DEVELOPMENT OF SAMPLE PREPARATION FOR DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES RESIDUE IN CRUDE PALM OIL USING GAS CHROMATOGRAPHY TECHNIQUE

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ABSTRACT

Organophosphorus pesticides (OPs) determination was accomplished using gas chromatograph equipped with nitrogen phosphorus detector. Crude palm oil was extracted with acetonitrile in an ultrasonic bath. A multi-cartridge system was developed in which, in a single step, performs the clean-up of the extractant from the oil. The extractant was loaded on to solid phase extraction column to which a Florisil cartridge and a C18 cartridge had been connected in series. The OPs pesticides residues were eluted with 15 ml of acetonitrile (saturated with hexane). The recovery of 5 OPs pesticides was in the range from 62.55 - 116.61% and the SD was in the range from 0.01 - 0.21.

KEYWORDS: Organophosphorus pesticides (OPs), Crude palm oil, Solid phase extraction (SPE)

1. INTRODUCTION

Palm oil has a high consumption rate people in producing countries; therefore, the continuous control of pesticides residues in palm oil is of great importance. Palm trees are treated with several types of pesticides. Those more extensively used belong to the class of organophosphorous pesticides (OPs) and are mainly fenthion, malathion, parathion-methyl, dimethoate and dichlorvos. Toxic residues in palm oil have been reported by several researchers [4], because pesticide residues in food constitute a significant health risk.

Due to the important of palm oil for the economy of Southern Thailand, a continuous effort is being made by the Ministry of Public Health to preserve its high quality characteristics. One of the most important quality criteria is low concentration or non-detectable pesticide residue. Therefore, palm oil that is obtained from the fruit of palm tree solely by mechanical or the physical method without any treatment is controlled by regulations.

To achieve accuracy and appropriate sensitivity for its measurement, many analytical methods for determination of OPs residues in palm oil have been studied. Analytical problems associated with fatty substrates are well known. With fatty substrates, rigorous clean up is necessary for satisfactory peak separation, sensitivity and overall performance of the chromatographic system. Lentza-Rizos [4] reported that low temperature used for fat precipitation (-78 °C) precluded its extended application. And also the report has shown that gravimetric fat removal through ice cooling gives sufficient clean-up for OPs determination when combined with solid phase extraction. Luca Rastrelli [5] analyzed 18 organophosphorus pesticide residues in virgin olive oil. After sample extraction with n-hexane and clean up by single-step multi cartridge system, recoveries of 18 pesticides at three fortification levels (0.5, 1.0 and 2.5 mg kg^{-1}) were in the range of 83-110%. Moreover, Di Muccio [2] developed a single step clean up method in which silica gel plus C18 cartridge were used in series, downstream of an extrelut column, to further reduce the co-extracted lipidic material in the final solution.

The aim of this work is to develop a simple, rapid and efficient clean up method suitable for routine analysis for the determination of OPs in crude palm oil by precipitation using easily achievable temperatures together with solid phase extraction.

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2. MATERIALS AND METHODS

2.1 Apparatus

A gas chromatography (GC) analysis was carried out on a Hewlett Packard HP 6890 gas chromatograph couple with nitrogen phosphorus detector (GC-NPD Hewlett Packgard HP6890). The GC was equipped with Rtx-5MS (5%phenyl 95%dimethylpolysiloxane) fused capillary column (30 m × 0.25mm i.d., × film thickness 0.25 μm) in helium carrier gas (1.2 ml min^{-1}) and with splitless injection system. The GC oven temperature was programmed at 70°C for 2 min then increased to 260°C at a rate of 9 °C min^{-1}. The inlet temperature and detector temperature were 270°C and 280°C, respectively. Nitrogen phosphorus detector was operated at a flow rate of hydrogen gas and air at 3.0 and 60 ml min^{-1}, respectively.

An ultrasonic bath was use for extracting OPs from crude palm oil. Solid phase extraction vacuum manifold (Supelco USA) was used for extractant clean-up. Rotary evaporation was used for removal of the solvent.

2.2 Materials

Five Organophosphorus pesticide standards were purchased from SAENGVITH SCIENCE CO., LTD. (Bangkok, Thailand). Acetonitrile, n-hexane, cyclohexane, isooctane and methanol were analytical grade (LABSCAN). Stock standard solutions of the pesticides were prepared in cyclohexane. Three ml of Florisil (Cat no. 2113) and six ml of C18 silica (Supelco Cat no. 57012) were used in the clean up step for sample preparation.

