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RESEARCH ARTICLE

Simplified method for derivatization of extractable glyphosate and aminomethylphosphonic acid and their determination by high performance liquid chromatography

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ABSTRACT

This work presents a simple procedure for pre-column derivatization of glyphosate and aminomethylphosphonic acid (AMPA) and their determination by high-performance liquid chromatography (HPLC). Derivatization was achieved by mixing a solution of 0.02 M FMOC-Cl, 0.05 M borate buffer and glyphosate or AMPA, then shaken for 1 hour, later washed with diethyl ether and ready for analysis. The quantification was performed by HPLC with fluorescent (FLD) or diode array detector (DAD). The result of the HPLC-FLD/DAD showed high linearity ($R^2 \geq 0.995$) of both compounds over eight point's concentration range and their high recovery from water compared to soil matrixes. The relative standard deviation (RSD) range from 0.1 to 30 % from the aforementioned matrixes. The limit of detection of HPLC-FLD for glyphosate from water, sandy and clay soil was 0.008 mg L^{-1}, 0.021 and 0.132 mg kg^{-1} respectively while that of AMPA was 0.004 mg L^{-1}, 0.74 and 0.224 mg kg^{-1}. Meanwhile, the limit of detection of HPLC-DAD for glyphosate from water, sandy and clay soils was 0.024 mg L^{-1}, 0.731 and 0.122 mg kg^{-1} respectively while that of AMPA for water sample was 0.076 mg L^{-1}. This study was unable to determine lower detection limit for AMPA from soil matrixes by HPLC-DAD thus suggested for more repeated extraction for increasing quantification of the compound.

Keywords: Glyphosate, aminomethylphosphonic acid, derivatization, analytical method, water, soil

1. INTRODUCTION

Glyphosate {N-(phosphonomethyl) glycine} is increasingly applied for weed control in agricultural and non-agricultural lands. The global estimate of application of glyphosate (GLY) active ingredient in 2014 was 8.2 million metric tons with the application rate of 2.13 and 1.94 kg ha^{-1} to agricultural and non-agricultural lands respectively [1]. This amount was 0.2% higher than estimated amount applied in 2013. Glyphosate is foliage applied herbicide thus not active while in soil and is completely mineralized by microbes with aminomethylphosphonic acid (AMPA) as its major metabolite [2–4]. Glyphosate had high efficacy in weed control [5] also, its residual impact on soil and water is of great environmental concern. Glyphosate was therefore, reported to have negative effect on soil microbes [4, 6], aquatic habitat [7, 8] and numerous health consequences to humans [9, 10]. In order to protect food contamination and health hazard, maximum GLY residue limit in food and water has been set up by various countries. For instance maximum acceptable limit of GLY in water was set as 0.7 and 0.28 mg L^{-1} by US environmental protection agency and health Canada respectively [11]. Monitoring GLY residue is therefore, achieve through the development of analytical technique for its determination. There are different analytical techniques used in determination of GLY and AMPA however, gas and liquid chromatography are the most widely used. Unlike other organic herbicides, GLY and its metabolite have unique properties of insolubility in organic solvent but highly soluble in water [4]. Moreover, due to none possession of chromophores and fluorophores in the molecular structure of GLY and its metabolite [12], there is difficulty in their direct detection with gas or liquid chromatography. In gas or liquid chromatography, GLY analysis is usually

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done after derivatization as it was reported to reduce polarity and its enhance volatility hence, easily detected when derivatized [13]. The feasible derivatization of GLY and AMPA in aqueous solution with requiring less or no sample pre-treatments coupled with compatibility of the derivatized sample with reverse-phase chromatographic separation makes liquid chromatography (LC) the preferred technique[14]. The GLY and AMPA are basically derivatized either by pre-column or post-column procedure. The pre-column is more preferred because it has fewer restrictions, easy in controlling reaction condition and can be perform manually because it does not require complicated equipment [15, 16].

