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Low temperature followed by matrix solid-phase dispersion-sonication procedure for the determination of multiclass pesticides in palm oil using LC-TOF-MS

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ABSTRACT

A simple and effective multiresidue method based on precipitation at low temperature followed by matrix solid-phase dispersion-sonication was developed and validated to determine dimethoate, malathion, carbaryl, simazine, terbuthylazine, atrazine and diuron in palm oil using liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS). Liquid–liquid extraction (LLE) followed by low temperature method were optimized by studying the effect of type and volume of organic solvent (acetonitrile, acetonitrile:n-hexane (3:2 v/v) and acetone) and time of freezing to obtain high recovery yield and low co-extract fat residue in the final extract. The optimal conditions for matrix solid-phase dispersion (MSPD) were obtained using 5 g of palm oil, 2 g of primary secondary amine (PSA) as dispersing sorbent, 1 g of graphitized carbon black (GCB) as clean-up sorbent and 15 mL of acetonitrile as eluting solvent under conditions of 15 min ultrasonication at room temperature. Method validation was performed in order to study sensitivity, linearity, precision, and accuracy. Average recoveries at three concentration levels (25, 50 and 100 $\mu g\,kg^{-1}$) were found in the range of 72.6–91.3% with relative standard deviations between 5.3% and 14.2%. Detection and quantification limits ranged from 1.5 to 5 $\mu g\,kg^{-1}$ and from 2.5 to 9 $\mu g\,kg^{-1}$, respectively.

1. Introduction

Malaysia is not only one of the leading countries in exporting palm (*Elaeis guineensis*) fruit, but also is the largest exporter of palm oil in the world. According to the World Bank and the Asian Development Bank, Malaysia is the world's second largest palm oil producer [1]. Palm oil is derived from the flesh of the palm fruit (mesocarp), while palm kernel oil is derived from the seed or kernel of the fruit. The palm oil obtained from the mesocarp of the palm fruit is widely used in various food products, such as margarines, shortenings, cooking oils, confectionery fats, and vanaspati without or with only minimal modification of palm oil composition, as well as in non food products such as oleochemicals, soaps, and biodiesel.

Palm trees are attacked by a variety of pests and unwanted plants that could reduce the quality and quantity of the palm fruits used to produce palm oil. In this respect, pesticides have played an important role in controlling and preventing these pests and harmful plants and hence, indirectly increasing the palm oil production. On the other hand, the excessive use of pesticides in agricultural activities has adverse effects towards human and other living organisms. Even when applied in accordance with Good Agricultural Practices (GAP), they can leave residues, which can be detrimental to food safety [2]. Therefore, there is a need of highly sensitive and selective analytical procedures to detect pesticide residues. The Codex Alimentarius Committee on Pesticide residues and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) have established maximum pesticide residue limits for some of the pesticides in palms destined for oil production [3]. However, it should be noted that there are no harmonized MRLs established for pesticide residues in palm oil yet. But the National Committee on Agricultural Commodity and Food Standards issued a Notification entitled the Thai Agricultural Standards on Pesticide Residues: Maximum Residue Limits (TAS 9002-2006) for palm oil on 31 July 2006 which was published in the Royal Gazette [4].

Most analytical procedures for the determination of various pesticides are based on gas or liquid chromatography [5,6], but electroanalytical techniques such as differential pulse polarography (DPP) have also been used for the determination and study of several pesticides in different matrixes like water, soils, plants, and food [7,8]. Recently, differential pulse voltametry measurement

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based on carbon nanotubes supported palladium nanoparticles-modified glassy carbon electrode, was successfully applied for the determination of organophosphates [9].

Modern trends in analytical chemistry are towards the simplification and miniaturization of sample preparation methods in order to save time, chemicals and cost of analyses, as well as to increase the sample throughput and improve the quality and sensitivity of the analytical methods, and if possible to hyphenate the different steps of the analytical process [2,10]. High fat food samples such as palm oil represent a particular analytical challenge for pesticide residue analysis due to the inherent complexity of the matrix that may have an adverse affect on the results of analysis [2].

