Journal of Chromatography
Separation Techniques

Fitri et al., J Chromatogr Sep Tech 2017, 8:1
DOI: 10.4172/2157-7064.1000346

Research Article Open Access

A Rapid Liquid Chromatography Method for Determination of Gylphosate in Crude Palm Oil with Fluorescence Detection

Fariq Fitri MSM[1]*, Halimah Muhamad[2], Dzolkhifli Omar[1] and Norhayu Asib[1]

[1]*Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Malaysia*
[2]*Analytical and Quality Development Unit, Malaysian Palm Oil Board, No. 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Selangor, Malaysia*
China

Abstract

A rapid and simple method for the determination of glyphosate in crude palm oil (CPO) was developed and validated using high performance liquid chromatography with fluorescence detector. Glyphosate was derivatized with 9-fluorenylmethylchloroformate (FMOC-Cl) and then separated using a C_{18} reverse phase column with potassium dihydrogen phosphate and acetonitrile as the mobile phase. A linear correlation was obtained for the concentration of glyphosate from 0.05-1.5 µg mL^{-1} with a correlation coefficient of 0.9998. The average recovery obtained for glyphosate ranged between 80% and 100% at five fortification levels with the relative standard deviation (RSD) of less than 3% of all cases. The limit of detection and limit of quantification for glyphosate were 0.05 and 0.1 µg/g, respectively. The method will facilitate palm oil trade through quality assurance in terms of glyphosate residues in palm oil products and also to counter any issues related to food safety for palm based products.

Keywords: Palm oil; Glyphosate; Herbicide; Recoveries; HPLC; Fluorescence

Abbreviations: CPO: Crude palm oil; FLD: Fluorescence detector; FMOC-Cl: 9-fluorenylmethyl chloroformate; RSD: Relative standard deviation.

Introduction

Nowadays, the use of pesticides is one of the methods to control pests effectively with low management cost. However, its impact towards the environment should be taken into account as the use of pesticides poses risk on the safety of food products. It is indeed a concern if the residues still remain in the crops for in a long-term period, besides affecting food products. Thus, the determination of pesticide residue in palm oil should be used as an important parameter in ensuring the quality of vegetable oil produced. Moreover, monitoring the levels of pesticide residue content in palm oil is among the important consensus from the study on methods development for the determination of pesticide residues. This should be a priority to ensure that palm oil is free from any chemical residue for human safety and to meet the standard of waste management for a significant palm oil importer [1].

According to Gibon et al. [2] crude palm oil (CPO), which is rich in minor components, has high value nutrients, such as tocopherol and tocotrienol (vitamin E), as well as carotenoid (α and β-carotene). Meanwhile, Nuzul Amri et al. [3] reported that the properties of crude palm kernel oil (CPKO) are unchanged over the last 17 years based on a survey carried out for a year. In addition, several analytical methods for the determination of pesticide in palm oil matrices had been reported. Halimah et al. [4] developed an analytical method for the detection of chlorpyrifos in pure olein oil sample. The developed method was a modification of the method reported by Cloborn et al. [5] regarding the detection of chlorpyrifos residues in tissues and cow's milk. In this study, the researchers used liquid-liquid extraction involving n-hexane and acetonitrile solvents before the clean-up step was preceded with sililic acid chromatography column. The gas chromatography with electron capture detector (GC-ECD) was selected as the quantification method as the percentage of the recovery study was 97%.

Although a lot of researches on pesticide residue determination in palm oil had been carried out, the analytical methods for determining the residue of glyphosate in palm oil matrices via pre-column derivatization with FMOC-Cl have limited reports. The use of pre-column derivatization provides numerous advantages in terms of the use of non-complicated instruments, rapid, fewer restrictions, and efficient. The method developed, thus, should be able to facilitate palm oil trade through quality assurance in terms of the absence of glyphosate residues in palm oil products, as well as to counter any issue related to food safety for palm-based products.

