A RAPID AND COST EFFECTIVE ULTRASONIC SOLVENT EXTRACTION METHOD FOR DETERMINATION OF λ-CYHALOTHRIN AND CYPERMETHRIN RESIDUES IN SOIL

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ABSTRACT

An effective, simple, and cost effective ultrasonic solvent extraction procedure was developed for the determination of λ-cyhalothrin and cypermethrin residues in soil by gas chromatography with electron capture detector (GC-ECD). Several extraction parameters were optimised with regard to the solvent type, solvent volume and duration of sonication. Under the optimum conditions, recovery studies were performed at five fortification levels (0.01, 0.02, 0.05, 0.1 μg g^{-1}). The recovery level ranged from 93.99% to 101.49% and 90.59% to 99.50% for λ-cyhalothrin and cypermethrin respectively. The relative standard deviation values were less than 4% in all cases. The sensitivitiy of the method was acceptable with limits of detection (LOD) of 0.0025 μg g^{-1} and 0.01 μg g^{-1} for λ-cyhalothrin and cypermethrin, respectively. Finally, the proposed method was applied successfully for residue determination of both λ-cyhalothrin and cypermethrin in soil samples from an oil palm plantation in Labu, Negeri Sembilan, Malaysia.

Keywords: λ-cyhalothrin, cypermethrin, gas chromatography, ultrasonic extraction, soil.

Date received: 5 January 2015; **Sent for revision:** 2 February 2015; **Received in final form:** 3 July 2015; **Accepted:** 28 September 2015.

INTRODUCTION

Pesticides are applied widely to protect plants from diseases, weeds, and insect damage. Consequently their residues usually come into contact with soil (Andreu and Picó, 2004). This is because pesticides can be displaced from their site of application via spray drift, volatilisation, and natural rainfall. As a result, a part of the amount applied reaches the target while the remaining part is deposited in the soil, where it is subjected to different processes that will determine the fate of these agrochemicals (Castro *et al.*, 2001). Slow degradation of pesticides in the environment and extensive or inappropriate usage of pesticides by farmers can lead to environmental contamination of water, soil, air, several types of crops and indirectly to humans (Goncalves and Alpendurada, 2005). This occurs because soil is the principle reservoir for pesticides being used in the environment. Therefore, soil becomes a source from which residues can be released to the atmosphere, ground water and living organisms.

The target compounds for analysis in soil and sediment samples have traditionally been highly

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hydrophobic in nature (Goncalves and Alpendurada, 2005). Pyrethroid insecticides such as λ-cyhalothrin and cypermethrin are highly hydrophobic with high octanol-water partition coefficient ($K_{o/w}$) value (4×10^6 for cypermethrin, 1×10^7 for λ-cyhalothrin) and practically insoluble in water (0.01 mg kg^{-1} for cypermethrin, 0.005 mg kg^{-1} for λ-cyhalothrin) (Kidd and James, 1991). The hydrophobicity of these compounds causes strong sorption to soil and sediment particles, which make the compounds less bioavailable (Oudou *et al.*, 2004). These compounds are strongly adsorbed on soil particles that they do not easily leach from the application point due to their low solubility in water and high lipophilicity (Fernandez-Alvarez *et al.*, 2008). For that reason, various extraction and clean-up procedures have been proposed for the removal of pyrethroid insecticides from soil matrix. These procedures include ultrasonic solvent extraction (USE) with various types of solvents (Castro *et al.*, 2001; Fenoll *et al.*, 2005; Goncalves and Alpendurada, 2005; Gu *et al.*, 2008; Lesueur *et al.*, 2008; Sánchez-Brunete *et al.*, 2004; You and Lydy, 2007; You *et al.*, 2004), differential pulse voltammetry (DPV) (Oudou *et al.*, 2004), headspace solid-phase microextraction (HS-SPME) (Fernandez-Alvarez *et al.*, 2008), accelerated solvent extraction (ASE) (Zhang *et al.*, 2008), and homogenous liquid-liquid extraction (HLLE) (Wang *et al.*, 2008).

Currently, an extraction procedure based on sonication technique is vastly applied for pyrethroid extraction in soil and sediment samples (Castro *et al.*, 2001; Fenoll *et al.*, 2005; Goncalves and Alpendurada, 2005; Gu *et al.*, 2008; Lesueur *et al.*, 2008; Sánchez-Brunete *et al.*, 2004; You and Lydy, 2007; You *et al.*, 2004). This technique was first introduced for pesticides extraction in soil by Johnsen *et al.* in 1972. Sonication is one of the popular techniques as its provides a more efficient contact between the solid and the extraction solvent. This good contact results in a greater recovery of the analyte (Babić *et al.*, 1998). In terms of cost, this technique is much cheaper since no specialised laboratory equipment is required compared to other extraction techniques such as DPV (Oudou *et al.*, 2004), SPME (Fernandez-Alvarez *et al.*, 2008), and ASE (Zhang *et al.*, 2008) which require specialised and expensive equipment. The disadvantage of this technique is that it is not easily automated as it involves manual steps of filtration using either filter paper or membrane filter.

