nm and the flow rate was 0.3 mL/min at ambient temperature.

### E. Method validation

Method validations were presented in terms of the limits of detection (LODs), accuracy, repeatability, reproducibility and calibration curve. The LODs were established as the lowest or minimum detectable concentration that provided the occurrence in peak area signals. Accuracy was calculated as the percentage of recovery based on peak area and calibration curve. The repeatability was carried out by injection of the same standard solution eight consecutive times in the same day. The reproducibility was determined on six days in which it was performed each day on eight consecutive times. The calibration curve was obtained by the construct peak area of each pesticide to concentrations of mixed standard solution. The calibration curve should be prepared to cover the range of pesticide levels likely to be found in orange sample peels. Quantitation of the pesticides was carried out by external standard method using the mixed standard solution prepared in

methanol. The pesticide concentrations were calculated by the differentiation of peak area between spiked sample and unspiked sample, in the other word, it was calculated by subtraction of unspiked sample.

### III. Results and discussion

#### A. Investigation of extracting solvent

The selection of extracting solvent is very important to pesticide analysis, so various organic solvents were investigated; ethyl acetate, acetone, ethanol and a mixture of ethyl acetate-acetone-ethanol (1:1:1, v/v)

The peak area of dimethoate obtained with acetone and a mixture of ethyl acetate-acetone-ethanol (1:1:1, v/v) were similar whereas with ethyl acetate and ethanol the peak areas were lower than or approached to zero. Although the ethyl acetate extract was clean and less color than other solvent but lipids and waxes were also co-extracted. In addition ethyl acetate has less polar property so that the dimethoate is not readily partition into it. In acetone extract both polar (dimethoate) and less polar (carbaryl and fenvalerate) pesticides could be recovered with acetone owing to the solubility property. Forcing evaporation of ethanol extract took an extended period of time and led to resulting in the loss of the analyte, particularly dimethoate. In order to increase the efficiency of simultaneous extraction of dimethoate, carbaryl and fenvalerate, a mixture of ethyl acetate-acetone-ethanol (1:1:1, v/v) was the most suitable extracting solvent because it extended the polarity range for extraction of different class of pesticides and presented the maximum peak area (Fig. 2).

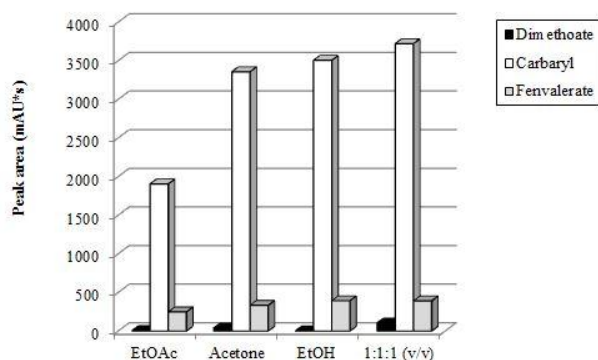


Figure 2 The effect of extracting solvents on pesticide extraction.

min. The similar response peak areas of dimethoate were obtained in the range of 5 to 15 min and had a tendency to decrease as the time expanding owing to volatile property. Increasing results in peak area of carbaryl and fenvalerate were obtained from initial time to 15 min after that the peak areas decreased. Sonication of the sample in the presence of solvents was much more effective. The ultrasonic disrupted the cell walls of orange peels and accelerated the washing pesticides out of the cell contents. A longer period of extraction time, the pesticide residues inside the orange peels were gradually released cause of more cells were broken. In addition, raised temperature caused by mechanical energy transfer to thermal energy also can profitably enhance the

mass transfer [22]. Based on the results obtained, 15 min was chosen for sonication time to achieve simultaneous determination of dimethoate, carbaryl and fenvalerate (Fig. 3).

