

Determination of Herbicide Diuron Levels in Palm Oil Matrices using HPLC-UV

(Penentuan Aras Herbisid Diuron dalam Matriks Minyak
Sawit Menggunakan HPLC-UV)

MOHD IZWARIE RAMLI, ZURIATI ZAKARIA, ISMAIL SAHID,
TAN YEW AI & HALIMAH MUHAMAD*

ABSTRACT

The objective of this study was to develop a method for the determination of diuron (3-(3,4-dichlorophenyl)-1,1-dimethyl urea) residue in crude palm oil (CPO) and crude palm kernel oil (CPKO) matrices. The method involves the extraction of the herbicide from the oil matrix using low temperature precipitation and solid phase extraction techniques, detected by high performance liquid chromatography-ultra violet (HPLC-UV). The HPLC separation was carried out on an Ascentis™ RP-Amide column and elution with acetonitrile (solvent A) and water-methanol (2:1, v/v) (solvent B) as a suitable solvent system, at ratio of 4:6 (v/v). The optimum volume of acetonitrile for the extraction of diuron was 30 mL and 4 mL was obtained as the optimum volume of the solvent for elution analyte through the SPE cartridge. A linear correlation was obtained for the concentration of diuron from 0.05–1.0 µg mL⁻¹ with a correlation coefficient of 0.99. The recovery of diuron from CPO was 83.2–101.4% with a relative standard deviation of 1.4–9.9% and 79.4–87.9% with relative standard deviation of 0.9–5.6% for CPKO. The method detection limit and limit of quantification obtained were 0.018 µg g⁻¹ and 0.058 µg g⁻¹, respectively. The method was used to determine diuron residues in palm oil from different refineries situated at different locations throughout Malaysia.

Keywords: Crude palm kernel oil; crude palm oil; diuron; herbicide

ABSTRAK

Objektif kajian ini adalah untuk membangunkan satu kaedah bagi menentukan residu diuron (3-(3,4-diklorofenil)-1,1-dimetil urea) di dalam matriks minyak sawit mentah (CPO) dan minyak isirong sawit (CPKO). Kaedah yang dibangunkan melibatkan pengekstrakan herbisid daripada matriks minyak sawit menggunakan teknik pemendakan bersuhu rendah dan pengekstrakan fasa pepejal yang dikesan dengan kromatografi tekanan prestasi tinggi-ultra lembayung (HPLC-UV). Pemisahan dengan HPLC-UV dibuat menggunakan turus Ascentis™ RP-Amide dan dielusi dengan asetonitril (pelarut A) dan air-metanol (2:1, v/v) (pelarut B) sebagai sistem pelarut yang sesuai, pada nisbah 4:6 (v/v). Isi padu pelarut asetonitril optimum untuk mengekstrak diuron adalah 30 mL dan 4 mL diperoleh sebagai isi padu pelarut optimum untuk mengelut analit melalui kartrij SPE. Kolerasi lurus diperoleh pada julat kepekatan diuron 0.05–1.0 µg mL⁻¹ dengan kolerasi koefisien 0.9983. Peratus perolehan semula diuron bagi CPO ialah 83.2–101.4% dengan sisihan piawai relatif 1.4–9.9% dan 79.4–87.9% dengan sisihan piawai relatif 0.9–5.6% bagi CPKO. Had pengesanan kaedah dan had kuantitatif yang diperoleh masing-masing adalah 0.018 µg g⁻¹ dan 0.058 µg g⁻¹. Kaedah yang dibangunkan digunakan untuk menentukan residu diuron di dalam minyak sawit daripada kilang penapisan yang berbeza di seluruh Malaysia.

Kata kunci: Diuron; herbisid; minyak isirong sawit; minyak sawit mentah

INTRODUCTION

Malaysia is the largest exporter (2000–2008) and producer (2000–2005) of palm oil in the world (MPOB 2009). Palm oil is obtained from the mesocarp of the oil palm fruit, while crude palm kernel oil (CPKO) is derived from the kernel of the fruit. Palm oil possesses highly beneficial, nutritional and culinary properties due to its unique composition, comprising fatty acids and antioxidants (Lin 2011). Crude palm oil (CPO) consists mainly of palmitic acid (45%), followed by oleic acid (40%), linoleic acid (10%) and stearic acid (5%), while CPKO contains mainly lauric acid (48%) (Lin 2011). The benefits of palm oil

for health, food production and cosmetics have created a demand for this product worldwide. Therefore, palm oil is one of the 17 edible oils that have been accepted as meeting the requirements of the FAO/WHO food standards under the CODEX Alimentarius Commission Programme (Zainudin et al. 2009).