Versatility of sonication technique is shown in method development of pesticides in soil samples. This is due to the possibility of selecting and optimising the solvent type or solvent mixture that allows the maximum extraction efficiency and selectivity. Solvent selection is the first step that an analyst usually has to engage in before moving to the next step of optimisation in sample preparation. Generally, selection of solvent depends on polarity properties of both the solvent and the analytes of interest. In this case, the extraction solvent should have polarity properties compatible with both cypermethrin and λ-cyhalothrin. To date, various solvents or combination of solvents have been investigated and applied for extraction of pyrethroids in soil matrix using USE technique such as ethyl acetate (Castro *et al.*, 2001; Goncalves and Alpendurada, 2005; Sánchez-Brunete *et al.*, 2004), petroleum ether: acetone (Gu *et al.*, 2008), acetone: dichloromethane (You and Lydy, 2007; You *et al.*, 2004), and acetonitrile: water (Fenoll *et al.*, 2005; Lesueur *et al.*, 2008).

This article presents a simple, efficient and cost saving method to determine λ-cyhalothrin and cypermethrin in soil based on USE without the need of a clean-up step. The effects of different extraction parameters that could contribute to the extraction efficiency such as solvent type, amount of solvent and duration of sonication were studied and optimised. The developed method was then applied to soil samples obtained from Labu oil palm plantation for monitoring study purposes.

MATERIALS AND METHODS

Chemicals and Materials

High performance liquid chromatography (HPLC) grade acetone, acetonitrile, and ethyl acetate were obtained from Merck (Darmstadt, Germany). Pesticide standards for cypermethrin and λ-cyhalothrin with >97% purity, were purchased from Dr Ehrenstorfer (Augsburg, Germany). Reagent grade anhydrous $MgSO_4$ was obtained from Supelco Inc. (Bellefonte, PA, USA). Soil extracts were filtered using filter paper and syringe filter (nylon, 0.45 μm) and both were purchased from Whatman (Maidstone, Kent, United Kingdom).

Apparatus and Glassware

Vortex mixer used in the sample extraction and partition step, was obtained from Barnstead/Thermolyne Inc. (Dubuque, IA, USA). Ultrasonicator used in samples extraction step was obtained from Branson 5510 (Danbury, CT, USA), and N-Evap nitrogen evaporator for sample concentration was obtained from Organomation Associates Inc. (South Berlin, MA, USA).

Microlitre pipettes, adjustable between 100 to 1000 μl, and pipettes tips (Eppendorf, Hamburg, Germany) and microvials (Agilent, Palo Alto, CA,USA).

Instrumentation

Sample extracts were analysed on an Agilent Model 6890 series gas chromatograph equipped with a 7683 auto-sampler, split/splitless injector, and an ECD operated at 280ºC (Agilent Technologies). The injection mode was splitless and operated at 250ºC

and the injection volume was 2.0 μl. A DB-608 column (30 m x 0.25 mm i.d. x 0.25 μm film thickness, Agilent Technologies) was used to separate the analytes. Nitrogen was used as carrier and make-up gas, with the flow rate for carrier gas and make-up gas at 1.2 ml min^{-1} and 60 ml min^{-1} respectively. The initial temperature was 150°C, with initial time of 2 min. The oven was heated to 250°C at 20°C min^{-1} and then held at that temperature for 25 min. Chemstation software was used for instrument control and data analysis. Calibration curve was carried out using seven external standards at concentrations of 0.01, 0.02, 0.05, 0.08, 0.10, 0.50, and 1.00 $\mu g\ g^{-1}$.

Preparation of Stock Standard Solution

Individual stock standard solutions of each pesticide were prepared in acetone at concentration of 2000 $\mu g\ g^{-1}$ by dissolving 0.1 g of cypermethrin or λ-cyhalothrin in 50 ml acetone and refrigerated at -20°C in amber glass-stoppered bottles in the dark. Intermediate working standard solutions were prepared by dilution of the stock solutions in acetone to give pesticide standards of 100 $\mu g\ g^{-1}$ and 10 $\mu g\ g^{-1}$. Serial dilutions of the working standard solutions were performed to give seven calibration solutions (0.01, 0.02, 0.05, 0.08, 0.1, 0.5, 1 $\mu g\ g^{-1}$) in acetone. All the standard solutions were stored in scintillation vials at 4°C in the refrigerator.