2.3 Method

2.3.1 Extraction

Two grams of crude palm oil were weighed in a 250-ml Erlenmeyer flask. Five of 3.33 mg kg^{-1} organophosphorus pesticide standards and then 30 ml of acetonitrile were added in the oil. The oils were extracted in an ultrasonic bath for 5 min. After agitation, the samples were stored in a freezer at –15°C and allowed to stand 24 hrs for lipid precipitation and separation [4]. 10 ml aliquot was taken into a pre-weighed 50 ml round bottle flask. The solvent was removed by rotary evaporation. At this stage, the flask was re-weighed in order to find the amount of oil co-extracted. The residue was made volume using 3 ml of cyclohexane for the clean-up step later.

2.3.2 Clean-up

The clean-up step was performed by solid phase extraction. The solid phase extraction consists of a single step clean-up in which Florisil plus C18 cartridge were used in series, to reduce further amount of oil co-extracted. Florisil cartridges were conditioned by successive elution of 3 ml isooctane, by mean of a gentle vacuum, to avoid drying-out during the procedure. Then the residue sample volume was loaded under gravity flow only. The cartridges were dried under nitrogen gas flow for 3 min. C18 cartridges were conditioned by passing 6 ml of methanol followed by 6ml of acetonitrile through the column. The C18 cartridge attached below the Florisil cartridge before elution (are shown in Figure 1). The cartridges were placed on a SPE vacuum manifold and were eluted with 15 ml acetonitrile (saturated with hexane) under gravity flow only [2]. The eluates were collected in a pre-weighed 50 ml round bottle flask and were evaporated to dryness. Then the flask was re-weighed in order to find the amount of oil co-extracted and re-dissolved with 900 μL cyclohexane before injected to GC-NPD. The exact concentration of five organophosphorus pesticide achieved by performing calibration curve of five organophosphorus pesticide standards. The exact concentrations were calculated % recovery from known concentrations (3.33 mg kg^{-1}).

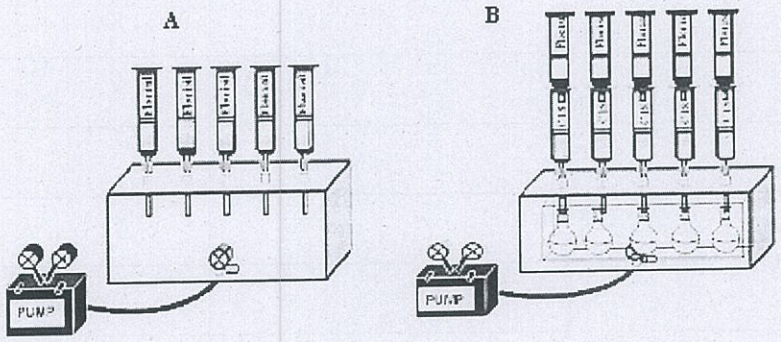

Figure 1 A = Solid phase extraction of florisil cartridges (3 ml, 500 mg)
B = Solid phase extraction of florisil cartridges (3 ml, 500 mg) couple with C18 cartridges (6 ml, 1 g)

3. RESULTS AND DISCUSSION

3.1 Linearity

Under the chromatographic conditions described in the materials and method section, the calibration graphs in 5 OPs were constructed by plotting the peak areas vs. concentrations. Good linearity was achieved in the range of 0.5 – 50 mg L^{-1} with correlation coefficients between 0.9977-0.9992. And calibration curves of 5 OPs were shown in Figure 2 (A-E).

3.2 Clean up efficiency

Lentza-Rizos [4] freezed the extracted at -20°C overnight, but our experiments were carried out at - 15°C and – 60°C. The results revealed that both of the aliquot, lipid and palm oil, were frozen at –60 °C. This gave an increasing in the amount of oil co-extracted in the process of thawing and filtration of the aliquot. So, in this research, we chose -15°C for lipid precipitation and separation.

For the solid phase extraction, Florisil and C18 cartridge were used in the clean up step with a low temperature. The efficiency of SPE depends on conditioning using an appropriate solvent. The preliminary studies used isooctane and n-hexane for conditioning of SPE. Since 5 OPs were quite non-polar, a compatible organic solvent had to be more non-polar. The best result was isooctane is enough non-polar solvent (polarity index: -0.4) for five OPs depositing on SPE while loading. N-hexane is less non-polar solvent (polarity index: 0.00) than isooctane. So 5 OPs could not deposit on SPE, n-hexane was used conditioning SPE. The efficiency of SPE depends on the type and quantity of sorbent and sample volume, as well as the extent of solvent strength .The performance of the tandem cartridge has been studied with respect to its ability to remove the amount of oil co-extracted and to recover OPs.

Table 1 reported the amount of oil co-extracted which was removed by four systems (i.e., low temperature without cartridge, low temperature with Florisil cartridge only, low temperature with Florisil connected to a 3 ml C18 cartridge, and low temperature with Florisil connected to a 6 ml C18 cartridge in series). The results show that greatest reduction oil co-extracted from eluate, compared with low temperature alone is achieved by the introduction of low temperature with Florisil connected to a 6 ml C18 cartridge which evidently removes highly non-polar components.

