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DETERMINATION OF GLUFOSINATE AMMONIUM IN CRUDE PALM OIL: USE OF THE MODIFIED QUECHERS METHOD AND LC-MS/MS DETECTION

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ABSTRACT

The herbicide, glufosinate ammonium is widely used as crop protection for food crops and also in oil palm plantations. The herbicide is used to control weeds and also as a dessicant agent for land clearing. In this work, glufosinate ammonium was extracted from crude palm oil (CPO) samples using a modified QuEChERS (quick, easy, cheap, effective, rugged and safe) method. This method was based on a simultaneous extraction and clean-up steps which required minimum sample handling. The extraction was performed using 5.0 ml deionised water, (0.1% formic acid) and dichloromethane. The glufosinate ammonium residue was extracted from CPO samples using a mini shaker and centrifuged for 10 min at 2500 rcf. The extracts, following pre-concentration were clean enough for direct injection into the LC-MS/MS. Quantitative recoveries from 84%-109% were obtained for samples spiked with three concentration levels of 0.005, 0.05 and 0.5 µg ml^{-1}. The correlation variation (CV) percentage for reproducibility and repeatability of replicate extractions were less than 13% and 10% respectively. The limit of detection and limit of quantification was 0.002 µg litre^{-1} and 0.025 µg kg^{-1} respectively. The results obtained demonstrated that the method has achieved the requirements of the criteria for acceptance of single laboratory method validation. Determination of glufosinate ammonium residues in Malaysian CPO samples were carried out and the residues were not detected in the analysed samples.

Keywords: glufosinate ammonium, QuEChERS, LC-MS/MS, crude palm oil (CPO), herbicide.

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INTRODUCTION

The use of herbicides in plantation sectors can cause contamination in agricultural crops. In Malaysia, glyphosate, paraquat, 2,4-D, metsulfuron methyl and glufosinate ammonium are among the common herbicides currently used in oil palm plantations. Glufosinate [ammonium-2-amino-4-(hydroxymethylphosphinyl) butanoate], a chemical name for ammonium salt, glufosinate ammonium (GA), was used as a broad spectrum contact herbicide. It is used to control a wide range of weeds or for total vegetation control on land which is not used for cultivation. GA was also used to desiccate (dry of) crops before harvesting the crops.

Several methods of analysis have been developed for the determination of dissociated organo-phosphorus pesticides in various matrices such as fatty food, water, soil, vegetable and human serum. Most of these methods are complicated, tedious, used large amounts of solvent and time-consuming (Baki *et al.*, 2004; Hiroyuki *et al.*, 1996; Sancho *et al.*, 1994; Maria *et al.*, 2005; Tsunoda *et al.*, 1993;Yashushi *et al.*, 2001; Vreeken *et al.*, 1998).

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The extraction procedure for determining GA residues in vegetables oil crops was published by Sochor (1991) (report A 48915). The author reported that the GA residues were extracted from plant and animal material with water and then cleaned-up by defatting with dichloromethane. After evaporation of the solvent, the residues were treated with trimethyl orthoacetate to form derivatives prior to gas chromatographic (GC) analysis with phosphorus-specific flame-photometric detection. However, this method is very tedious, solvents and time- consuming during the sample preparation steps.

Constantine *et al*. (2001) reported that the determination of GA at low levels of concentration was difficult mainly because of their high polarity and solubility in water. It often requires an extensive sample treatment including enrichment steps and laborious derivatisation to reach the low levels of the target compounds. The extraction procedures are also tedious and time-consuming. Therefore, there is a need for better methods of analysis and this article describes the novel application of a modified QuEChERS method for GA determination in food matrices. The problem of high oil content was overcome by the use of deionised water (0.1% formic acid) as the extraction solvent. The high solubility of GA in water gave an added advantage to this method because it eliminates the clean-up step with no additional time and expenses required. Method validation was also carried out by determining the recovery, repeatability and reproducibility of seven replicates, and seven injections of each spiked samples.