Several reagents are used in pre-column derivatization of GLY and AMPA, however, 9-flourenyl methyl chloroformate (FMOC-Cl) is the most widely used reagent. It has the ability in reacting with both secondary and primary amino group of GLY and AMPA respectively[15]. The use of FMOC-Cl in pre-column derivatization of GLY and AMPA was pioneered by Moye and Boning [12]. The authors performed the reaction under aqueous alkaline condition thus, it achieved through addition of 0.01 M FMOC-Cl solution, 0.025 M sodium borate solution and acetone to the GLY and AMPA standard solutions. The mixture was incubated for 20 minutes at temperature of 23oC on a laboratory bench without shaking or stirring. Later the derivatives were washed with ethyl ether to remove the excess reagents then analysed using ion exchange chromatography. Several other methods were reported afterward, which optimized Moye and Boning work for better resolution and detection of these compounds. One of the earlier work stirred the mixture for 30 minutes and acidified it with 0.1N HCl. Then FMOC-GLY derivatives was extracted with ethyl acetate and dried using rotor-evaporator before analysis[17]. Druart and co-workers [18] derivatized GLY and AMPA with solution of FMOC-Cl in acetonitrile under alkaline condition through addition of 0.05 M borate buffer. The mixture later agitated with magnetic stirrer for 1 hour then washed with ethyl ether before analysis. Recently, Skeff and co-workers [19] derivatized GLY and AMPA with 1 mM solution of FMOC-Cl in acetonitrile and 0.07 M borate buffer through vigorous shaking for 4 hours at room temperature.

These differences in the derivatization process necessitates a continuous optimization on GLY and AMPA pre-column derivatization. On the other hand, some of the methods of GLY and AMPA analysis by LC employed pre-treatments for sample clean-up prior to derivatization. These includes solid phase extraction [20], ions exchange [21] and column coupling [22] which all require additional sophisticated and costly apparatus. The FMOC-GLY and AMPA can be separated and detected using LC with mass spectrometry, fluorescent or ultraviolet detector. Mass spectrometry gives better resolution and separation however, it has very complex operating procedure thus require high skill. Fluorescent detector is widely used due to its simplicity in operation. Even though, Franz and co-workers [4] reported that, FMOC derivatives of GLY and AMPA had both fluorescent and ultraviolet properties but still less attention is given to the use of ultraviolet detector. It is of much interest therefore, to develop a method which is simple, sensitive and economically affordable for detection and quantification of GLY and AMPA in the environmental matrixes. In this study, our aim was to optimize FMOC-Cl pre-column derivatization of GLY and AMPA based on the previously reported studies, and to determine GLY and AMPA by LC with fluorescent and diode array detector (DAD).

2. MATERIAL AND METHODS

2.1. Chemicals

All the chemicals were of analytical grade unless otherwise stated and Millipore® Direct UV-Q water was used throughout in preparing solutions. Glyphosate (99.7%), AMPA (99%) and FMOC-Cl (97%) were purchased from Sigma Aldrich® (Seelze, Germany). Acetonitrile and diethyl ether were purchased from QREC®, Malaysia. Analytical reagent grade $CaCl_2$ and KH_2PO_4 were purchased from Emsure® Germany while $Na_2B_4O_7.10H_2O$ were purchased from Sigma Aldrich® (India). Stock solutions of GLY and AMPA (500 mg L^{-1}) and working standard were prepared Millipore water. A 0.05 M $Na_2B_4O_7.10H_2O$ (pH 9), 0.01 M and 0.05 M KH_2PO_4 solutions were prepared in Millipore water while 0.02 M FMOC-Cl was prepared in acetonitrile.

2.2. Instruments

The LC system Agilent 1100 series (Agilent Santa Clara, USA.) consisted of two detectors fluorescence (model G1321A) and DAD (model G1315B) detectors; it is equipped with a vacuum degasser (model G 1322A), quaternary pump of high pressure gradient (model G1311A), autosampler unit (model G1313A) and column compartment (model G1316A); all connected to Chemstat computer software. The analytical column was reverse-phase C_{18} Agilent® Zorbax Eclipse plus (4.6 x 150mm, 5µm).

2.3. Determination of Glyphosate and AMPA by HPLC-FLD

In other to identify the peaks of GLY and AMPA, a blank Millipore water and samples containing 1 mg L^{-1} of either GLY or AMPA standard solutions were derivatized and analysed. Afterwards, calibration curves were obtained by derivatization and analysis of samples containing GLY or AMPA solutions at 8 point different concentrations ranging between of 0 mg L^{-1} and 2 mg L^{-1}. Later calibration curves of GLY or AMPA was plotted from the peak areas versus their respective concentration.

