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Full Length Research Paper

Development of method for residue analysis of three herbicides in the soil by high performance liquid chromatography (HPLC)

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Contamination of soil and water resources by herbicides is an increasing environmental concern. Soil plays an important role in agro-ecosystem and the environment, but information for analysis of herbicides residue in the soil can be very difficult to come by. Laboratory experiment was conducted to simplify analytical methods of the residue of paraquat, glyphosate, and glufosinate-ammonium in soil through development and modification from previously published methods. The high performance liquid chromatography (HPLC) system used for analyzing paraquat-dichloride consisted of model 600 controller multi-solvent delivery system, model 717 plus auto-sampler and model 2996 photodiode array detector. The HPLC system used to analyze glyphosate and glufosinate-ammonium consisted of model 501 solvent delivery system, model 7125 manual injector equipped with a 20 µl loop, and model 470 scanning fluorescence detector. Results showed that developed procedures and HPLC instruments used were acceptable, simple, easy, accurate, safe and efficient in separating paraquat, glufosinate-ammonium and glyphosate as indicated by the calibration curve and recovery of spiked soil samples. Using short and small size of C_{18} column and high flow rate produced shorter retention time of paraquat. Adding acetone and washing with ethyl acetate was important derivatization steps for glufosinate-ammonium and glyphosate. The improved methods can be used in evaluating herbicide residues in the soil and water easily and accurately.

Key words: Herbicide residue, soil, HPLC, paraquat, glyphosate, glufosinate-ammonium.

INTRODUCTION

Herbicides are used quite extensively in most farming systems. Herbicides, when applied to the field do not only control targeted weeds, but may also leave unwanted residues in the soil, which are ecologically harmful (Haney et al., 2000; Derksen et al. 2002; Riaz et al., 2007). Although the efficacy of herbicide in controlling weeds is very high, its residual impact should also be considered for environmental safety. Preferred herbicides should not only have good efficacy, but also poses minimum adverse effects on crops, ecology, and the environment (Faheed and Abd-Elfattah, 2007).

Paraquat, glyphosate, and glufosinate-ammonium are among the most commonly used herbicides (DFP, 2011). Usage of these herbicides in the oil palm plantation

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causes damage on the environment (soil, water, and air) and adverse effects to non-targeted organisms (aquatic and terrestrial organisms) (Wahyu et al., 2010).

Contamination of soil, water resources, and agricultural products by herbicides is an increasing environmental concern (Ouyang et al., 2004; Akinloye et al., 2011). Bioassay and chromatography are among the several methods commonly used to determine pesticide residue (Wahyu et al., 2009; 2010). Various methods have been used for the analysis of paraquat, glyphosate and glufosinate ammonium, but the method and information for analysis of herbicides residue in the soil is very difficult to come by.

Analysis of paraquat residue in the soil was modified from many kinds of methods reported by Kirsten, 1966; Khan, 1974; Worobey, 1987; Chichila and Walters, 1991; Kennedy, 1992; Ahmad, 1993; Schuster, 1997; Kuntom et al., 1999; Grey et al. 2002 and Ouyang et al. 2004. Analysis of glyphosate and glufosinate-ammonium residues in the soil was improved from various methods developed by Deyrup et al. 1985; Roy and Konar, 1989; Eberbach and Douglas, 1991; Lovdahl and Pietrzyk, 1992; Schuster and Gratzfeld–Husgen, 1992; Alferness and Iwata, 1994; Sancho et al. 1994; Kataoka et al. 1996; Chang and Liao, 2002.

Because soils are important part of any agro-ecosystem and environment, simple, accurate, and safe methods should be conducted during its use. The expected methods are not only simple, accurate and safe but also more cost effective. The objective of this study was to simplify analytical methods of residue of paraquat, glyphosate, and glufosinate-ammonium in soils through modification from previously reported methods.