METHODOLOGY

Chemicals

All chemicals and solvents used were analytical and of the HPLC grade. GA and glyphosate, internal standard (IS) (99% purity) were purchased from Dr Erhenstofer Brand (Germany). Formic acid (98%), methanol and dichloromethane were obtained from Merck Company (Malaysia). Deionised water (DI) from Milli-Q system. All solutions prepared for LC were passed through a 0.45 µm nylon filter before being used as a dilution medium.

Solutions and Calibration Curves

An accurately weighed solid portions of GA and IS were dissolved separately in water (0.1% formic acid) in 100 ml volumetric flask: these served as stock solutions of 100 mg litre^{-1} and further diluted to form working solutions of 10.0 mg litre^{-1} and 0.5 mg litre^{-1} accordingly. A series of working standard solutions were prepared at the concentration levels of 0.001, 0.005, 0.05, 0.1 and 0.5 mg litre^{-1} in 10.0 ml volumetric flasks. The 2.0 ml of the IS at 0.5 mg litre^{-1} was also diluted to obtain 0.1 mg litre^{-1} IS in each level of the working standard solution.

Sample Extraction

A portion of the sample (5.0 g) was weighed with a precision of 0.1 mg and 5.0 ml of deionised water (0.1% formic acid) was added followed by 5.0 ml dichloromethane in polypropylene 15.0 ml centrifuge tubes. Then the sample was spiked with 50.0 ul of 10.0 µg ml^{-1} glyphosate solution as the internal standard and homogenised using tube shaker. The analyte was extracted using the following steps of incubation in a water bath at 60°C for 30 min with 10 s shaking for every 10 min. The mixture (10.0 ml) was then centrifuged (2500 rcf/ 10 min). In this way, two layers were obtained with the aqueous layer at the top and lipid layer at the bottom. An aliquot (1.0 ml) of the aqueous layer was promptly removed by pipette and filtered through a 0.45 µm filter into a 2.0 ml LC-MS/MS vial.

The efficiency of the extraction method was determined by adding known amount of GA to the blank oil sample. A 5.0 g of the uncontaminated oil was weighed with a precision of 0.1 mg in the 15.0 ml centrifuge tube. Then the oil sample was spiked with GA from stock solution of 10.0 µg ml and 1.0 µg ml^{-1} GA as well as 10.0 µg ml^{-1} of IS solution. Three levels of spiking were carried out by adding 250.0 µl and 25.0 µl from the stock solution of 10.0 mg litre^{-1} (equivalent to 0.5 µg kg^{-1} and 0.05 µg kg^{-1}) and 25 µl of 1.0 mg litre^{-1} GA stock solution which is equivalent to 0.005 µg kg^{-1}. Then 50.0 µl of IS (stock 10.0 µg litre^{-1}) was added each sample. The extraction procedure was carried out as described above.

Method Validation

Three spiked CPO and two blank samples were analysed at the spiking levels of 5, 50 and 500 ηg g^{-1}. The samples were analysed using the method described above over a period of three days. Each sample was analysed in seven replicates (n=7) and each sample was injected into the LC-MS/MS seven times in order to determine repeatability and accuracy of the method. Reproducibility study was also carried out by an experienced technical assistant in the laboratory.

Flow Diagram of GA Extraction

5.0 g sample in a 50.0 ml FEP tube
↓
Add 5.0 ml DCM, vortex briefly
↓
Add 5.0 ml DI water (0.1% HCOOH), vortex briefly and shake for 5 min
↓
Add internal standard, shake 30 s
↓
Centrifuge for 10 min at 2500 rpm
↓
Filter 1.0 ml of the upper layer with 0.45 µm filter paper into HPLC vial
↓
LC-MS/MS

LC-MS/MS

Extracts of samples and calibration standards (10 ul) were analysed on an Agilent 1100 liquid chromatograph coupled to 4000 Q Trap (Applied Biosystem) mass spectrometer equipped with Turbo VTM source operated in electrospray mode. Analytical separation was achieved using the Hypercarb (50 x 2.1 mm, 5 µm) column from Thermo Finnigan, Malaysia. Isocratic elution was achieved with a mobile phase of DI water (0.1% formic acid) – methanol (90:10) at a flow rate of 0.35 ml min^{-1}. Data acquisition was performed in the SIM mode using interface parameters: drying gas (nitrogen) flow of 1.0 ml min^{-1}, nebuliser pressure of 50 psi, drying gas and vaporiser temperature of 650°C, ESI negative voltage of -4200V.