Phenylurea herbicides such as diuron are widely used as selective pre- and post-emergence herbicides for the control of most broad-leaved weeds and annual grasses in many agricultural crops such as rice, corn, soybean, cotton and potato (Mou et al. 2008) as well as in palm oil plantations (Ainie et al. 2007). Diuron is commonly

used for the control of weeds in oil palm plantations and these comprise grasses (*Paspalum conjugatum* and *Ottocloa nodosa*), broadleaves (*Asystasia intrusa* and *Cleome rutidosperma*), legumes (*Desmodium triflorum* and *Mimosa pudica*) and ferns (*Lygodium flexuosum* and *Nephrolepis biserrata*) (Wahab 2001). Diuron is the common name for 3-(3,4-dichlorophenyl)-1,1-dimethyl urea ($C_9H_{10}Cl_2N_2O$) and trade names include Karmex, Crisuron, Di-on, Diater, Diurex, Toterbane and Unidron. In the pure form, it is an odourless crystal while a sample of 93% purity is a powder. Its melting point is in the range of 158-159°C. Diuron is relatively stable in nature. It has light solubility in water (42 mg L⁻¹) at 25°C and its molecular weight is 233.1 (Kidd & James 1991). Diuron is one of the phenylurea herbicides that causes the death of plants by blocking electron transport at photosystem II thus inhibiting photosynthesis. It is absorbed principally through the roots and has broad spectrum activity killing both broadleaf and grassy weeds.

Many reports have been published on the development of analytical methods for determining pesticide residues in vegetable oils especially olive oil, (Aramendia et al. 2007; Garcia-reyes et al. 2007; Guardia-rubio et al. 2006a, 2006b) but only a few researchers have reported on pesticide residues in palm oil. Yeoh et al. (2006) reported on the analysis of acephate, methamidophos and monocrotophos in CPO, while in a recent publication the analysis of organochlorine pesticide residues in CPO was reported (Muhamad et al. 2004). Methods on the determination of pesticide residues in oil palm matrices have been reported such as chlorpyrifos residue in refined palm olein, (Halimah et al. 1999) fluroxypyr in CPO and CPKO (Muhamad et al. 2008) and cypermethrin in palm oil matrices (Zainudin et al. 2009).

The analytical methods of determining pesticide residues in palm oil are challenging, due to inherent complexity of the matrix, mainly due to triglycerides (TAG). The preparation of oil samples for the determination of pesticide residues requires complete removal of the high molecular mass fat from the sample to maintain the chromatographic system (Halimah et al. 1999). Additional extraction and clean-up steps are also necessary in order to avoid damage to the different parts of the instruments used and potential interferences in the chromatographic analysis. The extraction procedure used has been based mostly on liquid-liquid extraction (LLE) while the clean-up procedure is based on gel permeation chromatography (GPC), solid phase extraction (SPE) and matrix solid-phase dispersion (MSPD). SPE was used in order to minimize interferences in chromatographic determination of pesticide in CPO and CPKO (Halimah et al. 1999; Muhamad et al. 2004, 2008). A classic LLE procedure was needed for more cost-effective extraction techniques such as low temperature precipitation (Zainudin et al. 2009). This technique was first introduced by Lentza-rizos et al. (2001a, 2001b) where it was used to determine the organophosphorus pesticide, endosulfan and five pyrethroid insecticides in olive oil and virgin olive oil.

The objectives of this study were to develop analytical methods for the determination of diuron in both CPO and CPKO and to monitor these samples from different refineries at different locations in Malaysia.

EXPERIMENTAL DETAILS

CHEMICALS AND MATERIALS

The HPLC grade methanol and acetonitrile obtained from Merck and water obtained from a Mili-Q water purification system (Milipore Corp., USA) were used to prepare all the aqueous solutions. Standard diuron was purchased from Dr. Ehrenstorfer (Augsburg, Germany). The micro liter pipettes, adjustable between 100 and 1000 µL and pipette tips were obtained from Eppendorf (Hamburg, Germany), the SPE vacuum manifold from Agilent (Palo Alto, CA, USA), the vortex mix from Barnstead/ Thermolyne Inc. (Dubuque, IA, USA), the GCB cartridges (500 mg/ 6 mL) from Altech Inc. (Deerfield, IL, USA), the PSA cartridges (500 mg/ 6 mL) from International Sorbent Technology (Hengoed, UK), the RP-18 cartridges (200 mg/ 3 mL) from Merck and the Florisil cartridges (1000 mg/ 6 mL) from Agilent Technology. The digital fridge thermometer was purchased from Libradi Trading Co. Ltd. (Connaught Rd, Hong Kong).