The extraction of GLY and AMPA from soil matrix was achieved through spiking 0.5 ml each of 1 mg L^{-1} of GLY and AMPA in 2 grams of either clay or sandy soils which later followed addition of 20 ml 0.01 M KH_2PO_4 then the mixture was shaken on rotary shaker for 2 hours. After which it was centrifuged at 10000 rpm for 10 minutes and the supernatants were filtered

using 0.45 µm syringe filter and kept for derivatization.

2.4. Determination of Glyphosate and AMPA by HPLC-DAD

A blank Millipore water and samples containing 5 mg L^{-1} of either GLY or AMPA standard solutions were derivatized and analysed for the identification of GLY and AMPA peaks. Later GLY or AMPA solution at 8 point different concentrations ranging between of 0 mg L^{-1} and 40 mg L^{-1} derivatized and analysed. This followed by plotting peak areas versus their respective concentration for the development of calibration curve.

A 0.5 ml each of 5 mg L^{-1} solutions of GLY and AMPA was spiked in 2 grams of either clay or sandy soils weighed into 50 ml centrifuge tubes then added with 20 ml 0.01 M KH_2PO_4 and shake for 2 hours on rotary shaker. This followed by centrifugation at 10,000 rpm for 10 minutes after which, the supernatants were filtered using 0.45 µm syringe filter and kept for derivatization.

2.5. Derivatization Procedure of Glyphosate and AMPA

A blank samples or samples containing GLY and /or AMPA from standard solution and soil extract were derivatized by addition of 1 mL of each into 25 mL centrifuge tubes then followed by addition of 1 mL 0.02 M FMOC-Cl and 2 mL 0.05 M borate buffer. The mixture was shaken at 180 rpm for 1 hour on end-to-end shaker after which 2 mL diethyl ether was added to each tubes and vortex for 2 minutes to remove unreacted FMOC. This resulted in formation of two layers; organic and aqueous layer thus the organic layer was discarded through a careful pipetting and the aqueous solution containing FMOC-GLY and/or FMOC- AMPA was transferred to HPLC vials for analysis.

2.6. Chromatographic Conditions

Mobile phase solvent was acetonitrile and 0.05 M KH_2PO_4 mixture (30:70 v/v) using isocratic mode. The running time was 20 minutes with flow rate of 0.7mL min^{-1} and column temperature of 40^oC while the injection volume was 20µl. For the HPLC-DAD analysis, two wave length were used, 206 and 210 nm while for the HPLC- FLD analysis, excitation and emission wave lengths were set at 270 nm and 315 nm respectively.

2.7. Validation of the Method

The methods was validated based on the following parameters; specificity, linearity, accuracy, precision, limit of detection and quantification. Specificity of the present method was achieved through comparative analysis of the blanks and samples containing GLY and/or AMPA. For the linearity, a calibration curves were obtained for eight concentration range of GLY or AMPA standard solutions. The precision was evaluated through replicate analysis of the eight concentration range spiked in water and soils then later relative standard deviation (RSD) was calculated as;

$$\text{RSD} = \frac{standard\ deiviation}{mean} \text{ x } 100 \quad (1)$$

The accuracy of this method was evaluated through calculated percent recovery of GLY and AMPA from water, as well as the GLY and AMPA extracted from clay and sandy soils spiked with eight different concentration range of GLY and AMPA. The limit of detection (LOD) was calculated as standard deviation of the concentration of three replicates multiplied by 3.3 while limit of quantification (LOQ) was calculated as standard deviation of the concentration of three replicates multiplied by 10 [23].