The MS system was operated in the negative Multiple Reaction Monitoring mode to give the highest sensitivity and selectivity. The transition ions monitored were mass to charge ratio, m/z 180.0 > 135.8 and 180.0 > 118.8 for glufosinate and m/z 167.9 > 149.7 and 167.9 > 123.9 for glyphosate. Both transitions were used for the quantification of glufosinate in the analysis of real samples.

Quantitation

GA was quantified using a linear calibration function that was established with standard solutions of glufosinate and glyphosate (IS) dissolved in deionised water at concentration levels 1.0, 5.0, 10.0, 50.0, 100.0 and 500.0 µg $litre^{-1}$. These concentration values were within the range encountered in the samples extracts. A calibration graph was constructed by plotting peak area ratios against the corresponding ratios of analyte amount. Thus, the glufosinate contents in sample extracts were calculated from the calibration slope and intercept values, taking into account the recovery calculated by the mean of glyphosate slope. In order to perform a good quantification, signal-to-noise ratio of the LC-MS/MS peak had to be greater than 3. When conducting quantification, the signal-to-noise ratio must be better than 10:1 whereas at the limit of detection, it is permissible to have a signal to noise ratio of 3:1.

RESULTS AND DISCUSSION

Chromatographic Separation and MS/MS Parameters

Table 1 shows the experimental mass conditions used in the LC-MS/MS analysis. Although glufosinate underwent very little fragmentation in ESI mode, two product ions were used to identify the compounds. For the analysis of GA in oil matrix, which has a very variable oil components, it is necessary to use suitable internal/surrogate standards to correct retention time shifts and compensate for losses in the sample preparation steps. In the described protocol, glyphosate was used as the internal standard. The response factor of GA in relation to glyphosate was between 0.5 and 5.0 over a concentration range from 1 to 500 ηg ml^{-1} and the results showed a linear response, with a coefficient of correlation above 0.999. The relative standard deviation of seven consecutive injections at a concentration of 5.0 ηg ml^{-1} was below 8%.

TABLE 1. ACQUISITION USING MRM MODE INDICATING RETENTION TIME WINDOWS, MOLECULAR MASS AND SPECIFIC IONS OF EACH ANALYTE

Name of analyte	Time (min)	First transition	Second transition
Glufosinate ammonium	5.5	180.0 >135.8	180 >118.8
Glyphosate (IS)	7.5	167.9 > 149.7	167.9 >123.9

Extraction. The extraction of trace residue from fatty foods or high lipid samples is problematic when the extract contains a large amount of lipids that need to be removed. Since GA is a highly polar compound with high solubility in water and very low solubility in oil matrix; deionised water with 0.1% formic acid was used to extract it from oil matrix. Formic acid was used to increase the ionisation of GA during the mass spectrometry detection. Dichloromethane was also used to separate and trap the lipid from the extract by separating the lipids into the DCM layer. Without DCM, oil droplets were still observed in the aqueous layer. Incubation of 30 min and shaking for every 10 min was necessary to ensure that all analytes were partitioned into the aqueous layer.

Method validation. Since no reference material were available for the validation of the method, spiked samples were prepared by adding 100.0 µl, 10.0 µl and 1.0 µl of the 500 µg litre^{-1} of GA standard to 1.0 g CPO sample to produce concentrations of 0.5, 0.05 and 0.005 mg kg^{-1} GA analyte. For each experiment, seven fortified replicates and one unfortified sample were analysed simultaneously. *Figure 1* shows the chromatogram of unfortified (blank) sample with no analyte detected in the sample. *Figure 2* shows mass spectrum of GA working standard solutions. *Figure 3* shows a linear standard calibration curve for concentration levels of 1.0, 5.0, 10.0, 50.0, 100.0 and 500.0 µg litre^{-1} of GA