Many multiresidue procedures employing different cleanup techniques and a variety of detection methods have been reported for the determination of pesticide residues in vegetable oils especially olive oil. Among these, the most commonly used methodology is based on gas chromatography (GC) after a comprehensive cleanup step and in most cases based on liquid–liquid partitioning extraction with solvents of different polarities [11] whereas, very few studies have been reported for the analysis of pesticide residues in palm oil. Liquid–liquid partitioning with acetonitrile followed by low temperature clean-up in order to precipitate the lipid has been reported for determination of the herbicides fluroxypyr, chlorpyrifos and organochlorine pesticides in crude palm oil (CPO) and crude palm kernel oil (CPKO) [12–14]. The use of a combination of two or more commercially available solid-phase extraction (SPE) adsorbents has been suggested to obtain high clean-up efficiency recently. In this case, solid-phase extraction (SPE) using dual layer graphitized carbon (GCB)/primary secondary amine (PSA) has been reported for the determination of cypermethrin in crude palm oil by gas chromatography [15].

Matrix solid-phase dispersion (MSPD) is a new solid-phase extraction based (SPE-based) and clean-up technique developed for multiresidue analysis by Barker et al. [16]. MSPD can be carried out simultaneously with sample homogenization, extraction and clean-up and it requires only a small sample size and small amounts of solvent [17–21]. Liquid–liquid extraction using acetonitrile saturated with petroleum ether followed by MSPD using aminopropyl as a dispersant material was reported by Garca-Reyes et al. [22] for the determination of herbicide residues in olive oil. The proposed methodology reported here is focused on the evaluation of a rapid, cheap and efficient sample preparation strategy consisting of low-temperature precipitation followed by MSPD-sonication coupled with LC-TOF-MS for the determination of simazine, atrazine, terbuthylazine, diuron, dimethoate, malathion and carbaryl in palm oil. The pesticides chosen represent different classes of compounds (organophosphates, carbmates, triazines and phenylureas) and chemical uses (insecticides and herbicides). To the best of our knowledge, no application of the MSPD method to the analysis of pesticides in palm oil samples has been published although it is currently being used in analytical laboratories for their analysis. For this purpose, MSPD-sonication method was evaluated in terms of cleanliness of the extracts, efficiency of the extraction (recoveries), analytical performance, matrix effects and sensitivity (limits of detection) for the analysis of multiresidue pesticides in palm oil samples collected from local markets in Kuala Lumpur, Malaysia. In this method, the co-extracted fat in organic phase was reduced by centrifugation and freezing based on the different of mass of palm oil and extracting solvent. Among different adsorbents tested in this study, PSA and GCB were used as a dispersant and clean up sorbents to remove fatty acids and clean-up sorbent respectively. This technique has some advantages such as low organic solvent consumption, low analysis time and analysis cost.

2. Experimental procedures

2.1. Standards, reagents and samples

The pesticide standards simazine, terbuthylazine, atrazin, diuron, dimethoate, malathion and carbaryl were obtained from Fluka (Buchs, Switzerland, HPLC grade 99.9%). Individual pesticide stock solutions at 1.0 mg mL^{-1} were prepared in pure methanol and kept in amber-colored bottles at 4 °C. All standard solutions were left for 2 h at ambient temperature prior to use. Working standard solutions of a mixture of pesticides were prepared by appropriate dilutions in methanol and stored at −20 °C. HPLC grade acetonitrile, n-hexane, acetone and methanol were purchased from Merck (Darmstadt, Germany). Primary secondary amine (PSA) sorbent was from Sigma–Aldrich (Steinheim Loius, MO, USA). Florisil, C_{18} and GCB cartridges, each with a configuration of 500 mg/6 mL was purchased from Alltech Inc. (Alltech Inc, USA). A Milli-Q-Plus ultrapure water system from Millipore (Milford, MA) was used throughout the study. Prior to LC-TOF-MS analysis, the oil samples were filtered through a 0.22 μm filter (Chromatography Research Supplies, Louisville, Kentucky, USA).