Experimental

Materials and methods

Glyphosate standard with purity at >97.5% was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Meanwhile, HPLC grade acetone, 9-fluorenylmethylcholoroformate, potassium dihydrogen phosphate, disodium tetraborate, dichloromethane, and acetonitrile were obtained from MERCK (Darmstadt, Germany). Micro liter pipettes, adjustable between 100 and 1000 µL, and pipette tips were obtained from Eppendorf (Hamburg, Germany). Microvials were purchased from Agilent (Palo Alto, CA, USA) and vortex mix from Barnstead/Thermolyne Inc (Dubuque, IA, USA). Besides, a sonicator (Bransonic, USA) was also used. The extracts were filtered by using syringe filter (nylon, 0.45 µm) and both were purchased from Whatman (Maidstone, Kent, UK). Blank CPO that was used as a control had been obtained from MPOB Labu refinery, while blank CPKO was obtained from Felda Pandamaran in Pelabuhan Klang refinery.

***Corresponding author:** Fariq Fitri MSM, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Malaysia, Tel: +60387694214; E-mail: fariqfitri@gmail.com

Received April 04, 2016; **Accepted** December 27, 2016; **Published** January 02, 2017

Citation: Fitri MSMF, Muhamad H, Omar D, Asib N (2017) A Rapid Liquid Chromatography Method for Determination of Gylphosate in Crude Palm Oil with Fluorescence Detection. J Chromatogr Sep Tech 8: 346. doi: 10.4172/2157-7064.1000346

Instrumentation

The sample extracts were analyzed on an Agilent 1100 HPLC system equipped with a quaternary pump (model G1311A), an auto sampler (model G1313A), and a degasser (model G1322A). The temperature of the column heater was maintained at 40°C with a column heater temperature control module. Meanwhile, the glyphosate derivatives were detected with a fluorescence detector with excitation at 370 nm and emission at 415 nm. The analytical column was Waters C_{18} reverse phase column (250 mm × 4.60 mm i.d., 5 µm, XBridge Waters). The system was controlled by HP ChemStation (Agilent Technologies), which also performed functions, such as 1453 data collection from the FLD detector and quantitative measurements. The mobile phase used was potassium dehydrogen phosphate (50 mM, pH 2.5) and acetonitrile in gradient mode. The flow rate was 1.00 mL min^{-1} and the volume injected was 15 µL. The analytical column was set at 40°C and the samples were run for 30 min.

Preparation of stock standard solution

A stock standard solution of glyphosate was prepared at a concentration of 2000 µg/mL by dissolving 0.1 g of glyphosate in 15 mL deionized water and 35 mL of acetone in a 50 ml volumetric flask. Then, intermediate working standard solutions were prepared by diluting the stock solutions in deionized water to obtain glyphosate standards of 100 and 10 µg/mL. Finally, serial dilutions of the working standard solutions were prepared to obtain seven calibration solutions (1.0, 0.8, 0.5, 0.3, 0.1, 0.08, and 0.05 µg/mL) in deionized water. All the standard solutions were kept in scintillation vials at 4°C in the refrigerator.

Preparation of mobile phase solution

The mobile system consisted of 0.05 M KH_2PO_4 buffer phase for pre-column. A 6.8 g amount of KH_2PO_4 was dissolved in 1 L of deionized water and the pH was adjusted to 2.5 with H_3PO_4. The solution was filtered through a 0.45 mm membrane and degassed. The flow rate of this mobile phase was optimized and maintained at 1.0 mL/min.

Crude Palm Oil (CPO) and Crude Palm Kernel Oil (CPKO) samples for fortification

CPO and CPKO which were free from glyphosate, used as control were melted at 60°C in an oven. After homogenization by shaking the samples, recoveries of glyphosate were determined at fortification levels of 0.05, 0.08, 0.3, 0.5, and 1.0 µg/g by using oil samples. Then, an appropriate amount of the fortification solution was pipetted into a screw cap centrifuge tube containing 5.0 g of the CPO and CPKO sample. The mixture was then vortexed for 5 min. The extraction was carried out without any clean-up process, as described below, prior to HPLC analysis.

Derivatization with FMOC-Cl analysis

A total of 1 mL of the upper layer from the 50 mL screw cap centrifuge tube was taken after extraction and prior to derivatization before injecting into HPLC. A molar ratio of disodium tetraborate, acetone, and 0.01 M FMOC-Cl was studied before added to complete the derivatization process, followed by 5 min of vortex, and it was left for few hours for optimum derivatization period study before analysis (0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 hours).