MATERIALS AND METHODS

Soil samples used for the analysis of paraquat, glyphosate, and glufosinate-ammonium residues were taken from MAB Agriculture-Horticulture Plantation Sepang, Selangor. These samples were air dried for 2-4 days at room temperature, pulverized, and passed through a 2mm sieve. These samples were placed into black polyethylene bags and then refrigerated at 5°C until use.

Soil texture used in the experiment is classed as sandy, clay and loam with a composition of 32.3% clay, 7.1% silt, and 60.6% sandy. Soil chemical properties are as follows: pH 5.03, C organic 1.14%, N 0.75 g/kg, P 0.05 g/kg, K 0.07 g/kg, Ca 0.02 g/kg, and Mg 0.03 g/kg.

Paraquat analysis

The standard of paraquat-dichloride was prepared according to procedures described by Ahmad (1983); Chichila and Walters (1991); Grey et al. (2002) and Ouyang et al. (2004). Analytical standard was heated in the oven for 3 to 4 h at 100 to 110°C and cooled in a desiccator. Stock solution (500 ppm) was prepared by dissolving 0.025 g paraquat-dichloride standard in 50 ml distilled water. The distilled water was processed using water purification system (ELGA, USA). The soil was fortified according to the procedures described by Khan (1974); Worobey (1987); Miles and Moye (1988) and Ouyang et al. (2004). Spiked soil samples for residue analysis were prepared in duplicates.

Extraction and clean-up were done on the fortified soil samples. The extraction and clean-up were adopted and modified based on the procedure described by Worobey (1987). The solution was transferred into chromatographic vials and ready to be injected into the HPLC for the detection of residues.

The HPLC analysis was prepared based on the procedure as described by Ouyang et al. (2004). The HPLC system used for analyzing paraquat-dichloride consisted of models 600 controller multi-solvent delivery system, model 717 plus auto-sampler and model 2996 photodiode array detector (PAD) (Waters, USA). Analytical column used was C_{18} column: 3.9 × 150 mm i.d. from Waters Ireland. The HPLC mobile phase was prepared by dissolving 5.0 g NaCl into 600 ml distilled water that was previously adjusted to pH 3.0 with HCl, and then the solution was mixed with 400 ml acetonitrile. Flow rate of the mobile phase was 1.0 ml/m. PAD detector wavelength was set at 257 nm. A 20 µl volume of each working standard and sample was injected into the chromatographic column by auto-sampler system. Recording of chromatograms and quantitative measurement of peak area were performed with a computer which was connected with Empower software (Waters, USA).

Glyphosate and glufosinate-ammonium analysis

The HPLC system used to analyze glyphosate and glufosinate-ammonium consisted of model 501 solvent delivery system (pump A and B), model 7125 manual injector equipped with a 20 µl loop (Rheodyne, USA) and model 470 scanning fluorescence detector (Waters, USA). A 100-µl micro-syringe (Hamilton, USA) was used to deliver sample to loop of injector. Analytical column was C_{18} column: 250 x 4.6 mm i.d. SS EXSIL ODS 5µm (SGE, Australia).

Stock and working standard solution of glyphosate and glufosinate-ammonium were prepared according to the procedures described by Miles et al. (1986); Lovdahl and Pietrzyk (1992) and Nedelkoska and Low (2004). Soil fortification was prepared according to the procedures described by Miles and Moye (1988) and Sancho et al. (1994). Spiked soils for residue analysis were prepared in duplicate. Extraction was conducted as procedures described by Miles and Moye (1988); Kataoka et al. (1996) and Sancho et al. (1994). Neutralized supernatant was centrifuged for 3 m at 5000 rpm prior to pre-column derivatization. Pre-column derivatization used aceton, acetonitrile, 0.025 M borate buffer (pH 9), 0.01 M FMOC-Cl, and ethyl acetate. Pre-column derivatization was modified from procedures as described by Miles et al. (1986); Schuster and Gratzfeld-Husgen (1992) and Nedelkosta and Low (2004). Un-reacted FMOC-Cl was removed from the medium by shaking the mixture with 1 ml of ethyl acetate for 1 m and let to stand until 2 layers were formed. The bottom layer portion was transferred into polypropylene micro-centrifuge tube. These samples were ready for chromatographic analysis on the C_{18} column.