Preparation and Fortification of Control Sample

Blank soil samples used as control, were obtained from MPOB-UKM Research Station, Selangor, Malaysia and free from both λ-cyhalothrin and cypermethrin residues. Top soil samples (0-15 cm) were collected at various locations where no oil palm were planted. This soil samples were separated from large debris (*e.g.*, gravel, woody materials). These soil samples were air dried and passed through 2 mm sieve. The samples were then homogenised and then stored in glass bottles and kept in a cold room at 5°C.

Recoveries of cypermethrin and λ-cyhalothrin from control samples were determined at fortification levels of 0.01, 0.02, 0.05, and 0.1$\mu g\ g^{-1}$. For each fortification levels, the solution was prepared by measuring an appropriate amount of pyrethroid reference standard into a known quantity of acetone solution. Then, 1.0 ml of the fortification solution was evenly pipetted into a 100 ml beaker containing 20 g of the soil sample. After homogenisation for 5 min using the vortex mixer, the fortified samples were allowed to stand for 30 min prior to extraction of pesticides from the soil samples.

Field Soil Samples for Monitoring Study

Soil samples were collected from the top layer (0-20 cm) from New Labu Plantation (Sime Darby Plantation) in Labu, Negeri Sembilan, Malaysia. This plantation regularly used pyrethroid group of pesticide including cypermethrin and λ-cyhalothrin to control insect pests. In this case, soil samples were randomly collected from three different points, 1 m away around each oil palm. The samples were then sieved (2 mm), homogenised using cone and quarter technique, and finally stored at -18°C before being analysed using the optimised USE method.

Analytical Procedures

In the method development, USE method was chosen as the extraction technique for both cypermethrin and λ-cyhalothrin in soil. Initial tests were carried out to optimise the extraction procedure. In this study, optimisation was divided into three experiments. Extraction parameters such as extraction solvent (acetonitrile, ethyl acetate, and acetone), solvent volume (15, 20, and 25 ml), and USE time (5, 10, 15, and 20 min) were comprehensively investigated and optimised. In general, homogenised soil (20 g) was weighed onto 100 ml beaker. Then, solvent was added and the sample was extracted by ultrasonic extraction. After the extraction period, the extract was decanted and filtered through a piece of Whatman filter paper (filled with approximately 1 g of anhydrous $MgSO_4$) into a 20 ml scintillation vial. The extract was then filtered again through a nylon syringe filter to remove additional fine soil particles to obtain a clear solution. An aliquot (equal to 20% of the original volume) was taken out into scintillation vial and evaporated to dryness using N-evaporator. The extract was then finally reconstituted with 1 ml of acetone and ready for GC analysis.

Selection of Solvent

In the first set of experiments, the extraction efficiencies of various organic solvents were compared: acetone, acetonitrile, and ethyl acetate. Homogenous soil sample, 20 g was spiked with a mixture of working standard solution to achieve final concentrations of approximately 0.02 and 0.1 $\mu g\ g^{-1}$. The spiked soil was extracted with 20 ml of extraction solvents using ultrasonic extraction for 20 min.

Selection of Solvent Volume

In the second set of experiments, the optimisation of solvent volume was carried out by using acetonitrile. For that, 20 g of a homogenous soil sample was spiked with a working standard solution to achieve a final concentration of approximately 0.05 $\mu g\ g^{-1}$. The spiked soil sample was then extracted with 15, 20, and 25 ml of acetonitrile by ultrasonic extraction for 20 min.

Selection of Optimum Duration of Sonication

In the final set of experiments, an optimum duration of sonication was investigated using 20 ml of acetonitrile. In order to achieve this, 20 g of a homogenous soil sample was spiked with a working standard solution to achieve a final concentration of approximately 0.05 $\mu g\ g^{-1}$. The spiked soil was extracted with 20 ml of acetonitrile by ultrasonic extraction for 5, 10, 15 and 20 min.

Quantification and Method Validation

In order to construct the calibration curve, seven working standard solutions (0.01, 0.02, 0.05, 0.08, 0.1, 0.5, 1 $\mu g\ g^{-1}$) were analysed using GC-ECD. The signal for each pesticide was measured for its peak area and an individual calibration plot for cypermethrin and λ-cyhalothrin was constructed. The linearity of the signals from the instrument was studied during the construction of the calibration curve. Since the gas chromatographic response for cypermethrin is known to be matrix dependent, quantification was also carried out by using standards in non-spiked residue free soil extracts obtained using a similar sample preparation method.

The accuracy and precision of the method were expressed in terms of recovery and RSD values in four replicate experiments. The specificity of the proposed method was assessed by analysing blank soil samples while the limits of detection (LOD) and limits of quantification (LOQ) of the proposed method were determined by considering a value of three and 10 times of the background noise obtained from blank samples.