Various commercial brands of palm oil were purchased from supermarkets in Kuala Lumpur, Malaysia.

2.2. Apparatus

2.2.1. Ultrasonic system

An ultrasonic water bath (Model: Power Sonic 405, Hwashin Technology, Korea) equipped by a generator with an output of 350 W and input of AC 230 V and 50 kHz was used in the extraction procedure.

2.2.2. LC-TOF-MS

The separation of the selected herbicides was carried out using an HPLC system (consisting of a vacuum degasser, an autosampler, and a binary pump-SL; Agilent Technologies 1200 Series) equipped with a reversed phase C_{18} analytical column of 50 mm × 2.1 mm and 1.8 μm particle size (Zorbax SB-C_{18}). Column temperature was maintained at 40 °C. The injected sample volume was 5 μL. Mobile phases A and B were acetonitrile and water with 0.1% formic acid, respectively. In the optimized chromatographic method, the initial mobile phase composition (10% A) was held constant for 5 min, followed by a linear gradient to 100% A after 30 min. The flow-rate was optimized at 0.25 mL min^{-1}. A 10 min post-run time was used after each analysis. This HPLC system was connected to an Agilent MSD QTOF (Agilent Technologies, 6530 Accurate Mass TOF), equipped with an electrospray interface operating in positive ion, using the following operation parameters: capillary voltage 4000 V; nebulizer pressure 40 psig; drying gas 9 L min^{-1}; gas temperature 300 °C; fragmentor voltage 190 V; skimmer voltage 65 V; octopole RF 750 V. LC/MS accurate mass spectra were recorded across the range 50–1000 *m*/*z*. The instrument performed the accurate-mass internal mass calibration automatically using a dual-nebulizer ion source combined with an automated calibrant delivery system, which introduced the internal.

2.3. Spiking procedure

A representative 200 g portion of palm oil sample was weighted and fortified homogeneously with appropriate volume of working standard solution to reach 25, 50, 100 μg kg^{-1} of the studied pesticides. The mixture was then gently blended in a mortar for 1 h, to asses the homogeneity of the sample. Then the sample was incubated at room temperature for 6 h, to make sure the solvent was completely evaporated. 5 g portion of the spiked sample was used for the extraction procedure described in Section 2.4.

Table 1
Removal efficiency of co-extracted fat from palm oil sample using different organic solvent by a freezing-lipid method.

	Mean value ± RSD%[a] (mg g^{-1})		
	Organic solvent		
	Acetonitrile	MeCN/n-hexane (3:2 v/v)	Acetone
Remaining fat after LLE	4.20 ± 0.35	10.70 ± 1.24	7.80 ± 0.43
Remaining fat after freezing	0.50 ± 0.05	3.90 ± 0.72	2.10 ± 0.06
Fat reduction ratio (%)	88.01	63.50	73.10

[a] $n = 3$.

2.4. LLE followed by low temperature precipitation procedure

5.00 ± 0.01 g homogenous palm oil samples were weighted in 50 mL screw capped centrifuge tubes. LLE was performed using 10 mL different organic solvents (acetonoitrile, acetonitrile/n-hexane (3:2 v/v), acetone) to optimize the efficiency of the pesticides extraction from palm oil for LLE and freezing process. The resulting mixtures were then shaken for 15 min using a vortex mixer and, after centrifugation at 4000 rpm for 3 min the centrifuge tube was horizontally kept in a freezer at −20 °C for 4 h. The organic phase containing the organic solvent and extracted pesticides remained as a liquid and rose to the top whereas the oil were frozen and precipitated to the bottom of the tube. The extracts were evaporated to dryness with rotary evaporator below 40 °C and the remaining fat weighted.