Mobile phase for glyphosate analysis was prepared as described by Sancho et al. (1994). The mobile phase consisted of acetonitrile – 0.002 M phosphate buffer pH 6.3 (7.5: 92.5, v/v). Before use, the mobile phase was filtered and soniced for 30 m. The mobile phase was delivered at 1.2 ml/m. The fluorescence detector was set with emission at 266 nm and extinction at 317 nm, attenuation at 64, and gain at 10 times. A 10 µl of solution obtained after derivatization were injected to a 20 µl loop. Recording of chromatograms and quantitative measurement of peak area were

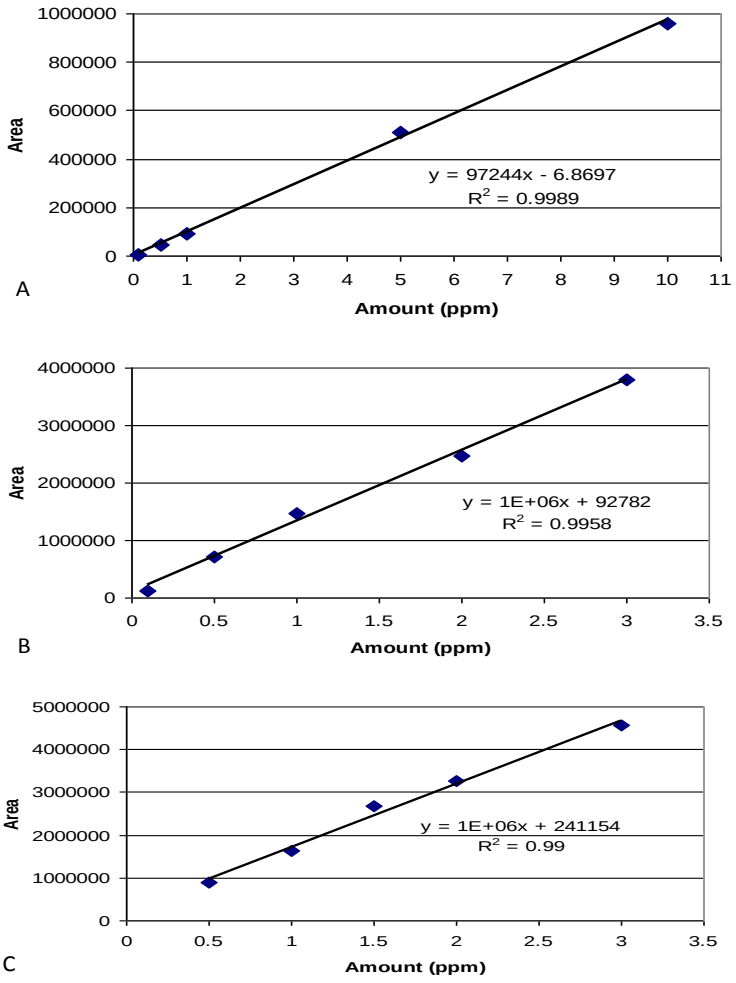

Figure 1. Calibration curve of paraquat (A), glyphosate (B), and Glufosinate-ammonium (C).

performed with a computer connected with Empower Software (Waters, USA). Mobile phase for glufosinate-ammonium analysis was prepared as described by Sancho et al. (1994). Before used, the mobile phase was filtered and soniced for 30 m. The mobile phase was delivered at 1.0 ml/m. The fluorescence detector was set with emission at 266 nm and extinction at 317 nm, attenuation at 64, and gain at 10 times. A 20 µl of solution obtained after derivatization were injected to a 20-µL loop. Recording of chromatograms and quantitative measurement of peak area were performed with a computer connected with Empower Software (Waters, USA).