Extraction optimization and Liquid-Liquid Extraction (LLE) analysis

Five g of CPO and CPKO samples were transferred into each 50-mL screw cap centrifuge tubes. Each sample was fortified with an appropriate volume of working standard solution in acetone: water (70:30, v/v) for the recovery experiment (based on fortification levels of 0.05, 0.08, 0.3, 0.5, and 1.0 µg/g). The spiked samples were mixed well by using a vortex mixer. Dichloromethane (10 mL) was added to the spiked sample in each tube and the mixtures were shaken for 3 min by using a vortex mixer before 5 mL of water was added. Then the mixture was shaken on the vortex mixer for about 5 min, and then, centrifuged for 30 min at 3000 rpm. The aqueous layer was separated from the oil layer for derivatization.

Method validation analysis

Typically, an analytical instrument must be determined of its efficiency level, linearity, and repeatability of injections before the instruments can be used for analysis. This is to ensure that the instruments are in good condition [6,7] Level of efficiency, linearity, and reproducibility for HPLC-FLD injections were determined by injecting a series of standard solution of glyphosate from 0.01, 0.05, 0.08, 0.1, 0.3, 0.5, to 1.0 µg/mL to sketch a calibration curve. Glyphosate standard calibration curve was prepared by plotting the peak area of the chromatogram as the y-axis against the concentration injected into the HPLC-FLD as the x-axis.

Repeatability and precision of the method developed are important criteria, in which the methods should be tested in the same condition, but by different operators and laboratories, known as inter-laboratory test. Intra-laboratory test was by two different operators/analysts at two different days. 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.

LOD and LOQ of instrument were determined by comparing the peak height of the chromatogram obtained with the height of instrument noise level (S/N). For LOD, peak height is three times higher than the level of noise (S/N ≥ 3) while the LOQ, the height of the peak is 10 times higher than the level of noise (S/N ≥ 10) [8-10].

Monitoring of CPO and CPKO samples analysis

As for the monitoring study, samples of crude palm oil were collected from 30 different refineries and producers (100 mL each), then obtained from Registration and Licensing Department, Wisma Sawit MPOB Kelana Jaya. Meanwhile, samples of crude palm kernel oil were obtained from 10 palm kernel oil refineries (100 mL each) throughout Malaysia. All samples were kept in a 100 mL brown bottle and stored in room temperature. All the samples were ready to be analyzed to determine the level of glyphosate residue by using the method that had been developed and optimized. Each sample was analyzed by 3 replicates.

Results and Discussion

Optimization of derivatization with FMOC-Cl

The derivatization process requires some reagents to react with the analyte to improve the physical and the chemical properties of the analyte. The main functions of the derivatization process are to change the molecular structure and the polarity of the analyte, to improve and to stabilize the separation of analyte, in addition to increase the detectability of the analyte. In some previous studies conducted by Bo et al. [11], Hanke et al. [12], as well as Nedelkoska and Low [13] several parameters, such as the optimization period of derivatization, temperature, concentration of reagents, and study on molar ratio, were carried out. This was to ensure that the process had been rapid and qualitative in order to minimize excess noise from FMOC-Cl.

In addition, a study on the optimization of the molar ratio of borate buffer, acetone and FMOC-Cl was successfully performed and gave a consistent, as well as good symmetry peak, as shown in Figures 1 and 2. On the other hand, the effect of derivatization period was studied and shown in Table 1. This study demonstrated that the optimum time was 30 min since there was no significant difference in intensity, retention time and the area for derivatization period longer than that. Hence, the relative standard deviation (RSD) values for peak areas, retention times and intensity by HPLC-FLD for standard solutions (0.5 µg/mL) were 0.4485%, 0.056% and 2.9453%, respectively. As the percentages of RSD were less than 3% the method developed had been satisfactory and could be repeated.

Extraction optimization and Liquid-Liquid Extraction (LLE)

In fact, various methods have been developed for determination of pesticides in environmental samples, either through the process of extraction or purification (separation and characterization). Some of the common extraction methods used are LLE and low temperature precipitation, while some of the common clean-up processes are SPE, MSPD, SPME, and GPC. However, 70% of the preparation methods that are often used to treat pesticide residues in fatty vegetable matrix are LLE, SPE, and GPC [14].