2.5. Matrix solid-phase dispersion-sonication

The procedure described in Section 2.4 was repeated, but this time, after the separation of the solvent and oil, a 7 mL aliquot of the acetonitrile extract, obtained from the freezing step was carefully evaporated up to a final volume of about 2 mL. This remaining extract was gently blended with 2 g of PSA as a dispersing phase in a glass mortar using a glass pestle until a homogenous mixture was obtained. The mixtures were introduced into a 100 mm × 20 mm I.D. glass column containing Whatman No. 1 and 1 g of GCB as a clean-up adsorbent placed at the bottom end of the column. The column was then set in a tube rack and closed with one-way stopcock and extracted with 15 mL of acetonitrile for 15 min at room temperature in an ultrasonic bath. The water level in the bath was adjusted to be at level with the solvent inside the column. After extraction, the columns were set on a vacuum manifold and the analytes were eluted and collected in graduated conical tubes. Elution step was carried out by gravity flow. With the purpose of improving method sensibility and obtain higher responses of the analytes, the extracts were concentrated to dryness, using a gentle stream of nitrogen. The residue was dissolved with 500 μL of acetonitrile/water (1:1 v/v) and then filtered through a 0.22 μm PTFE membrane filter (Millex FG, Millipore, Milford, MA) prior to LC-TOF-MS analysis.

3. Result and discussion

3.1. Low temperature precipitation study

Low temperature precipitation procedure was performed using three different organic solvents (acetonitrile, acetone, acetonitrile/n-hexane (3:2 v/v)) as described in Section 2.4 and the fat residue was weighted. Types of extracting organic solvent, the effect of volumes of solvent and the time of fat precipitation during freezing process on the efficiency of the extraction were evaluated. As can be seen in Table 1, the mean value ($n = 3$) of the remaining fat in the extract after LLE with different extracting solvents, expressed as mg g^{-1} of palm oil extracted was found to be 4.2 ± 0.4 mg g^{-1} using acetonitrile, 7.8 ± 0.43 mg g^{-1} using acetone and 10.7 ± 1.24 mg g^{-1} using acetonitrile/n-hexane (3:2 v/v). The remaining fat in the extracts after freezing step was found to be 0.50 ± 0.05 mg g^{-1} using acetonitrile, 2.1 ± 0.06 mg g^{-1} using acetone and 3.9 ± 0.72 mg g^{-1} using acetonitrile/n-hexane (3:2 v/v) as the extracting solvent. The mass of co-extracted fat in the extract when acetonitrile (MeCN) was used as extracting solvent after LLE and low temperature procedures was reduced to 88.1% whereas the reductions when using acetone and MeCN/n-hexane (3:2 v/v) were found to be 73% and 64% respectively. Therefore, acetonitrile was chosen as the extracting solvent in subsequent experiments. The mass of co-extracted fat after freezing step was considerably reduced. After LLE with acetonirile the extract still contained 82.8% of lipids, although a significant amount of lipid was eliminated by low temperature precipitation method. The results showed that freezing-lipid procedure was able to remove 90.2% of lipid from the matrix. The ratio of the volume of acetonitrile to that of the sample was tested (2:1, 3:1, 4:1). This study also revealed that the best recoveries were obtained by using acetonitrile: palm oil matrix at ratio 2:1 (10 mL MeCN and 5 g palm oil), and any further increased of the volume of acetonitrile did not improve the recovery of the pesticides studied. In evaluation of freezing time, different times in the range of 2–24 h were tested. The minimum time for satisfactory fat removal during low temperature precipitation was found to be 4 h. There was no significant difference in pesticides recovery with an increase in freezing time after 4 h, indicating the method is robust. However freezing time less than 4 h was not sufficient to remove the fat completely.

3.2. Optimization approach of MSPD conditions

The operating conditions for the matrix solid-phase dispersion procedure were evaluated in order to achieve the highest recoveries of selected pesticides from palm oil. Types and quantity of sorbents, and nature and volume of the eluting solvent are known to be key factors in MSPD, since they determine both the efficiency of the extraction and the purity of the final extracts [23]. In this study, two different dispersant sorbents, C_{18} and PSA were used while three clean up adsorbents namely alumina, Florisil and graphitized carbon black (GCB) were tested. MeCN was used as the eluting solvent in order to find the most suitable sorbent material with higher recoveries and lower fat levels transferred in the final extracts. The preliminary assays were performed without sonication-assisted extraction. The obtained mean recoveries and relative standard deviations (RSD%) ($n = 3$) of selected pesticides are shown in Table 2. These levels were selected based on the previous literature about MSPD extractions of pesticides from vegetable oil and food matrices [21,22].