The HPLC grade acetone was used to prepare the standard solutions. The stock solution of diuron (100 µg mL⁻¹) was prepared by weighing 0.0102 g of diuron to be dissolved in 25 mL of acetone solvent. The solution was then protected from light and stored in a freezer at -20°C. Working standard solutions for the calibration curve were prepared by drying aliquots of the stock solution under a gentle nitrogen gas stream and redissolving the residues in acetonitrile-water (1:1, v/v) to the required concentration prior to use.

OIL SAMPLES

Spiking of Oil Samples. The CPO and CPKO for the spiking tests were obtained from local mills. Both types of oil samples were spiked with the standard solutions of diuron.

Monitoring Studies. CPO (20 samples) and CPKO (10 samples) were taken from different refineries situated at different locations throughout Malaysia. They were kept in 1 liter dark bottles and stored in a cold room (temperature 4°C) prior to the extraction and clean-up processes via SPE. Each sample was analysed in triplicate.

LC System and Conditions. An Agilent 1100 HPLC system equipped with a quaternary pump (model G1311A), a degasser (model G1322A), an autosampler (model G1313A) and an ultra violet detector (model G1314A) were used for the chromatographic analysis. The system was controlled by the HP ChemStation (Agilent Technologies), which also performed functions such as

data collection from the UV detector and quantitative measurements. The UV detector was set at wavelength 254 nm. The chromatographic study was done using an Ascentis™ RP-Amide column (25 cm × 4.6 mm, 5 μm) obtained from Supelco (Bellefonte, USA).

Two mobile phase systems with different range of polarity were used. The first mobile phase system used was acetonitrile as solvent A and water as solvent B. The second mobile phase system was acetonitrile as solvent A and a mixture of water and methanol in the ratio of 2:1 (v/v) as solvent B. For both sets, the ratio of A : B in systems of 7:3, 5:5 or 4:6 (v/v) were tested. All the mobile phase used was filtered through 0.45 μm pore size cellulosic membrane filters and degassed by sonication prior to the usage with the water-methanol (2:1, v/v) solvent, while acetonitrile was prepared separately. The flow rate was 1.00 mL min⁻¹ and the volume injected was 100 μL. The analytical column was set at 25°C and samples were run for 15 min.

SAMPLE PREPARATION AND CLEAN-UP PROCEDURE

Liquid-liquid Extraction Procedure. Samples of CPO were melted at 60°C in an oven and then homogenized by shaking the samples. After homogenization, each of fresh CPO samples was weighed 5 g and placed in 50 mL screw cap test tubes. The CPO samples were homogenized using a vortex mixer for 1 minute and allowed to stand for 20 min to attain equilibrium after being spiked with 0.6 μg g⁻¹ and 1.2 μg g⁻¹ of diuron standard solution. The experiment was also conducted with triplicate unspiked samples namely control samples for each cartridges tested. Five different volumes of the acetonitrile solvent for the extraction process were tested: 10, 20, 30, 40 and 50 mL. A series of different volumes of acetonitrile were tested on the CPO samples. The extracted liquid was tested in triplicate for spiked samples. The method used for the extraction of diuron from CPO followed the procedure of Lentza-rizos et al. (2001a, 2001b). In this method, acetonitrile was used to extract the insecticide residues from olive oil and virgin olive oil. Therefore in the current study, a series of different volumes of acetonitrile were added to the tubes containing the spiked CPO sample and the mixtures were then shaken for 5 min using a vortex mixer. Each mixture was then allowed to stand until two layers were separated with the oil at the bottom of the test tubes. Each tube was stored horizontally in a freezer at -20°C for 2 h for oil precipitation. The freezer temperature was controlled by using digital fridge thermometer. The minimum time of 2 h for freezing was selected based on previous experiments conducted by Zainudin et al. (2009) where 2 h was found to be the optimum time for satisfactory fat removal by low temperature precipitation. A 10 mL of acetonitrile was transferred quickly into a flask through filter paper (Whatman No.4, 125 mm dia.) with the clean pipette (10 mL) to prevent the frozen oil from melting or re-dissolving. The filtration was conducted in room temperature under 1 atm. The 10 mL

pipette then rinsed 2 times with 2 mL acetonitrile solvent for each rinsed to avoid remains analyte in the pipette.

SPE CLEAN-UP PROCEDURE

Different types of sorbents were tested to accomplish this step, namely RP-18, Florisil, PSA, GCB and combination cartridges between Florisil+GCB and PSA+GCB. The GCB cartridge was attached to the top of Florisil and PSA cartridges using the PTFE adaptor. All the cartridges were tested for the ability to reduce the coeluted oil and lipids residue based on the control samples. The extract solution was dried using nitrogen gas equipped with water bath at temperature 40°C to obtain a final concentration of approximately 2 mL and then it was transferred to the SPE cartridges. The 2 mL extract was eluted through the cartridges by gravity flow and then gentle pressure was applied to achieve a flow of approximately one drop per second. Once all the aliquot was absorbed into the packed cartridges, the effluent was collected into a 20 mL vial (weight was recorded before used and after solvent evaporated).