RESULTS AND DISCUSSION

Optimisation of Ultrasonic Extraction

In this study, acetonitrile was selected as the extraction solvent for determination of λ-cyhalothrin and cypermethrin residues in soil. At first, acetonitrile, acetone, and ethyl acetate were the solvents considered for optimisation. Water, petroleum ether, and n-hexane were not considered because they represent the most extreme polar and non-polar solvents and hence the possibility of matrix co-extractives of very polar and very non-polar interferences from the soil. According to Vagi *et al*. (2007), dichloromethane minimised the influence of matrix co-extractives on the response of analytes. They reported that the extracts obtained with dichloromethane were cleaner, in comparison with ethyl acetate. For that reason they chose it for USE of organochlorine pesticides from marine sediments, providing a rapid extraction procedure without a clean-up step. Nevertheless, dichloromethane was not considered in this work due to its toxicity particularly related to central nervous system (Sánchez-Brunete *et al*., 2004). Furthermore, high volatility of dichloromethane makes it an acute inhalation hazard (Rioux and Myers, 1988).

Previous studies mostly used mid-polar solvents such as acetonitrile, acetone, and ethyl acetate as extraction solvents for pyrethroid residues in soil (Castro *et al*., 2001; Fenoll *et al*., 2005; Goncalves and Alpendurada, 2005; Gu *et al*., 2008; Lesueur *et al*., 2008; Sánchez-Brunete *et al*., 2004; You and Lydy, 2007; You *et al*., 2004). Their mid-polarity properties would minimise the co-extractives interferences in the chromatograms and balance the extraction between the analytes and interferences. *Table 1* summarises the recovery results of cypermethrin and λ-cyhalothrin obtained with acetonitrile, acetone, and ethyl acetate. From the ANOVA test, there was no significant difference among the mean recoveries at 95% confidence level and hence, the three extraction solvents gave equivalent results. Furthermore, the results showed that all extraction solvents gave satisfactory recoveries (70%-120%) except for acetone which gave recoveries of more than 120%. In the present study, acetone was discarded from the method development due to the presence of a high amount of matrix co-extractives in the final extracts. Our results regarding the final extract solutions were in agreement with other studies using acetone as extraction solvent (Vagi *et al*., 2007). Our finding showed that the organic extracts obtained by USE with acetone were light yellow coloured even after the filtration steps. Dirty extracts with even a small

TABLE 1. RECOVERY OF PYRETHROIDS OBTAINED BY USE (20 ml, 20 min) WITH VARIOUS SOLVENTS (n=3)

Analyte	0.02 $\mu g\ g^{-1}$						0.1 $\mu g\ g^{-1}$					
	Acetonitrile		Acetone		Ethyl acetate		Acetonitrile		Acetone		Ethyl acetate	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
λ-Cyhalothrin	103.15	2.79	125.65	10.19	102.51	9.05	95.72	2.01	109.60	4.77	96.94	4.18
Cypermethrin	104.19	6.04	122.52	14.39	107.11	15.51	104.71	3.84	118.37	6.26	107.81	6.25

Note: RSD - relative standard deviation.

amount of co-extractives may decrease the column activity and harm the detectors and hence affect the precision of measured data. To overcome this problem, an extraneous sample clean-up step prior to GC analysis is required and would result in the increase of solvents and materials used. Furthermore, acetone extracts also showed the highest matrix-induced response enhancement effect compared to acetonitrile and ethyl acetate. This effect can be seen from the high recovery values (> 120%) observed for acetone compared to acetonitrile and ethyl acetate.

Both acetonitrile and ethyl acetate performed acceptable extraction efficiencies as extraction solvents for USE of λ-cyhalothrin and cypermethrin from soil matrix. Our results obtained for both solvents were supported with earlier study by Goncalves *et al.* (2005). Their findings revealed that ethyl acetate gave the best recoveries while acetonitrile showed good properties as extraction solvent. In our study, both solvents gave almost similar recoveries for λ-cyhalothrin and cypermethrin as shown in *Table 1*. In addition, both solvents produced clear final extract solutions after filtration with no requirement for a clean-up step. Nevertheless, in this study acetonitrile showed better precision when compared to ethyl acetate for both analytes of interest. The comparison of different extraction solvents for USE showed that acetonitrile gave the best precision, the least matrix-enhancement effect, acceptable recoveries, and clear extracts. For these reasons, acetonitrile was selected as the extraction solvent in further optimisation experiments.

In the second experiment, different volumes of acetonitrile were investigated in order to optimise the extraction efficiency with minimum solvent consumption for USE. The 15 ml of acetonitrile was selected as the optimum volume for determination of λ-cyhalothrin and cypermethrin residues in soil. Before that, sample size must be taken into consideration to obtain more reliable data. Previous studies for pyrethroid analysis in soil sample used either 5 g (Castro *et al.*, 2001; Fenoll *et al.*, 2005; Goncalves and Alpendurada, 2005; Sánchez-Brunete *et al.*, 2004) or 20 g (Lesueur *et al.*, 2008; You *et al.*, 2004; You and Lydy, 2007) as the initial soil sample size. Increasing the amount of sample size would result in the larger extraction volume needed in USE. However, 20 g of sample was selected as the sample size in this research so that it could act as a more representative size of the bulk samples compared to 5 g of sample size.