In evaluating the type of dispersing sorbent, the extraction column was prepared with weak anion exchanger sorbent with polar capability such as PSA and palm oil blend which was packed with GCB as the clean up sorbent, produced the colorless extract with minimal interferences and the results were satisfactory for

Table 2
Mean percent recovery (50 μg kg^{-1}) ±RSD (%) (n = 3) of selected pesticides in palm oil samples with different dispersing/clean-up sorbents and influence of sonication assisted coupled with MSPD procedure on the pesticides recovery using MeCN as eluting solvent.

Compound	Recovery (%) (mean ± RSD)							
	C_{18}/Alumina	C_{18}/Florisil	PSA/Alumina	PSA/Florisil	PSA/GCB			
					Without sonication	With sonication		
						5	15	25 (mL)
Dimethoate	7.6 ± 6.4	57.3 ± 8.3	32.5 ± 7.4	41.2 ± 8.7	78.4 ± 9.6	62.3 ± 7.4	91.6 ± 6.4	86.2 ± 10.3
Malathion	17.3 ± 10.7	32.5 ± 12.1	35.8 ± 6.2	58.3 ± 9.2	69.2 ± 7.1	65.7 ± 12.5	84.7 ± 8.1	85.8 ± 7.6
Carbaryl	37.1 ± 9.4	42.6 ± 8.4	41.9 ± 8.5	65.6 ± 8.4	75.2 ± 6.2	51.4 ± 8.3	85.1 ± 10.2	87.5 ± 11.2
Simazine	31.8 ± 5.2	35.9 ± 7.4	128.1 ± 9.3	58.6 ± 11.4	77.6 ± 10.1	57.8 ± 9.4	94.7 ± 4.8	92.6 ± 8.4
Terbuthylazine	19.4 ± 7.8	41.4 ± 9.8	37.3 ± 11.4	73.9 ± 6.1	65.7 ± 7.6	65.2 ± 6.7	87.6 ± 11.5	86.1 ± 9.5
Atrazine	112.4 ± 7.2	47.3 ± 11.5	133.7 ± 7.8	72.4 ± 6.8	72.8 ± 12.1	54.1 ± 11.8	93.1 ± 7.5	92.4 ± 7.3
Diuron	38.5 ± 12.6	52.8 ± 7.2	121.4 ± 12.3	81.3 ± 8.3	73.1 ± 6.9	61.8 ± 6.4	77.5 ± 9.3	91.5 ± 6.2

MSPD column: 5.0 g palm oil + 2.0 g dispersant + 1.0 g clean up sorbent.
Eluation sovent: Acetonitrile (MeCN).

all pesticides studied as shown in Table 2. GCB has a strong affinity for planar molecules, and thus effectively removes pigments such as chlorophyll and carotenoids, as well as sterols present in foods [24]. The use of C_{18} as the solid support in MSPD extraction produced an extracts with maximal interferences for most of the pesticides studied. As can be seen from the results obtained, simultaneous extraction of all the studied compounds was unsuccessful with average recovery of 19.3% when alumina was packed in the base of reversed-phase materials (C_{18}): palm oil blend. In this case, the obtained chromatogram had a large number of other peaks because of co-extracted interfering substance. The extract obtained from the MSPD column including a mixture of PSA/palm oil blend and alumina as clean-up sorbent resulted the chromatogram with higher background and interfering peaks from the palm oil. In these assays, the occurrence of a high recovery of some pesticides could be attributed to the matrix effects and the presence of interfering endogenous compounds that enhance the chromatographic response to pesticides, indicating that perhaps this clean-up sorbent receives and retains more interferences which are subsequently desorbed from the column during the elution. The extraction column prepared with PSA/palm oil blend and Florisil as clean up sorbent, produced the clean extract and consequently the better chromatogram but with lower recoveries. Florisil is magnesium silicate containing 15% MgO and 85% SiO_2 that has often been used for the clean-up of apolar pesticides in fatty matrices due to its potential to retain polar matrix components such as lipids [25]. However, as can be seen in Table 2, the use of Florisil as a clean-up sorbent resulted in low recoveries for dimethoate, malathion and simazine.