A study on the volume of acetonitrile needed to elute the analyte retained on the sorbent was also carried out. The tests on each different volume of acetonitrile were carried out in triplicate. The components retained in the PSA and GCB cartridges were eluted with 2, 3, 4 or 5 mL acetonitrile and then the organic phase was evaporated under a gentle nitrogen stream equipped with water bath (40°C). The dried residue was redissolved using 1 mL of a mixture of acetonitrile-water (1:1, v/v) in a vortex mixer and filtered through 0.45 μm nylon filter, before being injected into the HPLC.

VALIDATION PROCEDURE

The linearity, method detection limits (MDL), limit of quantification (LOQ), accuracy and precision determined the efficiency of the extraction procedure. To determine MDL value, the method USEPA (1994) was followed. The CPO and CPKO samples were spiked with 0.03 μg mL of diuron standard solution. The oil samples used were previously analysed and found to contain no detectable level of diuron. It was then extracted using the optimum experimental conditions. Each concentration carried out seven replicates with a control sample to reduce memory effect. Relative standard deviation was used to assess the precision of the method. The MDL value was calculated from the standard deviation of the replicate studies results and multiplied by the appropriate Student's t value for 99% confidence interval. To determine LOQ value, MDL results were multiplied with 3.33.

Sensitivity and linearity of the detector response to the diuron was assessed by the calibration curve obtained from a series of six standard solutions of the pesticide in the range of 0.05 – 1.0 μg mL⁻¹. The calibration curve was obtained by plotting the peak area against the concentration. Accuracy and precision assessment of the HPLC were tested on the four replicates of the CPO and CPKO samples at each

of the six spiked levels of 0.06, 0.15, 0.3, 0.45, 0.6 and 1.2 $\mu\text{g g}^{-1}$ diuron.

INTRA-LABORATORY TESTING

Reproducibility was determined by intra-laboratory testing. Analysis was done in the analytical chemistry laboratory, Analytical and Quality Development Unit, Malaysian Palm Oil Board on CPO and CPKO samples. Intra-laboratory study on the extraction of diuron from the CPO and CPKO samples using the optimal method developed was analysed by two different experienced operators with three concentrations of diuron standard solution (0.3, 0.6 and 1.2 $\mu\text{g mL}^{-1}$).

RESULTS AND DISCUSSION

OPTIMIZATION OF THE HPLC SEPARATION

For the HPLC-UV analysis, the mobile phase consisted of an isocratic elution using two sets of system solvents with different polarity range. From set 1, it was found that higher recovery of about 200% was observed when acetonitrile-water was used. This could possibly be due to some coeluted fat from the oil samples which consequently caused an overlapping of the peaks. Therefore, in set 1, the ratio of 4:6 (v/v) of acetonitrile-water was not suitable for the determination of diuron, because of the long detection time of approximately 30 min.

In order to avoid the overlapping peaks as observed in set 1, a modification of system B in set 2 was undertaken. Preliminary experiments showed that the water-methanol ratio of 2:1 (v/v) in system B was the best mixture to use in order to avoid overlapping of the peaks (data not presented). Among the three ratios used in system A and B, the ratio 4:6 (v/v) gave the best peaks. Therefore, it was recommended that solvent A (acetonitrile) and solvent B (water plus methanol, 2:1 v/v) in set 2 at the ratio of 4:6 (v/v) of solvent A : B to be used for analysis of diuron residue in palm oil matrices.

LIQUID-LIQUID EXTRACTION

A study on the optimal volume of acetonitrile necessary for extraction of diuron from CPO was carried out. The recovery value for 0.6 and 1.2 $\mu\text{g g}^{-1}$ spiking levels for a series of volume extraction were very similar except

for 10 mL. Table 1 shows the recovery values of the five series of volume extraction. As shown in Table 1, when 30, 40 and 50 mL of the extracting solvent were used, recovery percentages were found to be higher in the range of 89.9–92.8% for both concentrations. However 20 mL gave only 85.1% and 87.1% recovery percentages for 0.6 and 1.2 $\mu\text{g g}^{-1}$ spiking levels, respectively. On the other hand, 10 mL extraction volume gave only 64% and 66.3% recovery for both concentrations. Recovery values were computed from the average of results at each spiking level from tests done in triplicate.