Table 2 summarises the recovery results of cypermethrin and λ-cyhalothrin which were obtained using three different volumes (15, 20, and 25 ml) of acetonitrile as the extraction solvent. From the analysis of variance (ANOVA) test, it was concluded that all the extraction volumes gave equivalent results at 95% confidence level and the increase of the solvent volume had no effect on the extraction efficiency using ultrasonic solvent extraction method. It was found that 15 ml of acetonitrile was enough to extract the pesticides with the recoveries of more than 100%. It has been established that, the more extensive the extraction procedure is, the more co-extracted interference can be expected. This will, increase the probability of matrix-enhancement effect, and consequently harm the ECD detector, not to mention the additional waste of the solvents discharged to the environment. Volumes of solvent less than 15 ml was not always sufficient to allow acceptable removal of the required aliquot from the mixtures since some of the solvents will be absorbed by the soil matrices. Therefore, 15 ml acetonitrile was selected as the optimum volume in further optimisation experiments in order to reduce the solvent consumption and the cost of the overall procedure.

TABLE 2. EFFECT OF ACETONITRILE VOLUME ON THE RECOVERIES OF λ-CYHALOTHRIN AND CYPERMETHRIN FORTIFIED AT 0.05 μg g^{-1} (n=4)

Analyte recoveries (%)	Acetonitrile volume (ml)		
	15	20	25
λ-Cyhalothrin	103	107	106
Cypermethrin	109	112	111

In the third optimisation experiment, different durations of sonication in USE were investigated in order to optimise the extraction efficiency with minimum time consumption. The 5 min was found to be the optimum time for USE method in determining λ-cyhalothrin and cypermethrin residues in soil. Previously, conventional methods such as Soxhlet and shake-flask normally require approximately 6 to 8 hr in order to extract the pesticides from the soil. To do this, bulky glassware or orbital shaker were needed and operated at certain duration of time (6 hr minimum). As a result, the amount of analysis per day was very minimal, time-consuming, labour intensive, less efficient and cannot meet current demand for analyses. Nowadays, modern methods have been proposed to save time and reduce solvent consumption as alternative to conventional methods (Berset *et al.*, 1999). Currently, most extraction method will not take more than 2 hr, any extraction method that takes a longer time is considered inefficient and impractical.

From the ANOVA test, it was concluded that there was no significant difference among the mean recoveries for both insecticides at 95% confidence level. Hence, the four sonication time gave equivalent results and the increase of the sonication time from 5 min to 20 min exhibited no substantial impact on the extraction efficiency. The effect of sonication time on the extraction of λ-cyhalothrin and cypermethrin can be seen in *Figure 1*. From *Figure 1*, it was found that the recoveries for both pesticides were quite

consistent although the sonication time varied from 5 to 20 min. Moreover, our results revealed the acceptable range of recoveries (70%-120%) for all the duration of sonication studied. However, a longer extraction time could result in the possibility of degradation of some analytes when using USE (Vagi *et al.*, 2007). Taking into consideration the above aspect, USE time was not evaluated at more than 20 min and optimum sonication time that gave efficient extraction and high recoveries was selected as 5 min. In this study, considering the basis that the recoveries of both pesticides obtained from one step extraction (15 ml of acetonitrile and 5 min of sonication) ranged from 104% to 117%, no repeated extraction cycle was required.

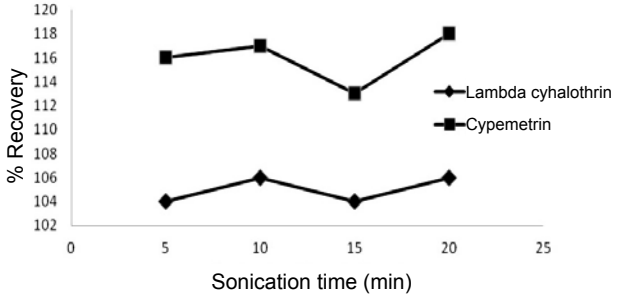

Figure 1. Effect of sonication time on the recoveries of λ-cyhalothrin and cypermethrin fortified at 0.05 μg g^{-1} (n=4).