Overall results indicate that the best results were obtained using 5 g of palm oil, 2 g of PSA as dispersion phase, 1 g of GCB as clean-up sorbent and acetonitrile as eluting solvent.

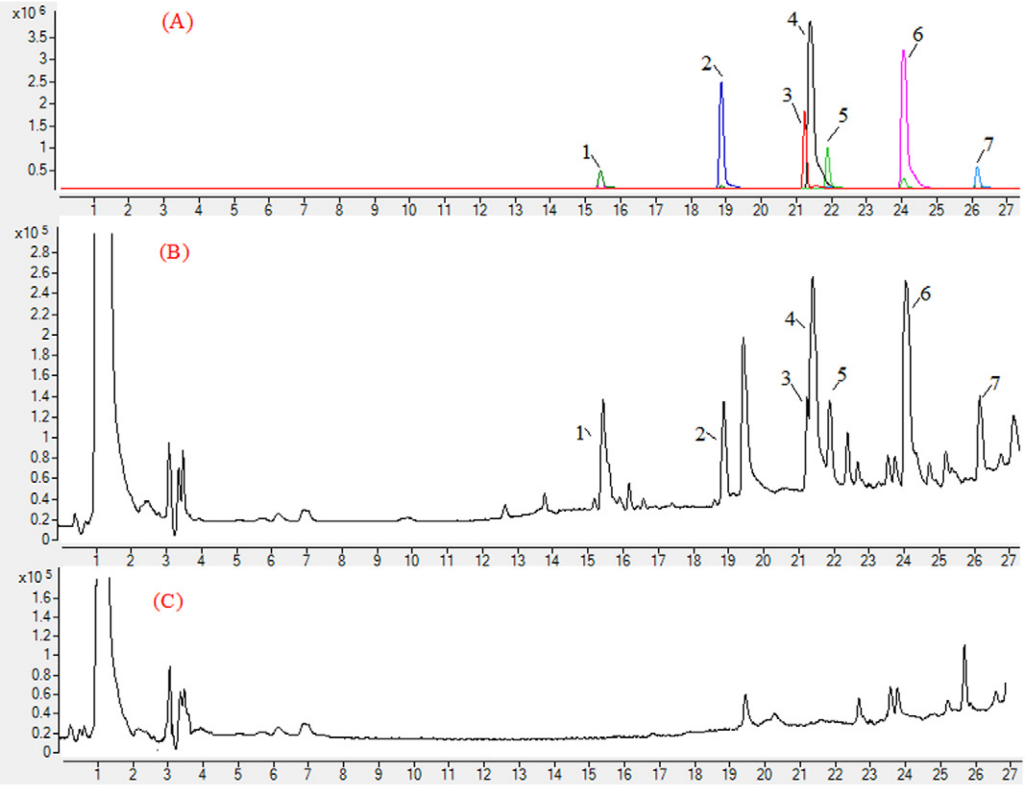

Fig. 1. Typical chromatograms obtained by LC-TOF-MS of: (A) Standard mixture solution of the pesticides in methanol, (B) Spiked palm oil samples with pesticides at 0.025 mg kg^{-1}, (C) Blank palm oil extract samples. Peak identification: (1) dimethoate; (2) simazine; (3) carbaryl; (4) atrazine; (5) diurone; (6) terbuthylazine; (7) malathion.