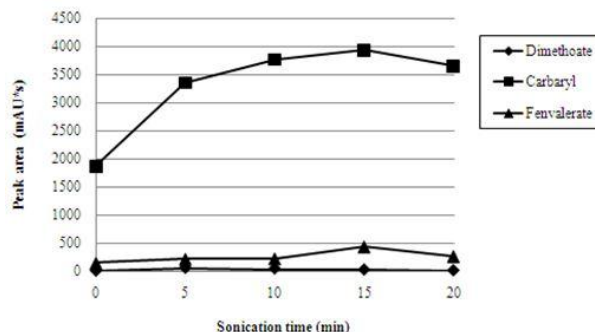


Figure 3 The effect of sonication time on pesticide extraction

#### C. Investigation of solid phase extraction

It is obvious that the direct injection of the crude extract produced unsatisfactory chromatograms, especially dimethoate (Fig. 4). This is because a mixture of ethyl acetate-acetone-ethanol (1:1:1, v/v) is capable to extract a wide range of compounds in orange peels including co-extractive compounds. It is not possible to analyze raw extracts without clean-up step by using HPLC-UV detection. Thus the additional SPE clean-up step was required in the extraction procedure.

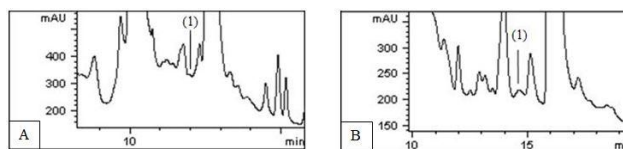


Figure 4 The chromatograms of dimethoate in the sample solution before (A) and after (B) SPE clean up step. Peak identification: (1) dimethoate.

The criterion concerned to select the eluting solvent is the ability to water miscible. Other solvents may have greater eluting power in reversed phase chromatography but are not water miscible. Therefore, in this research work, the eluting solvents; MeOH:H<sub>2</sub>O (7:3, v/v), ACN:H<sub>2</sub>O (7:3, v/v), Acetone:H<sub>2</sub>O (7:3, v/v) and acetonitrile were evaluated. The peak areas of dimethoate, carbaryl and fenvalerate in standard solution were calculated by the differentiation between standard solution and blank and the results are shown in Table 1. The unadsorbed dimethoate redissolved in deionized water resulted from the hydrophilic structure or due to relatively polar, thus dimethoate was preferred to soluble in deionized water rather than to retain on C18 sorbent. The elution of carbaryl and fenvalerate adsorbed on C18 sorbent was maximum accomplished, due to polarity property, with a mixture of acetone:H<sub>2</sub>O (7:3, v/v) and acetonitrile, respectively, while the co-extractives were retained by the sorbent.



**Table 1** HPLC peak data of dimethoate, carbaryl and fenvalerate in standard solution using different eluting solvents.

Eluting solvent	*Peak area of dimethoate (mAU*s)	*Peak area of carbaryl (mAU*s)	*Peak area of fenvalerate (mAU*s)
Un-adsorbed solution	362	47	17
MeOH:H <sub>2</sub> O (7:3, v/v)	86	3422	27
ACN:H <sub>2</sub> O (7:3, v/v)	102	3585	77
Acetone:H <sub>2</sub> O (7:3, v/v)	95	3804	98
ACN	57	3609	194

\*Peak area of triplicate results

The results obtained are related with Bushway [23] that the C18 sorbent proved to be better for carbaryl and fenvalerate retention than dimethoate due to their hydrophobic characteristics which provided high affinity for either less polar or non-polar compounds. Carbaryl and fenvalerate could be retained on C18 sorbent than dimethoate due to their hydrophobic characteristics which provided high affinity for either less polar or non-polar compounds as shown in Figs. 5-6.

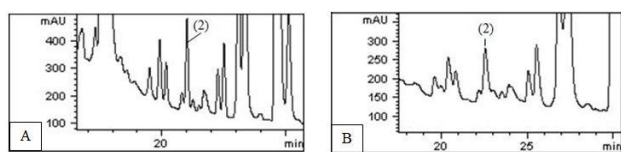


Figure 5 The chromatograms of carbaryl in the sample solution before (A) and after (B) SPE clean up step. Peak identification: (2) carbaryl.

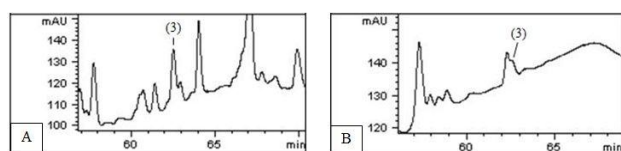


Figure 6 The chromatograms of fenvalerate in the sample solution before (A) and after (B) SPE clean up step. Peak identification: (3) fenvalerate.