Table 3 gives a summary of analytical methods used for quantification of λ-cyhalothrin and cypermethrin in soil. The table indicates that more than half of the current analytical methods used to extract pyrethroid insecticides involved ultrasonic solvent extraction, either with clean-up step (Gu *et al.*, 2008; You and Lydy, 2007; You *et al.*, 2004) or without clean-up step (Castro *et al.*, 2001; Fenoll *et al.*, 2005; Goncalves and Alpendurada, 2005; Lesueur *et al.*, 2008; Sánchez-Brunete *et al.*, 2004). From the results of this study, we propose a novel USE method that is rapid, with 5 min extraction time when compared to other techniques, thus facilitating a higher throughput of batches of extracted samples per day. This work utilised a single extraction cycle whereas in other studies (Sánchez-Brunete *et al.*, 2004; You and Lydy, 2007; You *et al.*, 2004; Castro *et al.*, 2001; Goncalves and Alpendurada, 2005; Gu *et al.*, 2008), the sonication extraction step was repeated on the soil matrix using fresh solvent. Repeated extraction was avoided in this study since it could contribute to errors in the analysis due to the increasing number of sample preparation steps.

TABLE 3. METHODS FOR THE ANALYSIS OF PYRETHROIDS IN SOIL

Sample size (g)	Extraction				Clean-up	Determination	Reference
	Technique	Time (min)	Solvent	Solvent volume (ml)			
10	Soxhlet extraction	480	*n*-hexane	125	-	Differential pulse voltammetry	(Oudou *et al.*, 2004)
0.5	Headspace solid-phase microextraction	30	Water	0.5	-	GC-μECD	(Fernandez-Alvarez *et al.*, 2008)
5	Accelerated solvent extraction	10	Acetonitrile	40	SPE (Florisil)	GC-ECD	(Zhang *et al.*, 2008)
4	Homogenous liquid-liquid extraction	30	Acetone	10	-	GC-ECD	(Wang *et al.*, 2008)
5	Ultrasonic extraction	15 (x2)	Ethyl acetate	4 (x2)	-	GC-ECD	(Castro *et al.*, 2001)
5	Ultrasonic extraction	15 (x3)	Ethyl acetate	5 (x3)	-	GC-MS	(Goncalves and Alpendurada, 2005)
50	Ultrasonic extraction	30 (x3)	Petroleum ether:acetone	50 (x3)	SPE (Florisil)	GC-ECD	(Gu *et al.*, 2008)
20	Ultrasonic extraction	5 (x3)	Acetone:ethylene chloride	50 (x3)	SPE (Florisil)	GC-ECD	(You *et al.*, 2004)
20	Ultrasonic extraction	5 (x3)	Acetone:ethylene chloride	50 (x3)	SPE (Florisil)	GC-ECD	(You and Lydy, 2007)
5	Ultrasonic extraction	15 (x2)	Ethyl acetate	4 (x2)	-	GC-MS	(Sánchez-Brunete *et al.*, 2004)
5	Ultrasonic extraction	15	Acetonitrile:water	30	-	GC-NPD	(Fenoll *et al.*, 2005)
20	Ultrasonic extraction	2	Acetonitrile:water	60	-	GC-MS and LC-IT-MS	(Lesueur *et al.*, 2008)
20	Proposed method	5	Acetonitrile	15	-	GC-ECD	-

These additional steps, will reduce the sample throughput and may adversely affect the accuracy of the method. From the evaluation of method performance using 15 ml of acetonitrile as extractant in USE for 5 min, it was concluded that these conditions exhibited excellent extraction capabilities, rapid analysis, environmental-friendly, efficient, less time and solvent consuming. Therefore, no further optimisation would be required particularly for the clean-up step in the sample preparation procedure.

Method Validation

Good linearity was obtained for both λ-cyhalothrin and cypermethrin calibration curves with regression coefficients of more than 0.99, indicating that the technique is quantitative for both pesticides. The selectivity of the analytical method in this work was determined by comparing the chromatograms of a blank matrix solution with the fortified matrix solution. *Figure 2* shows the chromatograms of pesticide standard solutions, blank soil samples, and fortified soil samples by GC-ECD. In the blank samples of soil, few interferences were present in the matrices at the analytes retention times of 15.0 min for λ-cyhalothrin and 26.0 min for cypermethrin. As a result, in the fortified samples, we can see that the analytes of interest were well separated from other compounds present in the soil matrix and hence allowed the differentiation and quantification of the analytes.

The GC-MS total ion chromatogram (TIC) for λ-cyhalothrin and cypermethrin spike and the mass spectrum of detected λ-cyhalothrin and cypermethrin from MS library are shown in *Figures 3* and *4*. The mass spectrum of pesticides detected in the pure standard acetone was confirmed by matching with mass spectrum from the MS library.

This indicates that the method developed has successfully removed most of the interferences in soil matrices and thus exhibited its selectivity. Additionally, from the chromatographic point of view, the method presented herein does not require a clean-up step of the soil extracts, based on this evident from the absence of interfering peaks in the blank (uncontaminated) samples. Nevertheless, a few interference peaks were present, but they somehow did not hinder the identification and quantification of analytes.