TABLE 2. RESULTS FOR SEVEN REPLICATES OF REPEATABILITY, REPRODUCIBILITY AND RECOVERY STUDIES

Analyte concentration (spiked sample) mg kg^{-1}	Repeatability (CV %) (N=7)	Reproducibility (CV %) (N=7)	Recovery (%) (Cv < 13%)
0.005	8	10	84 -109
0.05	13	6	77-107
0.5	4	9	84-95

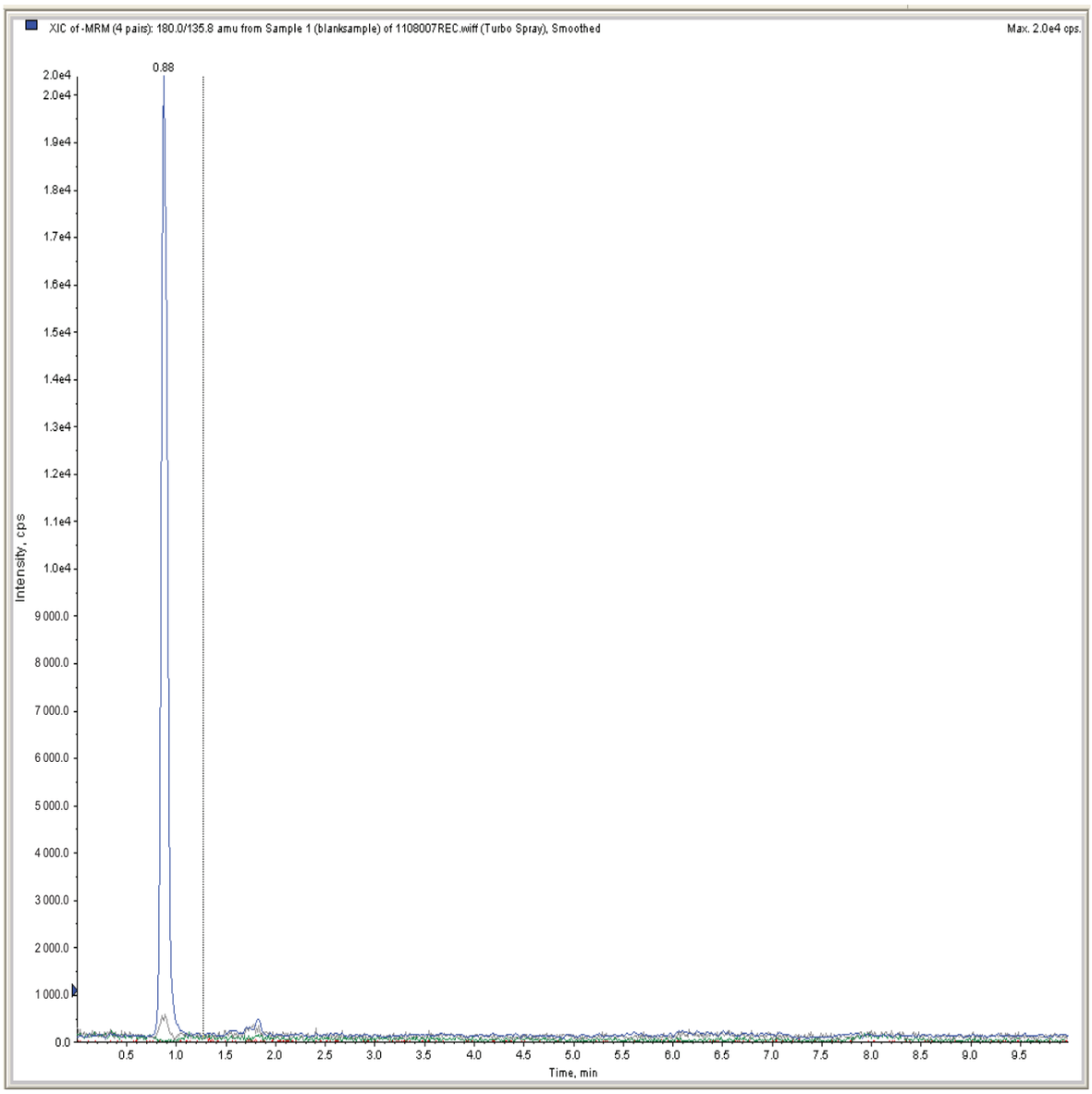

Figure 1. LC-MS/MS chromatogram of blank sample.