Statistical analyses using ANOVA (single factor) were undertaken to verify significant differences between the volumes of solvent tested. Variance testing was done with 20, 30, 40 and 50 mL of volume extraction for satisfactory recovery percentages. From the analysis results, $F_{\text{calculation}}$ value was found to be 21.03 and 8.44 for 0.6 and 1.2 $\mu\text{g g}^{-1}$ spiking levels, respectively, while F_{critical} was 4.07. Depending on F values, the results showed significant differences among the mean recoveries for both the concentrations. Based on the results obtained, 30 mL has deemed the optimum solvent volume for extraction.

SPE CLEAN-UP

A comparative study on the efficiency of RP-18, Florisil, PSA, GCB, Florisil+GCB and PSA+GCB was made to evaluate the feasibility of using these sorbents for extracting diuron from palm oil samples. Table 2 shows the weight of coeluted fat, percentage fat removed and the colour of final eluents solution. As observed, the weight of coeluted fat and RSD value for all the cartridges tested were 1.42±0.21 mg (PSA), 1.82±0.44 mg (Florisil), 3.21±0.32 mg (RP-18), 3.43±0.74 mg (GCB), 0.58±0.37 mg (PSA+GCB) and 1.57±0.24 mg (Florisil+GCB). From the results obtained, the removed fat percentage was calculated and found to be 99.7% (PSA), 99.6% (Florisil), 99.4% (RP-18), 99.3% (GCB), 99.9% (PSA+GCB) and 99.7% (Florisil+GCB). The results showed that the combinations of cartridges demonstrate more efficient rather than single cartridge in terms of the ability to remove the coeluted fat. However, based on the result obtained for both combination cartridges tested the combination of PSA+GCB was demonstrated to be more efficient compared with the combination of Florisil+GCB.

In addition, the colour of final eluents solution was also observed. The results show that, for the PSA, Florisil

TABLE 1. Recoveries of diuron from CPO using LLE technique with various volume of acetonitrile ($n=3$)

Concentration of diuron ($\mu\text{g g}^{-1}$)	Volume (mL)									
	10		20		30		40		50	
	%R	%RSD	%R	%RSD	%R	%RSD	%R	%RSD	%R	%RSD
0.6	64	0.8	85.1	2.1	92.3	1.7	92	0.6	90.6	0.5
1.2	66.3	0.5	87.1	0.4	92.8	0.8	91.3	2.7	89.9	1.0

%R = Recovery percentage
RSD = Relative standard deviation

TABLE 2. Weight of coeluted fat, percentage of removed fat and the colour of final eluents solution for various cartridges tested ($n=3$)

Cartridges	PSA	Florisol	RP-18	GCB	PSA+GCB	Florisol+GCB
Final weight (mg)	1.42	1.82	3.21	3.43	0.58	1.57
%RSD	0.21	0.44	0.32	0.74	0.37	0.24
Fat removed (%)	99.7	99.6	99.4	99.3	99.9	99.7
Colour final solution	Turbid yellowish	Turbid yellowish	Cloudy	Cloudy	Clear	Turbid yellowish

and Florisol+GCB cartridges turned the colour of eluents into turbid yellowish. For the RP-18 and GCB cartridges the colour of eluents was cloudy in nature while PSA+GCB cartridges gave the clearer final solution. This showed that the sorbents RP-18, PSA, Florisol, GCB and Florisol+GCB did not completely prevent the fat contents (compared with PSA+GCB) in the oil from coeluting with the solvent. Therefore, PSA+GCB were selected as the most suitable sorbent for diuron due to the ability to remove the fat contents and the clearer solution obtained after the clean-up. This result was consistent with the findings on the determination of cypermethrin in palm oil matrices as described by Zainudin et al. (2009) and the evaluation of a solid-phase extraction dual-layer carbon/primary secondary amine (PSA) used for clean-up of the fatty acid matrix components from food extracts described by Shimelis et al. (2007) in term of recovery study and clear final solution.

Statistical method namely Student's F test was used to analyze the data obtained from the single cartridge while t test was used for the combination cartridges tested. The $F_{\text{calculation}}$ and F_{critical} values obtained using F test for single cartridges tested were 20.48 and 3.34, respectively. It was found that there was significant difference in terms of their precision. Analysis using t-Test for the results obtained from the combination cartridges indicated that the $t_{\text{calculation}}$ value was lower than t_{critical} value which is -4.91 and 2.30,

respectively. The t-Test analysis results showed that there was no significant difference for both of the combination cartridges tested. However, the combination of PSA+GCB were chosen in terms of the higher percentage fat removed and the clearer final eluents solution.

The results showed that 4 mL of acetonitrile was sufficient for the elution of the analyte retained in the cartridges, with a recovery of 95%. It was found that for the 2 mL and 3 mL elution, the percentage recovery obtained was approximately 71% and 80%, respectively. Therefore, 4 mL of acetonitrile was selected as the correct volume to use in order to ensure complete elution of the compounds retained in the SPE cartridge.