RESULTS AND DISCUSSION

Calibration of working standard solution was used to test the ability of procedures and instruments for determination paraquat, glyphosate and glufosinate-ammonium. Linearity of calibration was assessed from a linear regression of response (area) versus concentration of paraquat, glyphosate, and glufosinate-ammonium in solution (ppm). Result showed that procedures and instrument used had good ability in separating paraquat, glyphosate and glufosinate-ammonium indicated by calibration curve (Figure 1). Response of paraquat was linear for working standard solution of paraquat at concentrations of 0.1, 0.5, 1.0, 5.0, and 10.0 ppm (R^2 = 0.999, n = 5). Response of glyphosate was linear for five working standard solutions (R^2 = 0.996, n = 5). Response of glufosinate-ammonium was linear for five working standard solutions (R^2 = 0.99, n = 5). Chromatograms of the working standard solution of paraquat (0.1, 0.5, 1.0, 5.0 and 10.0 ppm) were shown in Figure 2. Peak height and retention time of each standard solution was pointed out clearly by the overlaid chromatogram. Average retention time of paraquat was 1.31 m. Run time of paraquat standard solution was for 5.0 m. Recoveries of paraquat were 78.45 ± 0.46%, 87.80 ± 0.27, and 92.48 ± 6.87% at

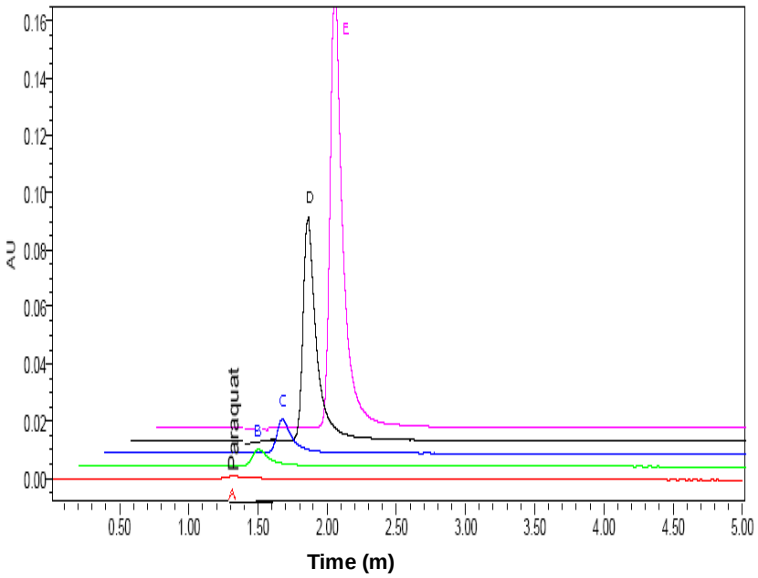

Figure 2. Overlaid chromatograms of paraquat standard solution at 0.1 ppm (A), 0.5 ppm (B), 1.0 ppm (C), 5.0 ppm (D), and 10.0 ppm (E).

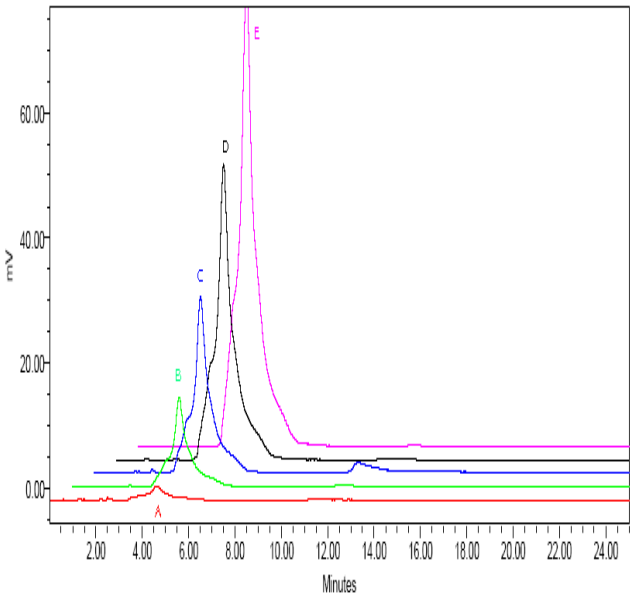

Figure 3. Overlaid chromatograms of glyphosate standard solution at 0.1 ppm (A), 0.5 ppm (B), 1.0 ppm (C), 2.0 ppm (D), and 3.0 ppm (E).