Although the chromatograms of orange sample peels extract after clean-up step was cleaner than those with direct injection the pesticides were still be found and not well separated from peak of co-extractives. Moreover the pesticide might be probably loss during evaporation and redissolution. In HPLC chromatogram, the average retention times of dimethoate, carbaryl and fenvalerate were approximately at 14.3, 22.8 and 63.7 min, respectively. Because of the target pesticides belong to different chemical classes thus the total analysis time was extended, although it seem to be longer analysis time used, but in many instance long time is needed to prevent interference. The ion used for identify dimethoate, carbaryl by MS (60 eV) were presented as the molecular ion  $[M+H]^+$  at m/z: 202 and 230 for carbaryl (Fig. 7) and dimethoate (Fig. 8), respectively. According to fenvalerate containing chlorine atom in structure, the characteristic of

chlorine isotopic pattern between Cl35:Cl37 in a ratio of 3:1 in the height unit of ammonium adducted ion,  $[M+NH_4]^+$  was observed which appeared at m/z: 437 and 439 (Fig. 9).

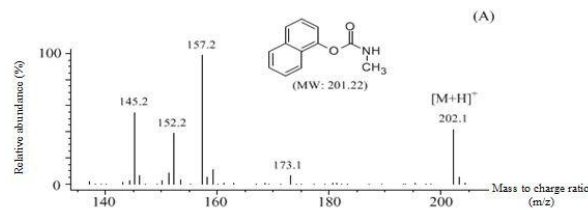


Figure 7 The mass spectrum of carbaryl.

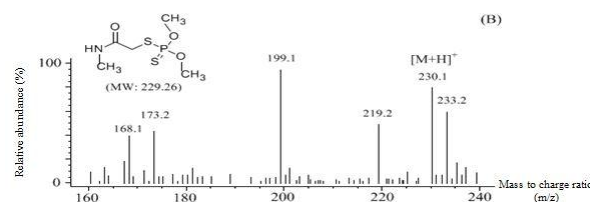


Figure 8 The mass spectrum of dimethoate.

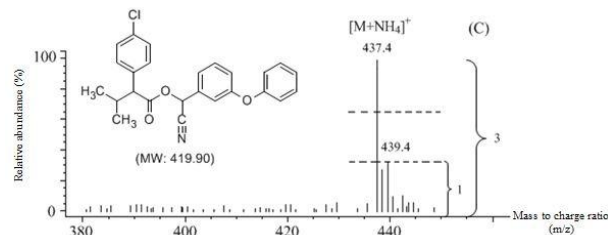


Figure 9 The mass spectrum of fenvalerate

## D. Validation of the method

Method validations were presented in terms of the limits of detection (LODs), accuracy, repeatability, reproducibility and calibration curve. The lowest detectable concentrations of dimethoate, carbaryl and fenvalerate were 0.2, 0.005 and 0.0002 mg/L, respectively. The percentage of recoveries using orange sample peels spiked with 5.00, 1.50 and 2.00 mg/L of dimethoate, carbaryl and fenvalerate were 91%, 80% and 97%, respectively. The intra-day (n = 8) precision was determined by injection of fortified sample at the concentration level of 6.0, 1.8 and 2.4 mg/L of dimethoate, carbaryl and fenvalerate, respectively, with the relative standard deviation (R.S.D) in the range of 0.63-4.19%. The inter-day (n = 8, 6 days) precision was determined on six successive days with R.S.D in the range of 0.57 - 4.90%.

## IV. Conclusion

This research attempted to fulfill the analysis of three pesticides, dimethoate, carbaryl and fenvalerate as representative from different classes, organophosphate, carbamate and pyrethroid, respectively, in a single step by using SPE technique couple with HPLC detection in orange

sample peels. Although the SPE C18 clean-up step is rapid and useful for matrix elimination when compared with direct injection of orange peel extracts but the interferences were still be found and produced a stronger decrease in the peak areas.

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