The LOD values of the proposed method were determined by analysing the decreasing concentrations of analytes spiked on soil until reaching a signal-to-noise (S/N) ratio of 3 while LOQ were derived from LOD values to give S/N of 10. The LOD for the proposed method was found to be 0.0025 $\mu g\ g^{-1}$ for λ-cyhalothrin and 0.01 $\mu g\ g^{-1}$ for cypermethrin. The LOQ values were 0.0075 $\mu g\ g^{-1}$ and 0.03 $\mu g\ g^{-1}$ for λ-cyhalothrin and cypermethrin respectively. Recoveries obtained for both pesticides ranged from 93.99% to 101.49% for λ-cyhalothrin and 90.59% to 99.50% for cypermethrin (*Table 4*). The overall RSD values were less than 4% in all cases.

Soil Sample Analysis for Monitoring Study

The developed method was applied to soil samples collected from an oil palm plantation in Labu, Negeri Sembilan, Malaysia. The main objective

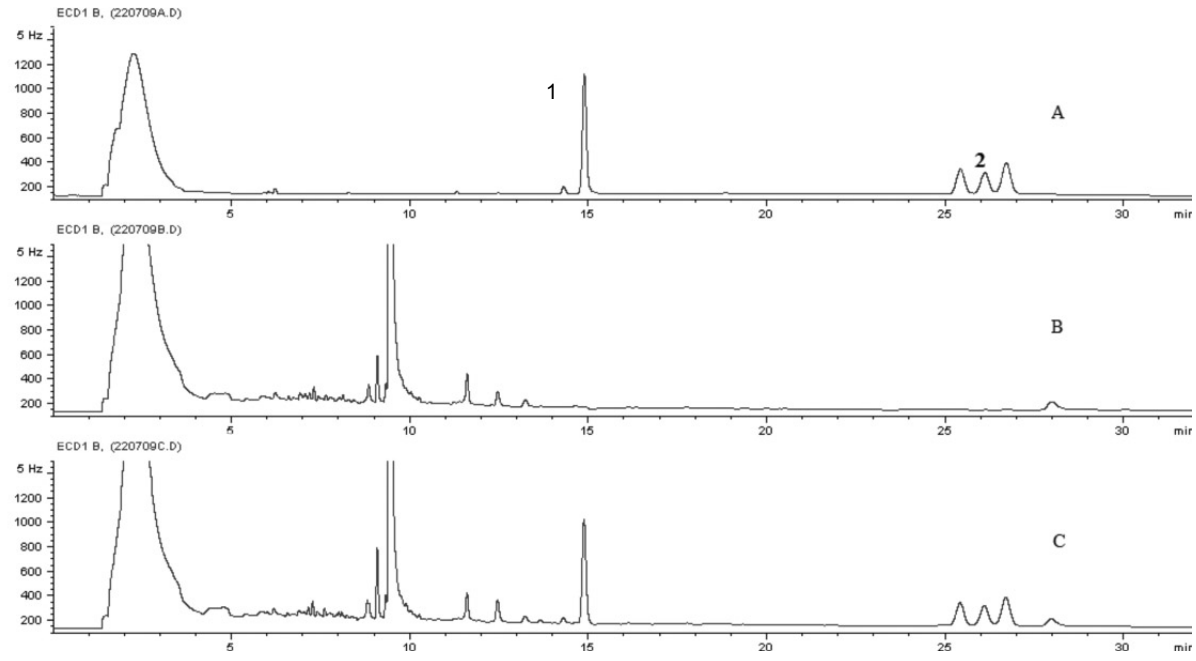

Figure 2. Gas chromatograms of: (A) pesticide standards in pure acetone (0.1μg g^{-1}) (1. Lambda cyhalothrin, 2. Cypermethrin), (B) blank soil sample (0.1 μg g^{-1}) and (C) blank spiked soil sample.

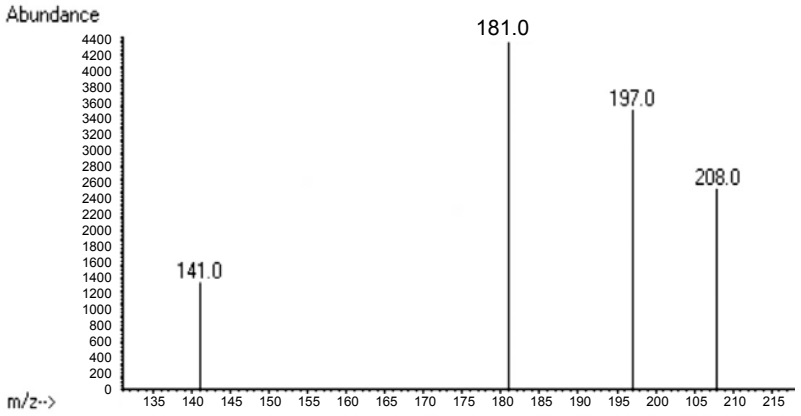

Figure 3. Mass spectra of λ-cyhalothrin.