VALIDATION

Linear relationship between the peak and concentration was observed for diuron analysis using HPLC-UV detector. The linear regression coefficient (r^2) was found to be 0.99 and the equation derived from the calibration curve was $y = 626.82x + 16.28$, where y is the area of the diuron peak obtained from the HPLC analysis and x is the concentration of diuron in $\mu\text{g mL}^{-1}$ (Figure 1). Since the linear regression obtained was 99.8%, the HPLC and detector were working properly.

The standard deviation obtained from determination of MDL testing was 0.6% multiplied with 3.143 (Student's t

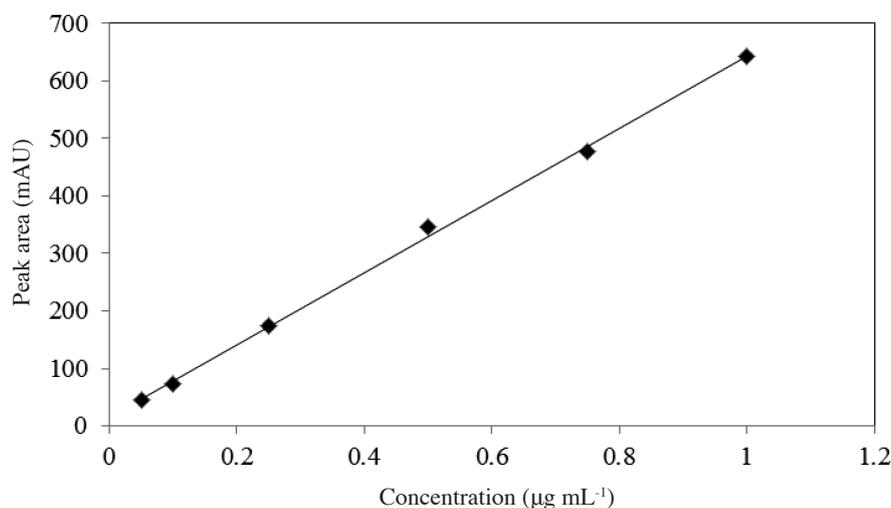


FIGURE 1. Calibration curve for diuron prepared in acetonitrile-water, 1:1 (v/v)

value at confidence level 99%) to get MDL value. Therefore, the MDL and LOQ for diuron residue in CPO and CPKO were $0.018 \mu\text{g g}^{-1}$ and $0.059 \mu\text{g g}^{-1}$, respectively. The MDL value achieved is 10 times lower than MRL value for palm oil that had been set by Food Act 1983 and Regulation Malaysia (2005a, 2005b) which is $0.1 \mu\text{g g}^{-1}$.

Four replicates of CPO and CPKO samples were analyzed to establish the accuracy of the extraction method. The method performance should meet the acceptable criteria of 70-120% mean recoveries and coefficient of variation not more than 20% (Pihlstrom et al. 2009). The recoveries of diuron from spiked CPO samples at levels of $0.06\text{--}1.2 \mu\text{g g}^{-1}$ were 83.2-101.4%

with a RSD of 1.4–9.9%, while the recovery of diuron from the spiked CPKO samples was 79.4-87.9% with a RSD of 0.9-5.6%. The values for the mean recovery and RSD are shown in Table 3. Since both the recovery and RSD values met the method performance criteria, these indicated the methods developed were satisfactory, reproducible, precise and accurate. Figures 2(a) - 2(c) show the chromatograms for the standard diuron solution, a blank sample of CPO and a spiked sample at $0.6 \mu\text{g g}^{-1}$ of CPO. Figures 3(a) - 3(c) show the chromatograms for the standard diuron solution, a blank sample of CPKO and a spiked sample at $0.6 \mu\text{g g}^{-1}$ of CPKO.