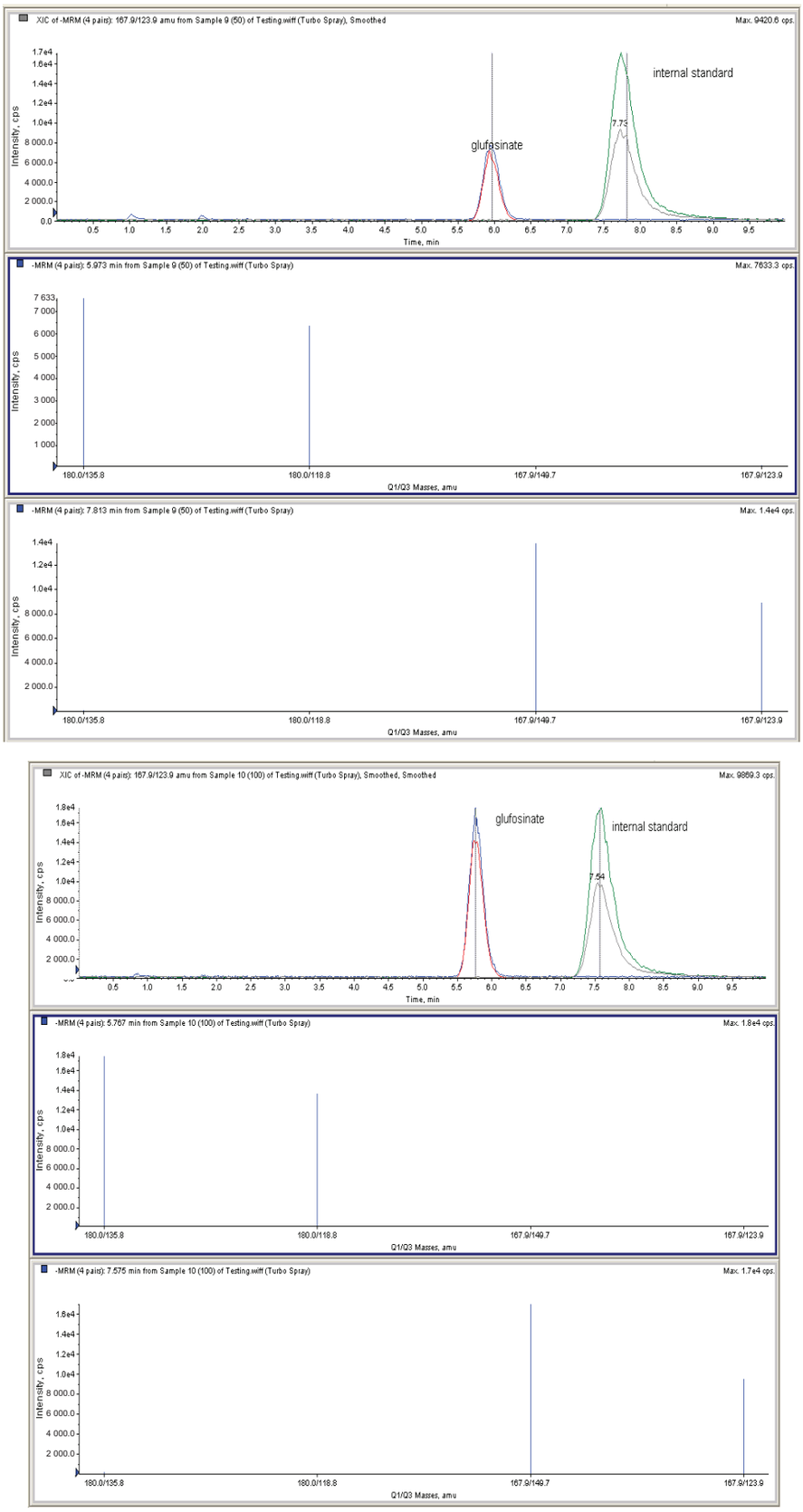

Figure 2. Mass spectrums of glufosinate ammonium working standard solutions.