Liquid-liquid extraction (LLE) is a traditional method that has long been applied. LLE is also known as liquid phase extraction (LPE) and solid-liquid extraction (LSE), as LLE extraction techniques typically use one or more types of solvents. LLE is applied either by shaking manually or by using high-speed homogenization for separating the analyte from the solid samples or semi-solid by using a suitable solvent. Besides, solvents that are commonly used for extraction of pesticides in samples are acetonitrile, ethyl acetate, acetone, and n-hexane. The extraction method that employs glyphosate in palm oil samples was carried out by testing several types of solvents, the optimum volume of solvents, and the optimum period of extraction.

The extraction of trace residues from fatty foods or high lipid samples is problematic when the extracts contain a large amount of lipids that need to be removed. Since glyphosate is highly polar compound with high solubility in water (12.0 g/L) and very low solubility in oil matrix, the standard solution for fortification was prepared in the acetone: water solution because of high solubility of acetone towards oil. Based on solubility of glyphosate in non-polar solvent, acetone have the highest solubility (0.078 g/L) compare to ethyl acetate (0.012 g/L) and toluene (0.036 g/L). Besides, several ratios of dilution of acetone with water had been tested to determine the most suitable ratio that can meet the requirement to mix well with glyphosate. In this study, the ratio of 70:30 (acetone:water) had been identified as the most suitable.

To obtain a high percentage of recovery, the appropriate solvents selected to enhance of extraction is necessary. Extraction of glyphosate residues in palm oil was carried out by comparing the usage of organic solvents, such as dichloromethane and chloroform. For each selected solvents, some concentrations of 0.1, 0.5, and 1.0 ug/g were used to