3. RESULTS AND DISCUSSION

3.1. Derivatization and Determination of Glyphosate and AMPA

Derivatization of GLY or AMPA occurs in alkaline condition and is based on the reaction between FMOC-Cl and amino functional group of GLY or AMPA thus, is achieved through a substitution of H atom from GLY or AMPA with aromatic ring of FMOC-Cl yielding FMOC-GLY or FMOC-AMPA and HCl [24] as shown in Fig 1. These resultant derivatives are compounds with both polar and non-polar properties. They derived their polar properties from GLY or AMPA while the non-polar characteristics was from FMOC-Cl. These FMOC-derivatives of GLY and AMPA resembles each other except that in AMPA there is presence of H atom instead of CH_2COOH functional group in GLY. Borate buffer (pH 9) increases the pH of the mixture to alkaline condition which help in promoting the reactivity of amines functional groups of GLY or AMPA as well stabilizing the solubility of FMOC-Cl in acetonitrile [25]. Ghanem and co-workers [21] reported that, the ratio between analyte, borate buffer and FMOC-Cl affects derivatized product formation Hence, Bernal and co-workers [14] obtained best result using the volume ratio of 1:2:1 of analyte, borate buffer and FMOC-Cl (v/v/v) which makes the adoption of this ratio in the present work. Adequate homogenization of the mixture gives good derivatives due to better interaction of FMOC and GLY or AMPA, which is why Druart and co-workers [18] used magnetic stirrer for homogenizing analyte, borate buffer and FMOC-Cl solution. However, the magnetic stirrer is only for one sample at a time hence the stirring can be difficult and time consuming when dealing with many samples. The present study therefore, used end-to-end shaker which handled many samples at a time. Adequate time is required for complete reaction hence replacement of Cl from FMOC-Cl by amino functional groups of GLY or AMPA. However, no standard time limit was set [24] but, Druart and co-workers [18] noticed no significant increase in reaction between FMOC-Cl and GLY or AMPA above 1 hour. This makes it possible for the present study to choose 1 hour reaction time for achieving complete reaction. The excess un-derivatized FMOC-Cl do interfere with the analyte

during analysis. The function of ethyl ether therefore, was to removed/reduced this excess solvent [26] which helps in increasing absorption of fluorescence or UV light by the analyte for good peaks and better separation of the compounds.