	Sample Name	Sample ID	Sample Type	File Name	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Analyte Concentration	Standard Query Status	IS Peak Area (counts)	IS Pe
1	1		Standard	90807\Testing.wiff	1.13e+003	1.47e+002	1.00	N/A	3.48e+005	1.60e-
2	5		Standard	90807\Testing.wiff	9.49e+003	7.63e+002	5.00	N/A	3.54e+005	1.62e-
3	10		Standard	90807\Testing.wiff	2.05e+004	1.53e+003	10.0	N/A	3.63e+005	1.62e-
4	50		Standard	90807\Testing.wiff	1.09e+005	7.35e+003	50.0	N/A	3.55e+005	1.62e-
5	100		Standard	90807\Testing.wiff	2.40e+005	1.65e+004	100.	N/A	3.90e+005	1.69e-
6	500		Standard	90807\Testing.wiff	1.08e+006	7.24e+004	500.	N/A	3.43e+005	1.55e-

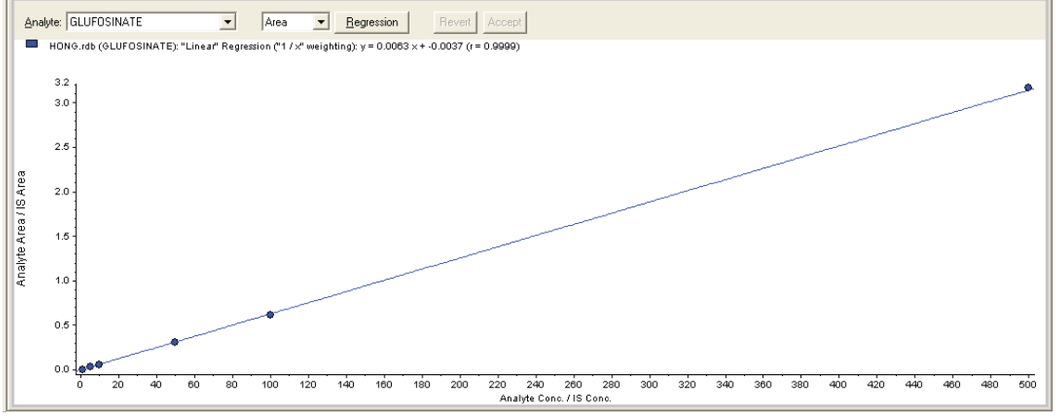

Figure 3. Linearity of glufosinate ammonium standard solutions calibration curve.