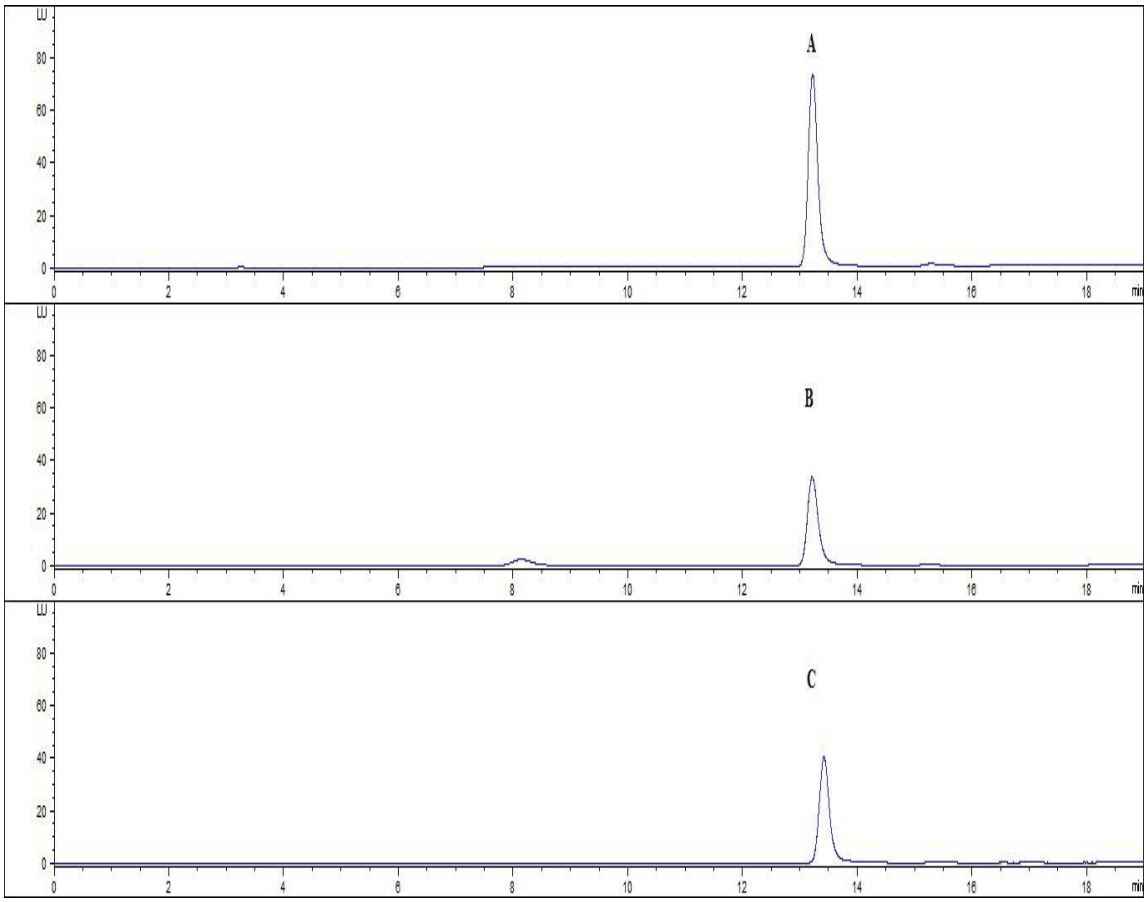

Figure 1: Glyphosate chromatogram (1.0 µg/ mL) at flow rate of 1.0 mL with molar ratio of borate buffer, acetone and FMOC-Cl (A) 1:1:0.5 (B), 1:0.5:0.5 and (C) 0.5:1:0.5.

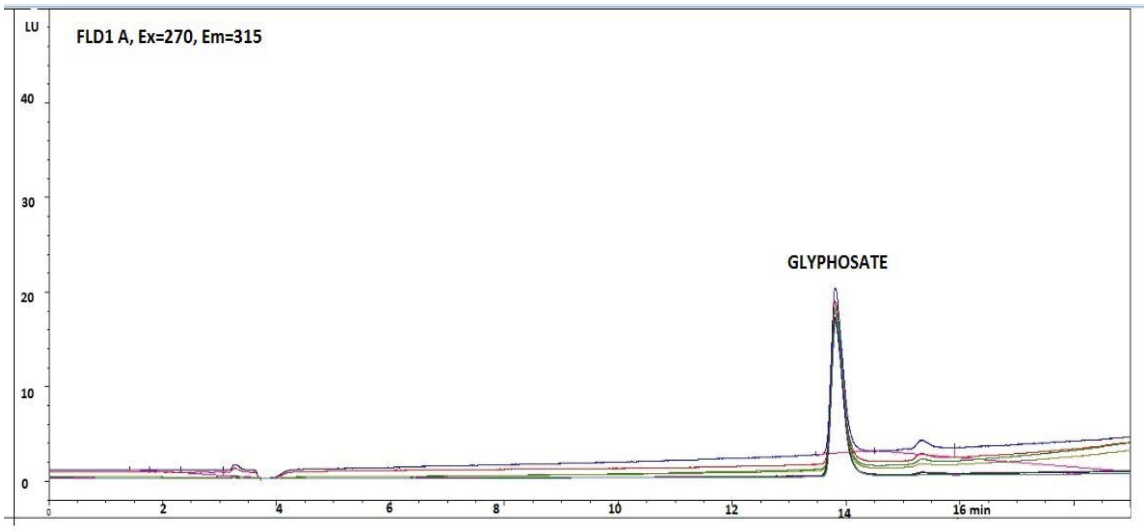

Figure 2: Overlay chromatogram of eight glyphosate standards at same concentration (0.5 µg/mL), with molar ratio of borate buffer, acetone and FMOC-Cl at 1:1:0.5.

Time (hour)	Area	Retention Time (min)	Intensity of peak
0.5	256.5399	13.82	17.50582
1	255.9935	13.821	17.16232
1.5	253.7324	13.823	17.07647
2	255.5514	13.825	16.17608
2.5	253.4689	13.814	16.58685
3	254.0001	13.81	16.36814
3.5	254.0706	13.819	16.63254
4	254.5403	13.836	16.16225
RSD (%)	0.4485	0.056	2.9453

Table 1: Optimum derivatization period for pre-column analysis (n=3).

compare the percentage of recovery. The result from the Table 2 shows that both solvents can be used in the extraction of glyphosate in palm oil samples, in which the percentage of recoveries ranged from 70-100%. The use of chloroform solvent gave a percentage of recovery at 74-83% with RSD range between 1.34% and 7.8% in contrast to the use of dichloromethane, which gave the percentage recoveries between 87 and 100% with RSD ranged from 1.11%-1.87%.