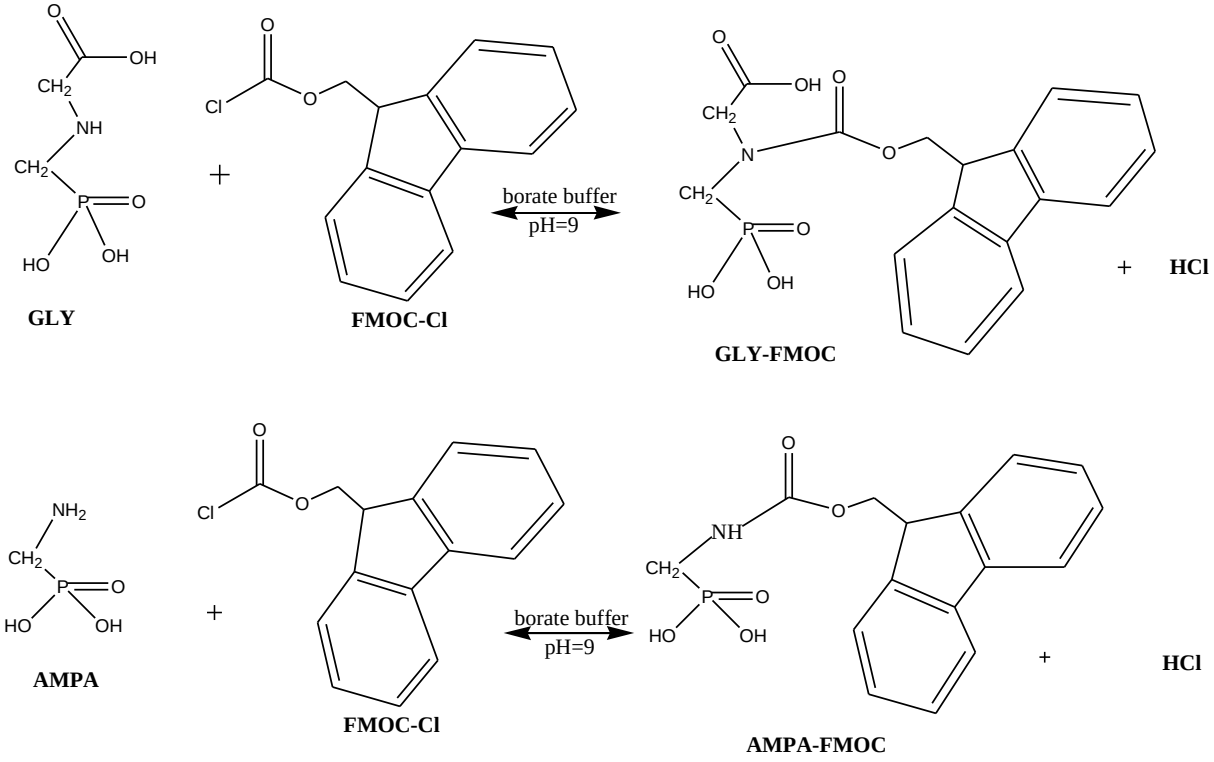

Fig 1. Derivatization reaction of glyphosate and AMPA with FMOC-Cl

The separation of FMOC-GLY and/or FMOC-AMPA was achieved by using the C18 column as a stationary phase while a mobile phase was acetonitrile and a solution buffer of 0.05 M KH_2PO_4 in an isocratic elution. Acetonitrile is a polar solvent with polarity index of 5.8, it controls the separation of these compounds while 0.05 M KH_2PO_4 buffer solution aids in neutralizing FMOC-GLY or FMOC-AMPA for increased retention on the stationary phase. The 40^{o}C temperature is high enough to reduce viscosity between the analyte and mobile phase thereby enhancing the solubility and chromatographic efficiency [27]. The chromatograms produced by the column used in the present work shows that, apart from FMOC-GLY or FMOC-AMPA derivatives, there was also FMOC-OH and unknown by-products. Nedelkoska and Low [28] reported co-elution of FMOC-OH with GLY which was presented by a large peak in front of GLY thus interfere in its separation. However, in this work we recorded success in early elution of FMOC-GLY or FMOC-AMPA before FMOC-OH as can be observed from the FLD-chromatograms but there was appearance of FMOC-OH large peak before that of FMOC-GLY or FMOC-AMPA from the DAD-chromatograms.

3.2. HPLC-FLD Method Performance and Validation

As shown in the Fig 2-a, there was no peak of either GLY or AMPA in the chromatogram of blank neither does it produced a large peak of FMOC-OH as expected, this might be due to low or no reaction between FMOC-Cl and water. The chromatograms of the standard solutions (Fig 2-b and c) shows the retention time of 2.54 and 5.23 minutes for GLY and AMPA respectively. There is also a presence of large peak of FMOC-OH eluted very late (10 minutes) than that of GLY or AMPA hence no overlapped or interference between the FMOC-OH and compounds of interest. The absence of any peak from control chromatograms and the presence of peaks in the chromatograms of GLY and/or AMPA standard solutions indicate selectivity and specificity of this method.