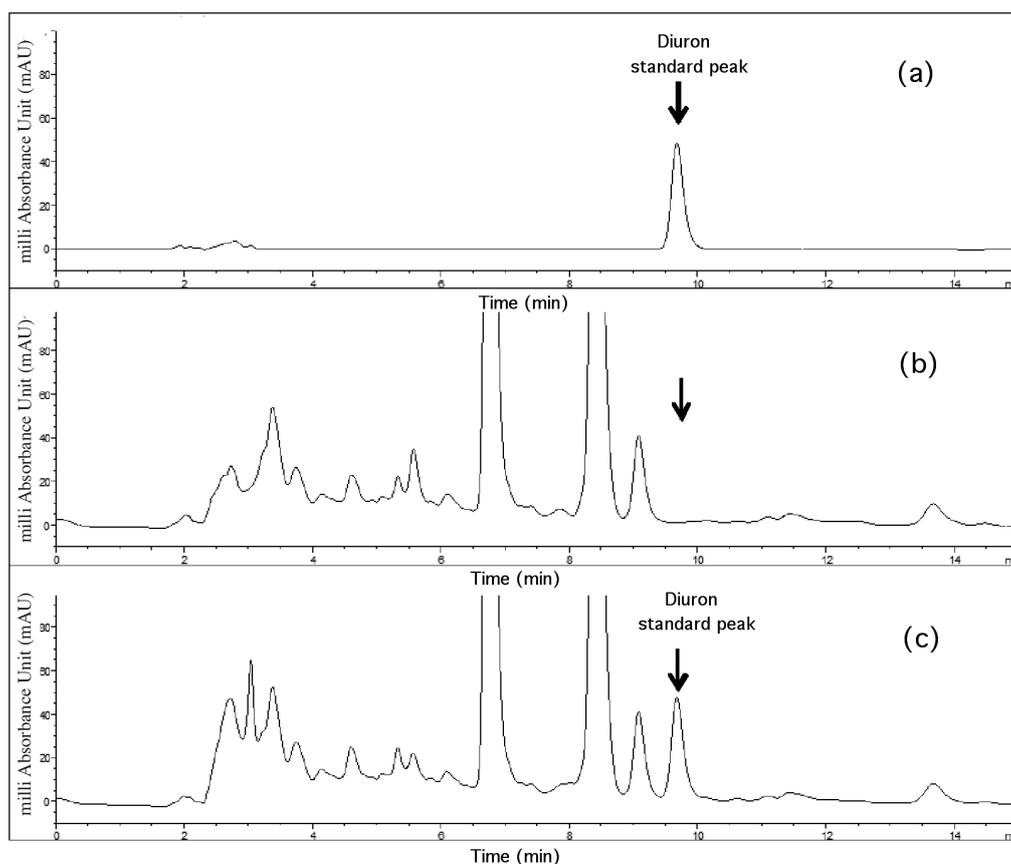


FIGURE 2. Chromatograms obtained for (a) diuron standard solution, (b) blank CPO sample and (c) spiked CPO sample ($0.6 \mu\text{g g}^{-1}$)

TABLE 3. Percentage recovery of diuron from CPO and CPKO after treatment ($n=4$)

Concentration of diuron ($\mu\text{g g}^{-1}$)	CPO		CPKO	
	%R	RSD (%)	%R	RSD (%)
0.06	98.5	1.4	81.6	2.9
0.15	85.3	5.6	85.0	3.5
0.3	90.2	9.9	87.9	5.6
0.45	101.4	1.4	79.4	0.9
0.6	83.2	2.7	80.8	3.2
1.2	93.1	3.7	81.7	1.8