In addition, Chen et al. [15] agreed that the use of dichloromethane to replace the chloroform usage can reduce carcinogenic risk. Thus, dichloromethane was selected as the extraction solvent for this extraction study. According to Gelsomino et al. [16], the use of dichloromethane and acetone in extraction can completely remove the co-extractive hydrophilic that interferes with the analysis because of its properties. Dichloromethane was also used to separate and trap the lipid from the extract by separating the lipids into the dichloromethane layer. Without dichloromethane, oil droplets were still observed in the aqueous layer. Hence, centrifugation for 30 min after vortex for 5 min was necessary to ensure that all analytes were partitioned into the aqueous layer.

Furthermore, the method for extracting glyphosate in food matrices with high fat content was carried out by using DCM and water. Glyphosate with its polar nature dissolves in water, while the long chain fatty component dissolves in DCM solvent. This is because; the fat component with high hydrocarbon chains is likely to dissolve in non-polar solvents, such as DCM [14]. Therefore, based on the results and observations obtained, a simple and efficient method of extraction for determining glyphosate residues in CPO and CPKO was successfully developed.

Method validation

Linearity: Typically, an analytical instrument must be determined of its efficiency level, linearity, and repeatability of injections before the instruments can be used for analysis. This is to ensure that the instruments are in good condition [6,7]. Level of efficiency, linearity, and reproducibility for HPLC-FLD injections were determined by injecting a series of standard solution of glyphosate from 0.01, 0.05, 0.08, 0.1, 0.3, 0.5, to 1.0 µg/mL to sketch a calibration curve. Glyphosate standard calibration curve was prepared by plotting the peak area of the chromatogram as the y-axis against the concentration injected into the HPLC-FLD as the x-axis (Figure 3). The equation derived from the standard calibration curve was y=572.03x+3, and the regression coefficient (R^2) was=0.9998. This showed that the response of the HPLC-FLD detector to glyphosate residues had been very good where the linearity factor was at R^2>0.999.

Limit of Detection (LOD) and Limit of Quantification (LOQ): Anon [17] reported that limit of detection (LOD) is necessary to verify the analytical method that had been developed. LOD is also defined as minimum concentration at which the analyte can be identified and reported at the level of 99%, where the analyte concentration exceeds zero and it is determined from the analysis of samples containing the analyte [18]. Moreover, LOD statistically determines or explains the measurement of compound analyte carried out by using analytical protocols to distinguish the measurement control (blank) with interference (background noise) [19]. On the other hand, limit of quantification (LOQ) is the result obtained at a certain confidence level that is greater than the quantitative results. Therefore, LOD and LOQ were determined for glyphosate in this study. The method used in this study to determine the LOD and the LOQ was based on the methodology proposed by the EPA, as reported by Corley [20] and EPA [21] For the determination of LOD for all the methods developed, three lowest concentrations expected, which were 0.01, 0.03, and 0.05 ppm, had been added into all the crude palm oil and crude palm kernel oil samples. Each concentration was then analyzed with seven replicates with a control (blank sample) to ensure the consistency of the instrument. The analysis of both CPO and CPKO showed that the

Glyphosate	0.1 µg/g		0.5 µg/g		1.0 µg/g	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Dicholoromethane (DCM)	92	1.47	87	1.12	90	1.87
Choloroform	79	1.34	83	5.76	74	7.80

Table 2: Recoveries of glyphosate with two different solvents (n=3) at three different concentrations (0.1 µg/g, 0.5 µg/g and 1.0 µg/g).

values of LOD and LOQ were 0.01 µg/g and 0.05 µg/g, respectively. Based on these results, it can be concluded that the developed method had been suitable for detecting residues of glyphosate in palm oil.