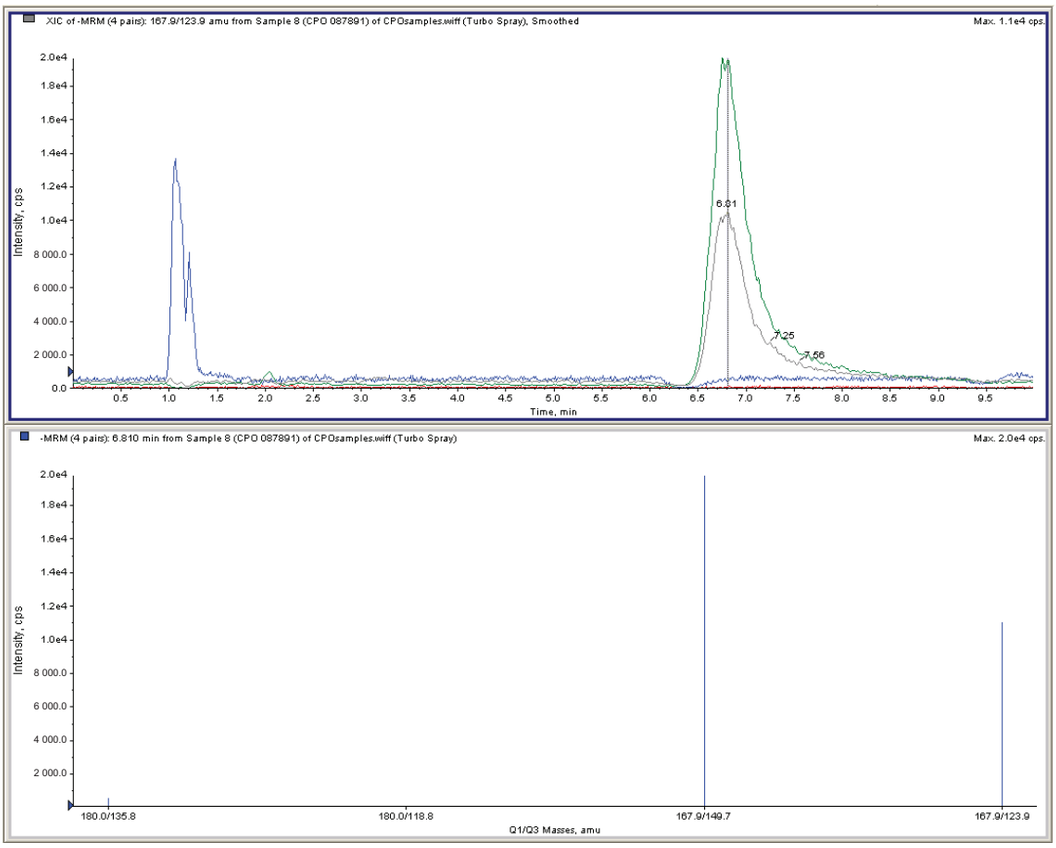

Figure 4. Mass spectrum of crude palm oil sample.