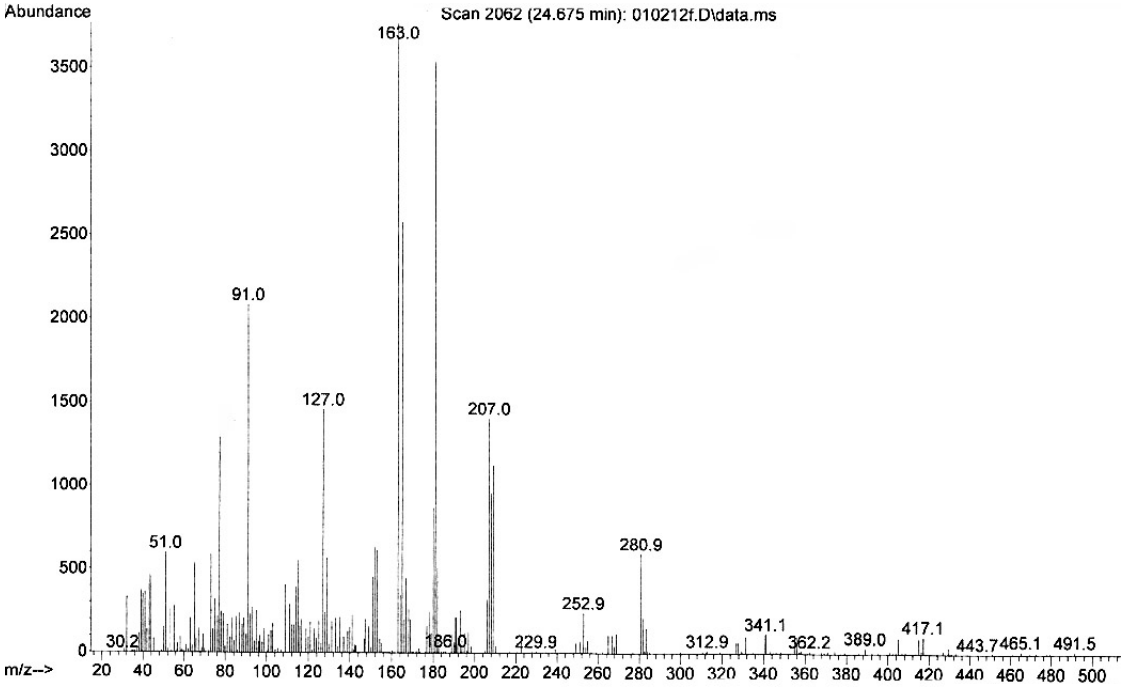

Figure 4. Mass spectra of cypermethrin.

TABLE 4. PERCENT RECOVERY OF λ-CYHALOTHRIN AND CYPERMETHRIN IN SOIL SAMPLES USING CALIBRATION STANDARDS IN PURE SOLVENT AND MATRIX-MATCHED STANDARD (n=4)

Pesticides	Spike level ($\mu g\ g^{-1}$)	Standard in acetone		Matrix-matched standard	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
λ-Cyhalothrin	0.01	107.51	3.59	101.49	3.95
	0.02	109.16	2.18	93.99	2.19
	0.05	98.37	0.66	100.92	0.67
	0.1	108.17	2.57	100.15	2.57
Cypermethin	0.01	104.01	2.86	99.45	2.86
	0.02	117.83	2.50	90.59	2.50
	0.05	101.94	0.46	98.36	0.45
	0.1	134.63	1.70	99.50	1.70

Note: RSD - relative standard deviation.

was to identify and quantify λ-cyhalothrin and cypermethrin in that particular soil. From the study, it was found that λ-cyhalothrin and cypermethrin residues were not detected in the analysed soil samples.

CONCLUSION

The major advantage of ultrasonication is the speed of extraction and the elimination of clean-up stage involving additional chemicals and apparatus. This method gives a good extraction efficiency, precision, and recovery of both λ-cyhalothrin and cypermethrin, combined with fast and simple sample preparation procedures. Additionally, the method proposed does not require any clean-up step since in the chromatogram there were no interfering peaks in the blank samples at the analytes retention time. The method also involves a low solvent consumption and it therefore reduces the risk to human health and the environment. Furthermore, it is an improvement in comparison to other USE methods. This method, although not automated, allows simultaneous extraction of up to eight samples thus making it an ideal technique for laboratories having a large number of samples for these particular analyses.

ACKNOWLEDGMENT

The authors would like to thank MPOB and the Universiti Malaya (PGO47-2013B) for financially supporting this work, and the Director-General of MPOB for permission to publish this article. The authors are also highly indebted to Rosilawati Yusoff and Rosliza Razak for their excellent technical assistance.

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