0.5, 5.0 and 10.0 ppm fortification concentrations, respectively.

Figure 3 showed chromatograms of the five working standard solutions of glyphosate (0.1, 0.5, 1.0, 2.0 and 3.0 ppm). These chromatograms displayed the retention time and peak height clearly. Average of retention time of glyphosate was 4.64 m. Run time of glyphosate standard solution was for 25.0 m. Recoveries of glyphosate were93.3 ± 0.64% at 0.1 ppm and 92.60 ± 7.85% at 0.5 ppm fortification concentrations.

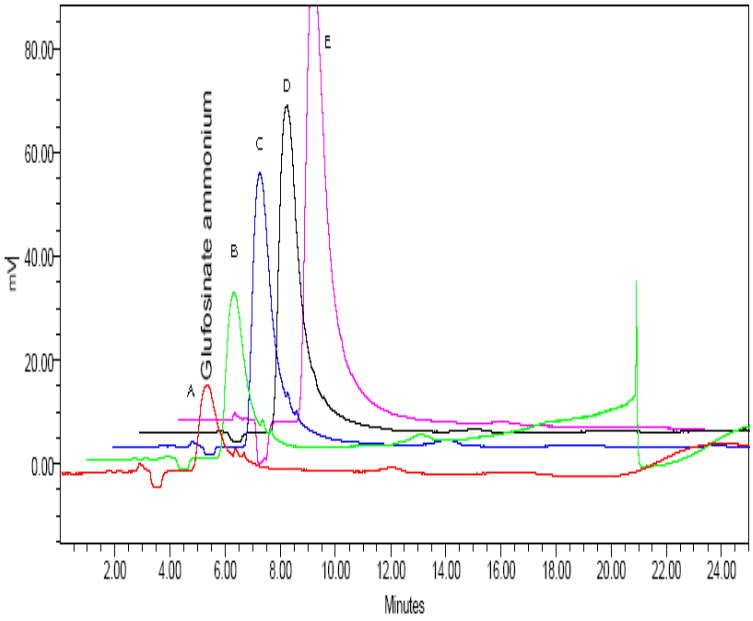

Figure 4. Overlaid chromatograms of glufosinate-ammonium standard solution at 0.5 ppm (A), 1.0 ppm (B), 1.5 ppm (C), 2.0 ppm (D) and 3.0 ppm (E).

Figure 4 showed chromatograms of the five working standard solutions of glufosinate-ammonium (0.5, 1.0, 1.5, 2.0 and 3.0 ppm). These chromatograms clearly illustrated retention time and peak height. Average of retention time of glufosinate-ammonium was 5.36 m. Run time of glufosinate ammonium standard solution was for 25.0 m. Recoveries of glufosinate-ammonium from sandy, clay and loam soils were 84.27 ± 1.04% at 0.5 ppm and 95.78 ± 15.38% at 2.5 ppm fortification concentrations.

Procedures and instruments used were acceptable for determination of paraquat, glufosinate-ammonium, and glyphosate in the soil indicated by linearity of calibration curve and percentage recovery of spiked soil samples (Table 1). Regression between response (area) and concentration of the herbicides (ppm) had R square value of 0.999, indicating that 99.90% variance of area could be explained by the concentrations of the herbicides (ppm).