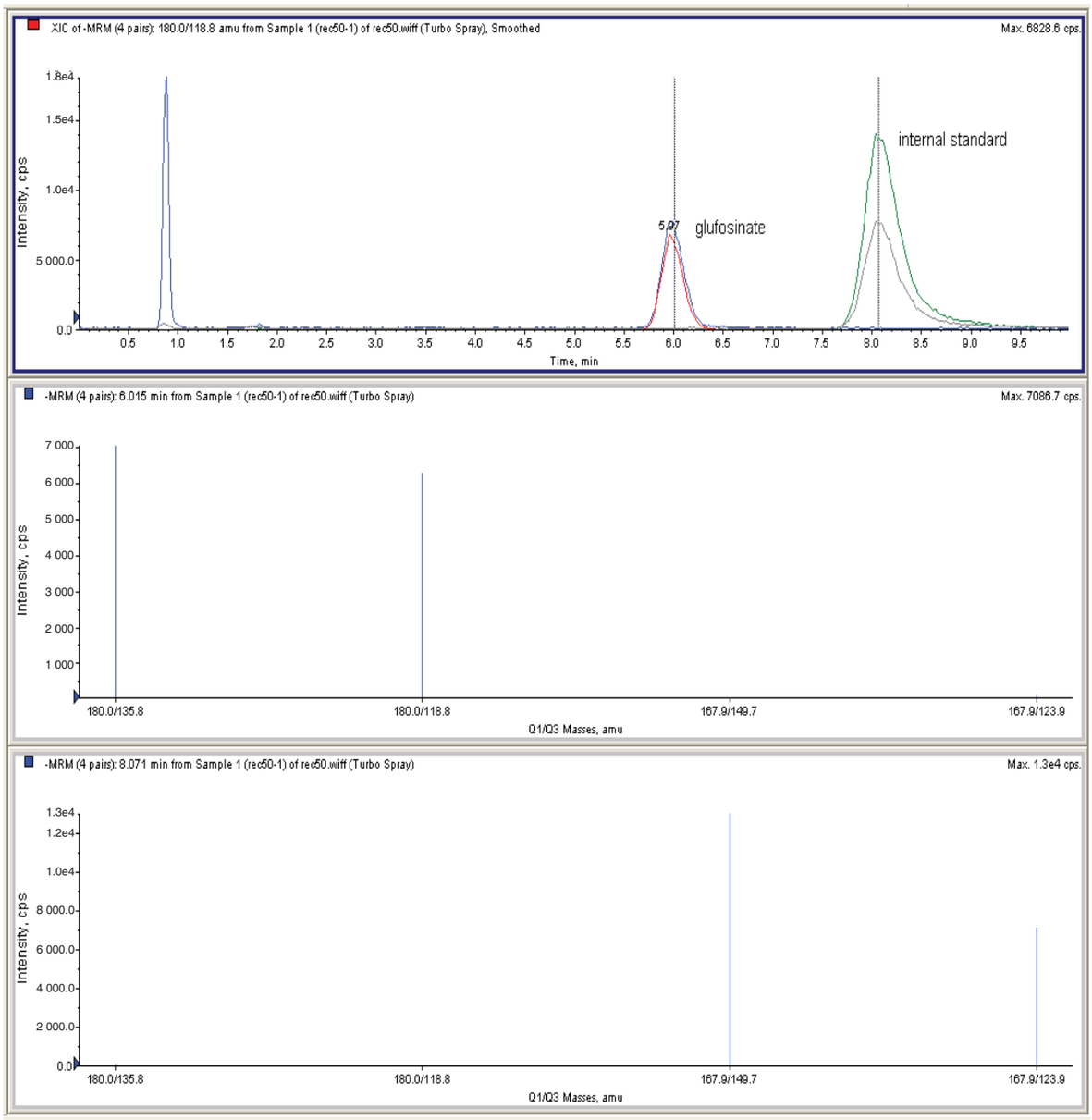

Figure 5. Mass spectrums of spiked sample at 50 µg kg^{-1} sample.

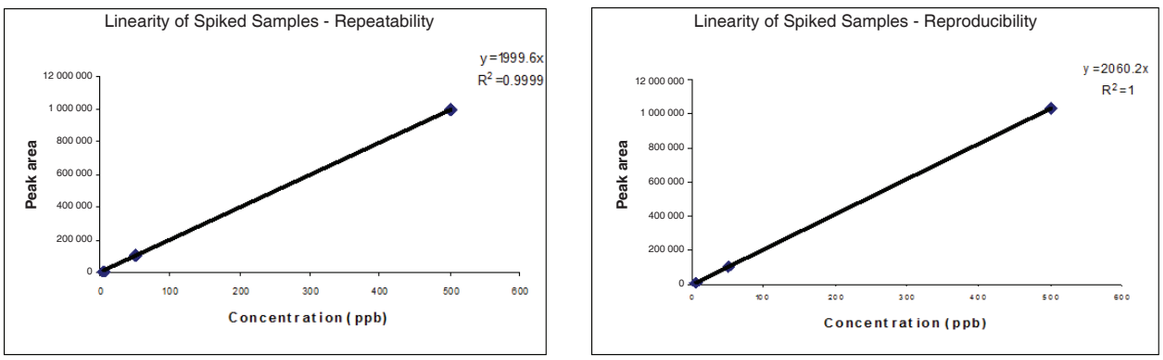

Figure 6. Matrix-matched calibration curves.

solutions. The regression coefficient (R^2) was 0.9999 which was acceptable for trace analysis.

Results for recovery studies, repeatability and reproducibility of the method are shown in *Table 2.* Recoveries from spiked samples were in the range of 77%-109% which were acceptable for trace analysis requirements with CV less than 10%. *Figure 5* shows the mass spectrum of spiked sample at 50 µg kg^{-1}. Results for repeatability and reproducibility for n = 7 showed the CV were less than 13%. The linearity of the matrix-matched was studied by plotting a linear recovery graph for the three levels of concentration as shown in *Figure 6*. The LOD and LOQ for this method were 0.002 mg $litre^{-1}$ and 0.025 mg kg^{-1} respectively. Thirty CPO samples were analysed for GA content and no residue was found in the sample as shown in *Figure 4.*

CONCLUSION

A modified QuEChERS-based method has provided a fast and effective way to prepare the oil matrix samples. This study successfully showed that this method was effective in providing just enough sample clean-up to avoid matrix interferences, while still maintaining low level analyte detection. The method was validated for recovery, repeatability and reproducibility. The results demonstrated that the modified method has achieved acceptable quantitative recoveries of 84%-109% with CV for repeatability and reproducibility of < 13% and 10% respectively for LC-MS/MS detection. The detection limit of 0.002 µg kg^{-1} obtained below the regulatory maximum residue limits for the herbicide was achieved.

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