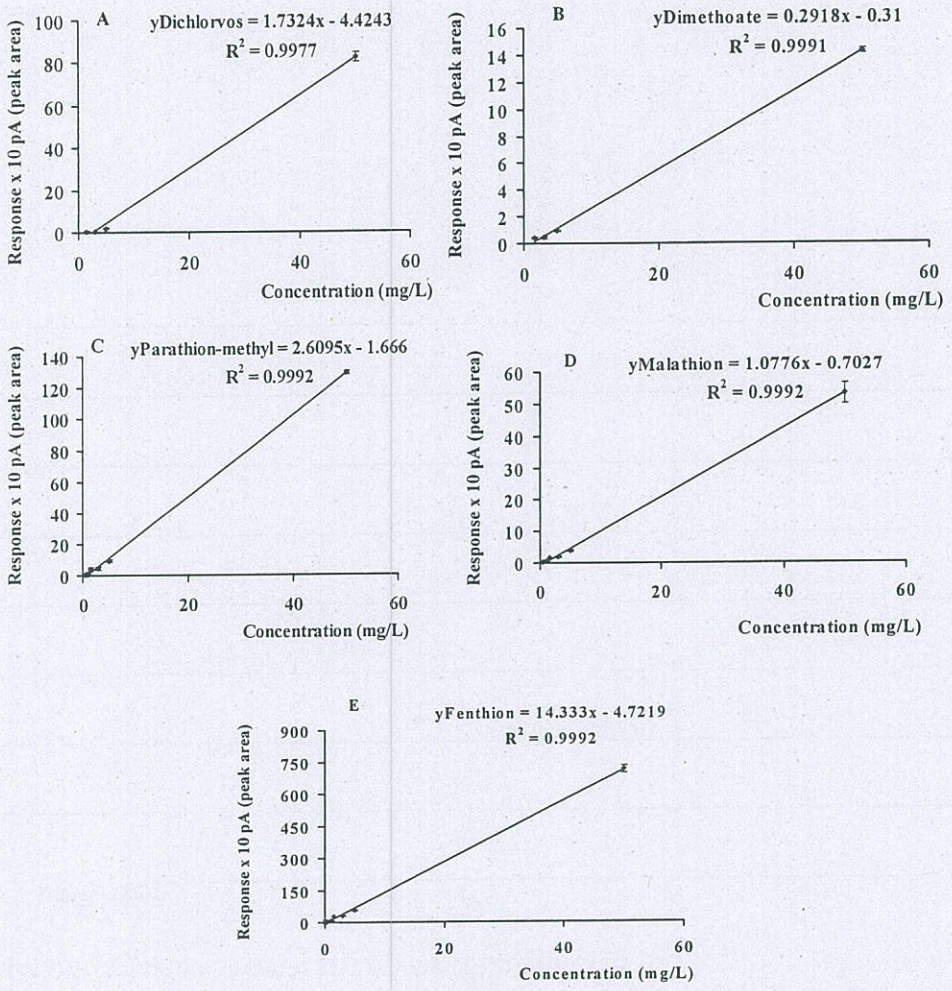

Figure 2 Linearity of 5 OPs: A=dichlorvos, B= dimethoate, C= parathion-methyl, D=malathion and E=fenthion

Table 1 Mean (n=2) amounts of oil co-extracted released into the eluate with 2 grams of the oil applied to the four system investigated.

The amount of oil co-extracted in the eluate, mg (n=2) ± SD			
Low Temperature	Low Temperature Florisil	Low Temperature Florisil + 3ml C18	Low Temperature Florisil + 6ml C18
25.1 ± 14.1	17.5 ± 7.1	5.1 ± 3.5	4.6 ± 4.3

The advantages of solid phase extraction clean up were not only removing the amount of oil co-extracted from extractant, but also giving qualitative parameters such as retention time, reproducibility and minimizing ghost peak chromatogram (Figure 3).