The extraction conditions in terms of with and without sonication were evaluated. In addition a series of experiments were designed at three volumes of acetonitrile (5, 15, 25 mL). As can be seen in Table 2, 15 mL of acetonitrile and 15 min sonication assisted extraction in small column containing PSA as dispersing phase and GCB as clean up sorbent presented the cleanest extracts for all analytes of interest extracted from the palm oil matrix and they were recovered quantitatively with good reproducibility. According to these results, the elution of pesticides using 5–15 mL of acetonitrile showed a response enhancement ranging from 51.4% to 94.7%. No significant increase in the recoveries was observed when the volume of elution solvent increased. In subsequent experiments, the volume of acetonitrile was set at 15 mL and all the MSPD elution was conducted by vacuum flow.

3.3. *Performance of the analytical procedure*

3.3.1. *Matrix effect and recovery study*

Once the parameters that affect the MSPD procedure were optimized, a method validation process was performed by establishing the basic analytical requirements of the performance to be appropriate for quantitative determination of selected pesticides in palm oil.

The use of matrix-matched standards provides reliable quantitation capabilities for food analyses [26]. Matrix effect was evaluated by comparing the detector response for pesticide standards prepared in methanol with that for standards prepared in palm oil extract.

When standards were prepared by spiking blank palm oil extract samples with known amounts of pesticides, lower peak areas were obtained for the same pesticides concentration. The different responses obtained from pesticide standard solutions and palm oil-matched standard spiked at 0.025 mg kg^{-1} using LC-TOF-MS are shown in Fig. 1. The results revealed that, there was an obvious matrix effect that decreased the chromatographic response of these pesticides. Therefore, quantitation of pesticides was performed with matrix-matched calibration using the same matrix as the sample analysed.

Recovery studies were performed by spiking untreated palm oil samples with the appropriate volumes of composite working standard solution at three different concentration levels: 25, 50, 100 μg kg^{-1}. Five replicates were carried out at each spiking level to determine the mean recovery (%) and relative standard deviation (RSD%). Most values of the relative standard deviations of the analysed samples were in general less than 10% that could be attributed to the experimental error. The obtained results for mean recoveries and RSD (%) of all pesticides at three concentration levels are shown in Table 3. For all compounds in all samples, the mean recoveries lie within an acceptable range from 72.6% to 91.3% with relative standard deviation value from 5.3% to 14.2%.

Table 3
Mean percent Recovery ± RSD (%) ($n = 5$) obtained by MSPD-sonication procedure of the spiked palm oil sample for the pesticides studied.

Mean recovery ± RSD (%)			
Pesticide	Concentration level (μg kg^{-1})[a]		
	25	50	100
Dimethoate	78.4 ± 8.1	105.4 ± 14.2	81.3 ± 8.6
Malathion	83.5 ± 11.3	85.2 ± 7.1	74.3 ± 6.5
Carbaryl	89.4 ± 7.3	75.4 ± 9.4	72.6 ± 7.7
Simazine	91.3 ± 7.2	92.1 ± 7.3	87.1 ± 13.4
Terbuthylazine	90.4 ± 9.2	96.1 ± 11.6	76.1 ± 7.4
Atrazine	89.1 ± 5.7	91.2 ± 5.3	107.4 ± 9.2
Diuron	88.3 ± 10.7	77.4 ± 8.2	82.7 ± 10.3

[a] $n = 5$.

Table 4
Retention times, *m/z* ions, established EU MRLs, method precision expressed as the RSD%, calibration data, LOD and LOQ of the pesticides analysed in palm oil samples by LC-TOF-MS (font bold: *m/z* ions selected for quantification and confirmation of pesticides by LC-TOF-MS).