Recovery and precision: According to Anon [22], the percentage of recoveries value accepted at the global stage is between 70% and 110% with the RSD value <20% in certain conditions. APVMA [23], on the other hand, outlined several criteria that need to be considered in determining the percentage of recovery, such as sample matrix, sample processing procedure, and analyte concentration. The accuracy and the precision of the methods were tested by adding five series of standard concentration of glyphosate into CPO and CPKO. Both CPO and CPKO were then prepared by using the optimized method before they were ready to be analyzed with HPLC-FLD. The percentage of recovery obtained from the five replicates for each concentration in CPO and CPKO are shown in Tables 3 and 4. The percentage recovery of glyphosate in CPO added with concentration of 0.05 - 1.0 ug/g were 85-90%, while for CPKO sample, it was 87-92%. Meanwhile, the percentage of RSD obtained for CPO was 1.13 - 2.47%, whereas for CPKO, it was 0.04-0.90%. As the percentages of recovery achieved for both CPO and CPKO had been above 80% with % RSD less than 3%; the method developed had been satisfactory and could be repeated.

Conclusion

Method development for determination of glyphosate in palm oil matrices had been conducted via HPLC-FLD optimization with three parameters, which were selection of mobile phase, FLD wavelength, and flow rate of mobile phase. From the results obtained, it had been discovered that the use of acetonitrile and KH_2PO_4 with a flow rate at 1.0 mL/min was the most suitable, while the selected FLD wavelength was at excitation of 370 nm and emission of 415 nm. Besides, optimization of HPLC-FLD was carried out to determine the best operation condition to achieve the most apt glyphosate chromatogram in the analysis of glyphosate residue.

Moreover, the accuracy and the precision of the method had been based on the percentage of recovery and the percentage of relative standard deviation. For CPO, the recovery and % RSD obtained were in the range of 85-97% and 1.1-2.5%, meanwhile 87-92% and 0.04-0.9% for CPKO, respectively. Besides, repeatability of the method was measured with percentage of recovery and % RSD via intra-laboratory test. The percentage of recovery and % RSD obtained from both the operators were in the range of 91-96% and 3.3-4.5% for CPO, while 90-95% and 0.5- 1.3% for CPKO.

Meanwhile, the values of limit of detection (LOD) and limit of quantification (LOQ) obtained for CPO and CPKO were 0.01 and 0.05 ug/g for both, respectively. This proved that the analytical method developed had been precise, simple, accurate, and efficient. Therefore, this method had been determined as suitable to be used to analyze and monitor glyphosate residue in CPO and CPKO.

Acknowledgements

The authors would like to thank the Malaysian Palm Oil Board (MPOB) for financial support and the Director General of the MPOB for permission to publish this paper. The authors are also indebted to Puan Rosilawati and Puan Rosliza for their excellent technical assistance and discussion.

Level of Spiking (µg/g)	Mean Recovery (%)	RSD (%)
0.05	**86**	2.47
0.08	**97**	1.18
0.3	**85**	1.81
0.5	**87**	1.13
1.0	**90**	1.87

Table 3: Recovery and statistical data obtained from analysis of glyphosate in CPO samples (n=5).

Level of Spiking (µg/g)	Mean Recovery (%)	RSD (%)
0.05	**87**	0.44
0.08	**91**	0.45
0.3	**90**	0.90
0.5	**88**	0.61
1.0	**92**	

Table 4: Recovery and statistical data obtained from analysis of glyphosate in CPKO samples (n=5).

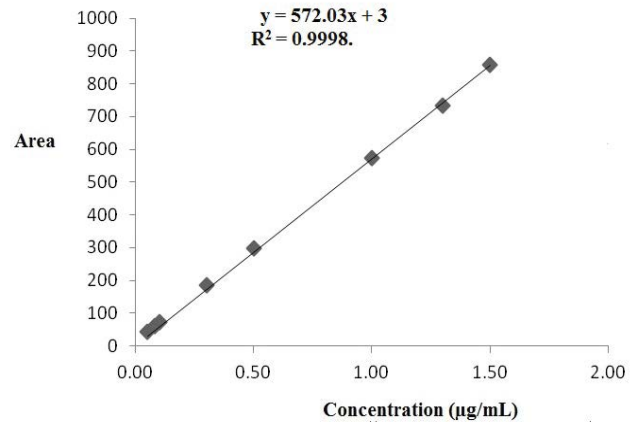

Figure 3: Calibration curve for glyphosate prepared in water (0.1-1 µg/mL).