%R = Recovery percentage

RSD = Relative standard deviation

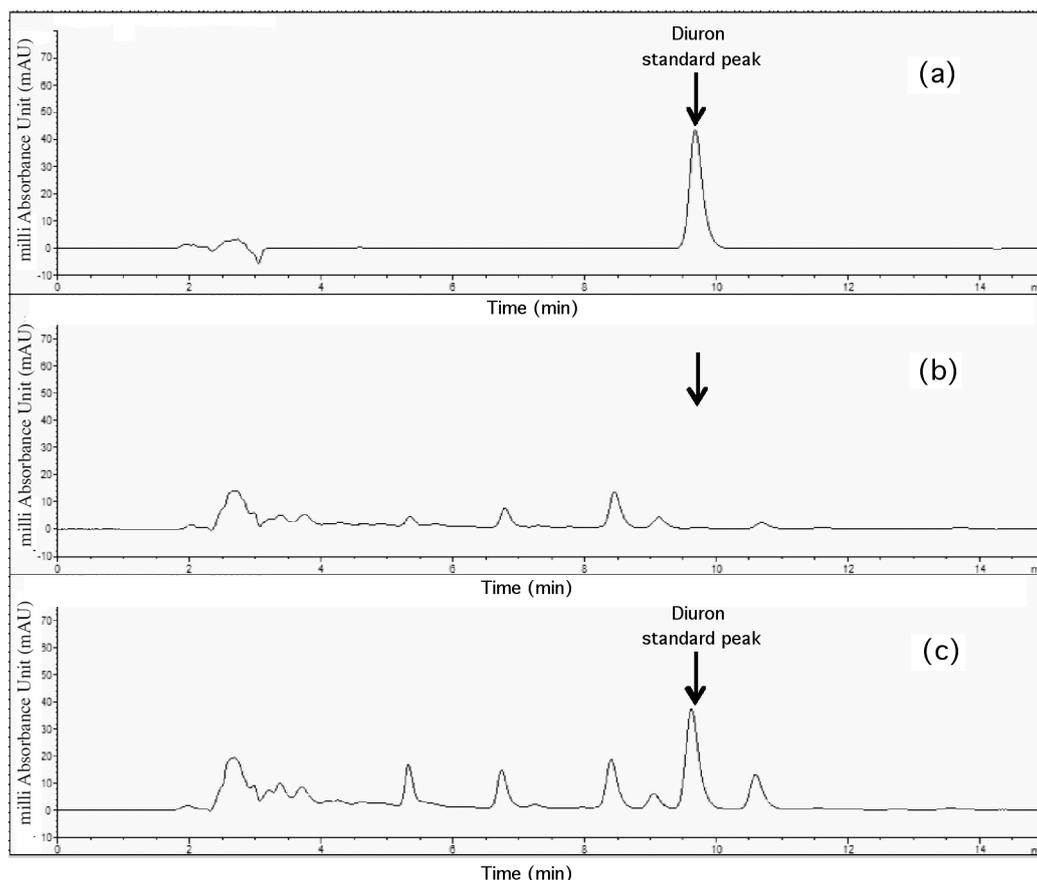


FIGURE 3. Chromatograms obtained for (a) diuron standard solution, (b) blank CPKO sample and (c) spiked CPKO sample ($0.6 \mu\text{g g}^{-1}$)

INTRA-LABORATORY (REPRODUCIBILITY)

Intra-laboratory results which were done by different experienced operators are shown in Table 4. The test was conducted with three concentrations of diuron standard solution which is 0.3 , 0.6 and $1.2 \mu\text{g mL}^{-1}$. The range of recovery percentage and %RSD that obtained by both operators for three concentration were in the range of 78 - 104.9% and 0.6 - 9.1% for CPO while 88.7 - 100.9% and 0.7 - 15.2% for CPKO. The results showed an effectiveness of the method that was developed to extract diuron from CPO and CPKO.

Statistical methods namely F test (ANOVA single factor) was used to analyze the results obtained. It was found that there was no significant difference in the analyses of their

precision (F test). The values ($F_{\text{calculation}}$) obtained from both operators for CPO and CPKO samples were 0.22 and 0.30 , respectively. The F_{critical} value for each of the samples was 7.71 , respectively. Since the critical value is higher than the calculation of F value, it could be concluded that there is no significant difference in the analytical results obtained.

MONITORING RESULTS

Based on the above method, analysis for the level of diuron in CPO and CPKO samples from mills and refineries located at various parts of Malaysia was carried out. A total of 20 CPO and 10 CPKO samples were tested for diuron levels.

TABLE 4. The percentage recovery of intra-laboratory analysis conducted by two difference experienced operator ($n=3$)

Concentration of diuron ($\mu\text{g g}^{-1}$)	Operator I				Operator II			
	CPO		CPKO		CPO		CPKO	
	%R	%RSD	%R	%RSD	%R	%RSD	%R	%RSD
0.3	98.9	0.9	90.3	3.7	78	9.1	88.7	1.4
0.6	81.3	3.3	93.8	9.9	102.5	3.4	90.1	0.7
1.2	104.9	0.6	100.9	15.2	90.5	0.8	98.9	5.6

%R = Recovery percentage
RSD = Relative standard deviation

Diuron residues were not detected in any of the CPO and CPKO samples tested.

CONCLUSION

The method developed was relatively rapid, sensitive, reproducible and accurate for the quantification of diuron residue in palm oil matrices with an effective baseline separation and method detection limit that allows determination below the maximum permissible residue limit for the pesticide. Extraction of the diuron from the samples was performed using a small volume of organic solvent, without the use of chlorinated solvents, thus reducing human health risks and contamination to the environment. The SPE clean-up steps using PSA combined with GCB cartridges was a suitable purification process for determination of diuron residue. Therefore, the method proposed is suitable for analysis and monitoring of traces of diuron residue in palm oil and its products.

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Mohd Izwarie Ramli, Tan Yew Ai & Halimah Muhamad*
Analytical and Quality Development Unit
Malaysian Palm Oil Board
No. 6, Persiaran Institusi, Bandar Baru Bangi
43000 Kajang, Selangor
Malaysia

Mohd Izwarie Ramli & Zuriati Zakaria
School of Chemical Sciences and Food Technology
Faculty Science and Technology
Universiti Kebangsaan Malaysia
43600 Bangi, Selangor
Malaysia

Ismail Sahid
School of Enviromental and Natural Resource Sciences
Faculty Science and Technology
Universiti Kebangsaan Malaysia
43600 Bangi, Selangor
Malaysia

*Corresponding author; email: halimah@mpob.gov.my

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