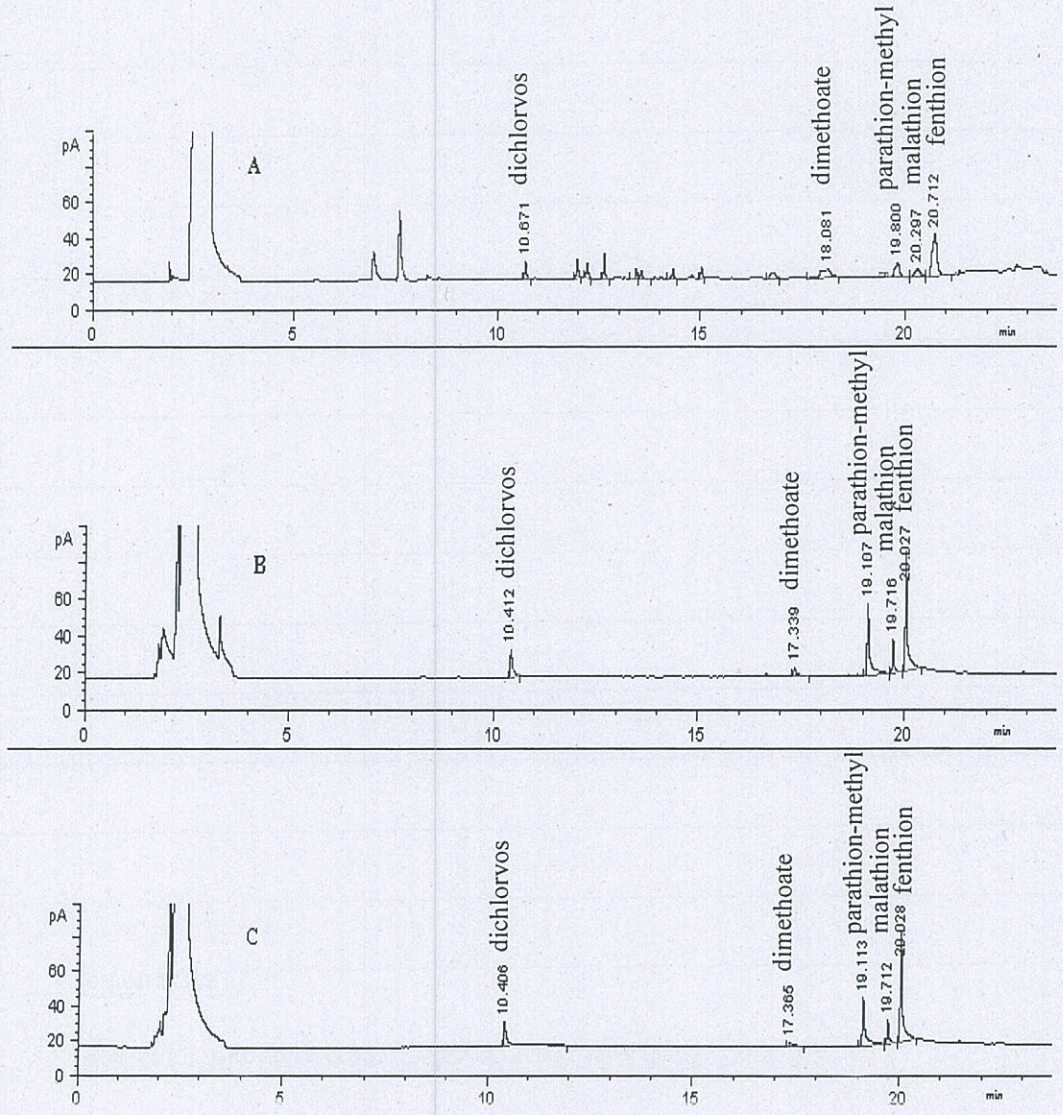

Figure 3 Chromatograms of organophosphorus pesticides from crude palm oil. A= low temperature without cartridge, B= low temperature with solid phase extraction clean up (Florisil plus C18 (6ml) cartridge) and C= standard of five organophosphorus pesticides

Table 2 Mean (n=2) recovery value of 5 OPs from 2 grams of crude palm oil spiked at different level

Pesticides	Recovery % mean (n=2) ± SD	
	I	II
Dichlorvos	77.95 ± 0.01	78.21 ± 0.13
Dimethoate	116.61 ± 0.13	80.73 ± 0.10
Parathio-methyl	77.78 ± 0.02	76.56 ± 0.21
Malathion	71.80 ± 0.01	82.69 ± 0.14
Fenthion	62.55 ± 0.02	73.75 ± 0.14

Table 2 presents the results for the recovery experiment on five OPs obtained with the system described (consisting of a low temperature Florisil connected to a 6 ml C18 cartridge in series). The recovery was studied at spiking levels ranging from 1(level I) – 3(level II) mg kg^{-1}. Data for the recovery experiments are the mean plus the standard deviation for duplicates carried out using 2 grams of spiked crude palm oil. All five OPs investigated were recovered with values ranging from 63-117%.

4. CONCLUSION

Acetonitrile was used for extraction of five OPs from crude palm oil. Low temperature (-15 °C) was used for lipid precipitation and separation. Solid phase extraction on a Florisil coupled 6-ml c18 cartridge was used for clean up of the oil co-extracted.

The development sample preparation overcomes for the amount of oil co-extracted removal and the minimum requirement for reagent. The addition clean up on the tandem column does not significantly influence the recoveries of five OPs and improves chromatographic performance by minimizing matrix effect.

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