References

1. Ainie K, Tan YA, Norman K, Yeoh CB (2007) Pesticide Application in oil palm plantation. Oil Palm Bulletin 54: 52-67.
2. Gibon V, De Greyt, Kellens M (2007) Palm Oil Refining. European Journal of Lipid Science and Technology 109: 315-335.
3. Nuzul Amri I, Ainie K, Tang TS, Siew WL (2003) Current status of Malaysian crude palm kernel oil characteristics. Oil Palm Bulletin 47: 15-27.
4. Halimah M, Osman H, Ainie K, Tan YA, Md Pauzi A (1999) Determination of chlorpyrifos in refined palm olein by GC-FPD and GC-ECD. Journal of Oil Palm Research 11: 89-97.
5. Cloborn VH, Mann HD, Oehler DD (1968) Dursban determination in milk and body tissues of cattle. J AOAC int 51: 1243-1245.
6. Halimah BM (2000) Pembangunan kaedah analisis residu klopirifos di dalam minyak sawit. Tesis Sarjana, Pusat Pengajian Sains Kimia & Teknologi Makanan, Fakulti Sains & Teknologi, Universiti Kebangsaan Malaysia.
7. Halimah BM (2006) The environmental fate of of fluroxypyr and chlorpyrifos in oil palm agroecosystem. Tesis Ijazah Doktor Falsafah, Pusat Pengajian Sains Kimia & Teknologi Makanan, Fakulti Sains & Teknologi, Universiti Kebangsaan Malaysia.

8. Cesnik BH, Gregorcic A (2006) Validation of the method for the determination of dithiocarbamates and thiram disulphide on apples, lettuce, potato, strawberry and tomato matrix. Acta Chemica Slovenica 53: 100-104.

9. Blasco C, Font G, Pico Y (2004) Determination of dithiocarbamate and metabolites in plants by liquid chromatography-mass spectrometry. Journal of Chromatography A 1028: 267-276.

10. Walia S, Sharma RK, Parmar BS (2009) Isolation and simultaneous LC analysis of thiram and its less toxic transformation product in DS formulation. Bulletin Environmental Contamination Toxicology 83: 363-368.

11. Bo L, Xiaojun D, Dehua G, Shuping J (2007) Determination of glyphosate and aminomethylphosphonic Acid Residues in Foods Using High Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry. Chinese Journal of Chromatography 25: 486.

12. Hanke I, Singer H, Hollender J (2008) Ultratrace-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural water by solid-phase extraction followed by liquid chromatography-tandem mass spectrometry: performance tuning of derivatization, enrichment and detection. Analytical and Bioanalytical Chemistry 391: 2265-2276.

13. Nedelkoska TV, Low KCG (2004) High performance liquid chromatography determination of glyphosate in water and plant material after pre-column derivatisation with 9-fluorenylmethyl chloroformate. Analytica Chimica Acta 511: 145.

14. Gilbert-Lopez B, Garcia-Reyes JF, Molina-Diaz A (2009) Sample treatment and determination of pesticides residues in fatty vegetable matrices. Talanta 79: 109-128.

15. Chen IS, Shen CSJ, Sheppard AJ (1981) Comparison of methylene chloride and chloroform for the extraction of fats from food products. JAOCS, pp: 599-601.

16. Gelsomino A, Petrovicova B, Tiburtini S, Magnani E, Felici M (1997) Multiresidue analysis of pesticide in fruits and vegetables by gel permeation chromatography followed by gas chromatography with electron-captured and mass spectrometry detection. Journal of chromatography A 782: 105-122.

17. Anon (2002) Method detection limit (MDL) and reporting limit (RL/DLR) requirements for ELAP certification.

18. USEPA (1994) Method detection limit-Definition in in 40 CFR. Part 136, Appendix B, Washington: USEPA.

19. Anon (1996) Analytical detection limit guidance and laboratory guide for determining method detection limits.

20. Corley J (2003) Best practices in establishing detection and quantification limit for pesticide residues in food. Philip WL, Aldos CB, John JM (eds.), Handbook of Residue Analytical Methods for Agrochemicals. USA: John Wiley & Sons Ltd.

21. EPA (Environmental Protection Agency) (1996) Results of the EPA method 1631 validation study.

22. Anon (2008) European Commission. Guidance document on residue analytical methods.

23. APVMA (Australian Pesticides and Veterinary Medicines Authority) (2004) Guidelines for the validation and analytical methods for active constituent, agricultural and veterinary chemical products.