In this experiment, retention time of paraquat was achieved at 1.31 m (Figure 2). Using short and small size of C_{18} column (3.9 × 150 mm from Waters, Ireland) and high flow rate (1 ml/m) produced shorter retention time than retention time reported by Schuster (1997) (3.0 m) and Ouyang et al. (2004) (4.35 m). Ouyang et al. (2004) used silica analytical C_{18} column (250 × 4.6 mm i.d. from Alltech Associates, IL) with a flow rate of 1.0 ml/m, whereas Schuster (1997), used analytical C_{18} column (100 × 2.1 mm i.d. Hypersil ODS) with a flow rate of 0.4 ml/m. Various retention time of paraquat were reported, namely, 4.2 m (Ahmad, 1983), 29 m (Worobey, 1987), 5.6 m (Chichila and Walters, 1991), 3 m (Schuster, 1997), and 4.35 m (Ouyang et al., 2004). Retention time was affected by many factors such as size and types of column, pH and composition of mobile phase, and flow rate used (Win and Brian, 2003).

Retention time of glyphosate standard solution was achieved at 4.64 m (Figure 3). Various retention time of glyphosate were reported, namely, 4.0 m (Glass. 1983), 6.0, 10.0, and 16.0 m (Miles et al., 1986), 11.0 m (Miles and Moye, 1988), 15.0 m (Kawai et al., 1991), 8.0, 14.0, and 22.5 m (Lovdahl and Pietrzyk, 1992), 14.0 and 17.0 m (Schuster and Gratzfeld-Husgen, 1992), 14.0 m (Spann and Hargreaves, 1994), and 7.3 m (Chang and Liao, 2002). Many factors affected retention times, such as stationary phase, size and type of column, number of column used (single or coupled), derivatization (pre or post-column), pH and solution composition of mobile phase, and injection volume (Win and Brian, 2003). Adding acetone and washing with ethyl acetate was important derivatization steps. Glyphosate is soluble in water and insoluble in organic solvents. FMOC-Cl is soluble in acetonitrile, not soluble in water but highly reactive with water to form FMOC-OH.

Pre-column derivatization modified from many authors, which was used in this analysis, gave clear and sharp peaks. Nedelkoska and Low (2004) stated that one of the main disadvantages of using FMOC-Cl is the interference of FMOC-OH, which is represented by the large peak in

Table 1. Recovery of paraquat, glyphosate, and glufosinate-ammonium residue.

Amount added to spiked samples (ppm)	Recovery (%)*	Standard Deviation
Paraquat		
0.5	80.02	0.39
5.0	87.80	0.27
10.0	92.48	6.87
Glyphosate		
0.1	93.30	0.64
0.5	92.60	7.85
Glufosinate-ammonium		
0.5	84.27	1.04
2.5	95.78	15.38

*Mean of duplicate with three readings for each replicate.

front of glyphosate chromatogram. Derivatization without adding acetone, and washing with ethyl acetate as described by Sancho et al. (1994). Schuster and Gratzfeld-Husgen (1992) produced interference and broad peaks of glyphosate standard solution. Average of retention time of glufosinate-ammonium was 5.36 m (Figure 4). Retention time usually can be increased by the decreasing ionic strength of the buffer. An increasing pH of mobile phase decreased the retention time (Miles et al., 1986). Kawai et al. (1991) reported that increased in the percentage of acetonitrile, decreased the retention time.

Conclusion

Procedures and HPLC instruments used were acceptable in separating paraquat, glufosinate-ammonium, and glyphosate as indicated by calibration curve (R^2 = 0.99, n = 5; R^2 = 0.99, n = 5; and R^2 = 0.996, n = 5, respectively) and recovery of spiked soil samples. Adding acetone and washing with ethyl acetate was important derivatization steps for glufosinate-ammonium and glyphosate. Size and type of column, pH and solution composition of mobile phase, and injection volume affected retention times.

Using short and small size of C_{18} column (3.9 x 150 mm from Waters, Ireland) and high flow rate (1 ml/m) produced shorter retention time of paraquat. These methods have been developed to be more simple, easy, accurate, safe, and efficient in using chemical and ready to be adopted.

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