Pesticide	t_R (min)	*m/z*	MRL (μg kg^{-1})[*]	Calibration data		RSD (%)						LOD	LOQ
				Equation	Correlation coefficient	Speaking level (μg kg^{-1})						(μg kg^{-1})	(μg kg^{-1})
						50		500		2500			
Dimethoate	15.5	230.0063, 198.9610, 170.9694, **124.9778**	50	$y = 10501.3X - 612.13$	0.9992	5.3[a]	6.5[b]	3.4[a]	5.4[b]	3.1[a]	4.3[b]	3.5	5.2
Malathion	26.1	**331.0397**, 284.9880, 127.0354, 124.9810	nr	$y = 873.9X + 25110$	0.9987	6.1[a]	8.2[b]	5.1[a]	10.4[b]	4.0[a]	10.4[b]	1	2.5
Carbaryl	21.2	202.0865, **145.0485**	20	$y = 53889.10X + 12351.2$	0.9991	4.7[a]	5.9[b]	2.7[a]	7.3[b]	1.9[a]	3.1[b]	1.5	3
Simazine	18.8	**202.0854**, 174.0541, 132.0323	nr	$y = 48{,}956X - 35{,}879$	0.9994	5.0[a]	7.0[b]	4.3[a]	5.5[b]	3.8[a]	6.8[b]	2	7
Terbuthylazine	24.04	**230.1167**, 174.0541	nr	$y = 190{,}134X - 223{,}041$	0.9991	8.3[a]	10.6[b]	8.7[a]	9.0[b]	6.5[a]	11.7[b]	1.5	5
Atrazine	21.4	**216.1010**, 174.0541	100	$y = 173{,}967X - 128976$	0.9998	9.5[a]	13.2[b]	5.0[a]	12.8[b]	3.7[a]	8.2[b]	1.5	3.6
Diuron	21.8	**233.0243**, 255.0063, 72.0444	nr	$y = 15{,}973X + 2648$	0.9986	4.8[a]	6.7[b]	4.1[a]	6.7[b]	2.0[a]	4.6[b]	5	9

nr: non reported.
[a] Intra-day repeatability ($n = 5$).
[b] Inter-day reproducibility ($n = 5$).
[*] Ref. [26].

3.3.2. *Precision, linearity and lower limit values*

Repeatability of the developed analytical method to obtain precision were calculated by running five extractions of palm oil samples spiked at the three concentration levels (50, 500, 2500 μg kg^{-1}) in five-replicate in single day and in five different days, as intra-day and inter-day precision study. Table 4 shows the recovery values and the RSDs (%) obtained from these assays.

Linearity was determined by matrix-matched standard solutions of palm oil (in triplicate) at seven concentration levels between 5 and 500 μg kg^{-1}. The slope and intercept values, together with relative standard deviations were estimated using regression analyses. The responses of all compounds were linear in the range under study with the regression coefficients higher than 0.994 (see Table 4).

The instrumental limit of detection and limit of quantitation were determined from the injection of matrix-matched standard solutions with low concentration levels giving a signal-to-noise ratio of 3 and 10, respectively. The obtained results are summarized in Table 4. These LOD and LOQ levels are considerably low since they are far below the maximum residue level regulations established for selected pesticides in this study. These results demonstrate the high sensibility of the proposed method based on MSPD and LC-TOF-MS for the detection and quantification of the selected pesticides in palm oil.

3.3.3. *Application of the method to real samples*

The proposed method based on low temperature precipitation followed by matrix solid-phase dispersion-sonication was applied for the determination of seven multiclass pesticides in palm oil. Four different brand palm oil samples obtained from local markets in Kuala Lumpur city, Malaysia. The results showed that, no pesticide residues were found at concentrations above the detection limit and the permitted MRL published by Thai Agricultural Standards on Pesticide Residues (TAS 9002-2006) for palm oil (see Table 4) [2].

4. Conclusions

An efficient, fast and easy to perform analysis method based on low temperature followed by matrix solid-phase dispersion-sonication was successfully applied to determine seven multiclass pesticides in palm oil. The MSPD procedure using PSA (dispersant) and GCB (clean-up sorbent) was carefully optimized to maximize recovery of the pesticides contained in palm oil samples while eliminating most of the interfering matrix components. The results demonstrate that the accuracy, linearity, and selectivity of the proposed method are acceptable with good obtained recoveries and low LOQ between 2.5 and 9 μg kg^{-1} allowing application of the procedure for detection below the levels imposed by existing regulations. In addition, the method offers considerable saving in terms of solvent consumption, cost of materials, sample manipulation and analysis time.

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