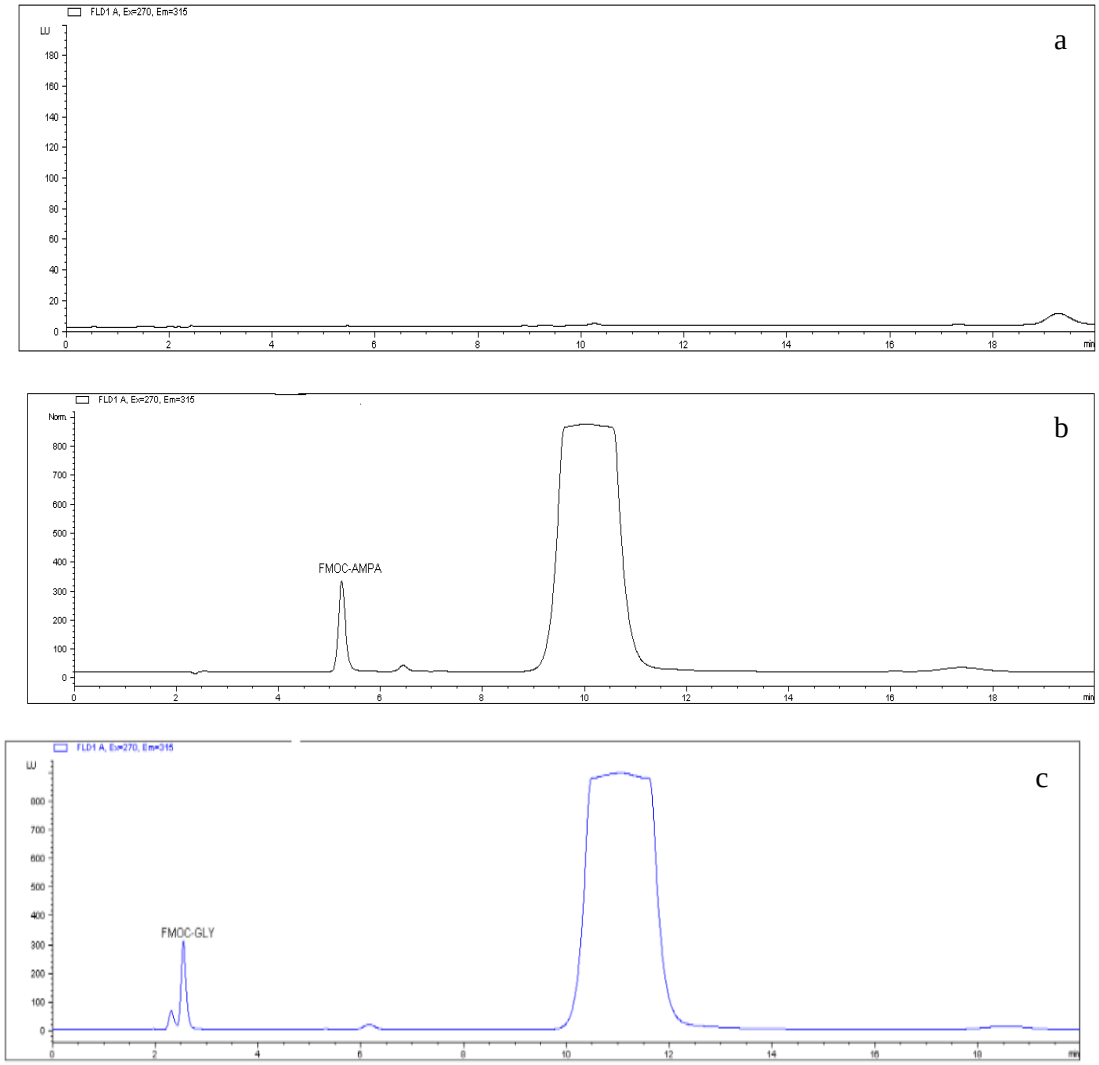

Fig 2. Chromatograms of control (a), 1 mg L-1 AMPA (b) and glyphosate (c) standard solutions by LC-FLD

Eight different concentrations ranging between 0 mg L^{-1} and 2 mg L^{-1} of GLY or AMPA standard solution were analysed for linear calibration curve. The response values shows high linear regression (Table 1) which indicated the linearity and reliability of this method.

Fig 3 is the chromatograms of GLY and AMPA in water and extracted residues from the spiked soils. The retention time of GLY and AMPA from water was 2.45 and 5.23 minutes respectively while from soil it was 2.42 and 5.37 minutes for GLY and AMPA respectively.

The percent recovery (Table 2) of eight different concentrations ranging between 0 mg L^{-1} and 2 mg L^{-1} of GLY and AMPA in water indicated a very good recovery for GLY (80-110%) and AMPA (73-103%) hence the accuracy of this method. The calculated LOD and LOQ for GLY in water was 0.008 mg L^{-1} and 0.028 mg L^{-1} while AMPA had 0.004 mg L^{-1} and 0.015mg L^{-1} respectively. Moreover the RSD for these eight concentration was 0.2-1% and 0.1-0.7% for GLY and AMPA respectively. This indicated that the method is repeatable, efficient and have a very good precision. The recovery of GLY (34-74%) and AMPA (37-51%) from clay soil was low compared to that sandy soil which shows 49-105% for GLY and 32-70% for AMPA.

The recovery of both compounds in both water and soil samples increased with increasing concentrations. The LOD and LOQ of GLY in clay soil was 0.132 mg kg-1 and 0.399 mg kg^{-1} while AMPA had 0.224 mg kg^{-1} and 0.678 mg kg^{-1}. The sandy soil shows low LOD and LOQ of both compounds compared to clay thus, LOD and LOQ from this soil was 0.021 mg kg^{-1} and 0.064 mg kg^{-1} for GLY while AMPA had 0.074 mg kg^{-1} and 0.331 mg kg^{-1} respectively. Furthermore, the RSD of the analysed extracted solutions was 12-24% for GLY and 10-26% for AMPA in clay soils while sandy soil had 6-30% and 10-28% for GLY and AMPA respectively.

Table 1. Linearity AMPA and Glyphosate standard solutions by LC-FLD

Compounds	Regression equation (n=8)	R^2
AMPA	y= 2886.9x+43.001	0.998
Glyphosate	y= 1265.8x+84.005	0.994

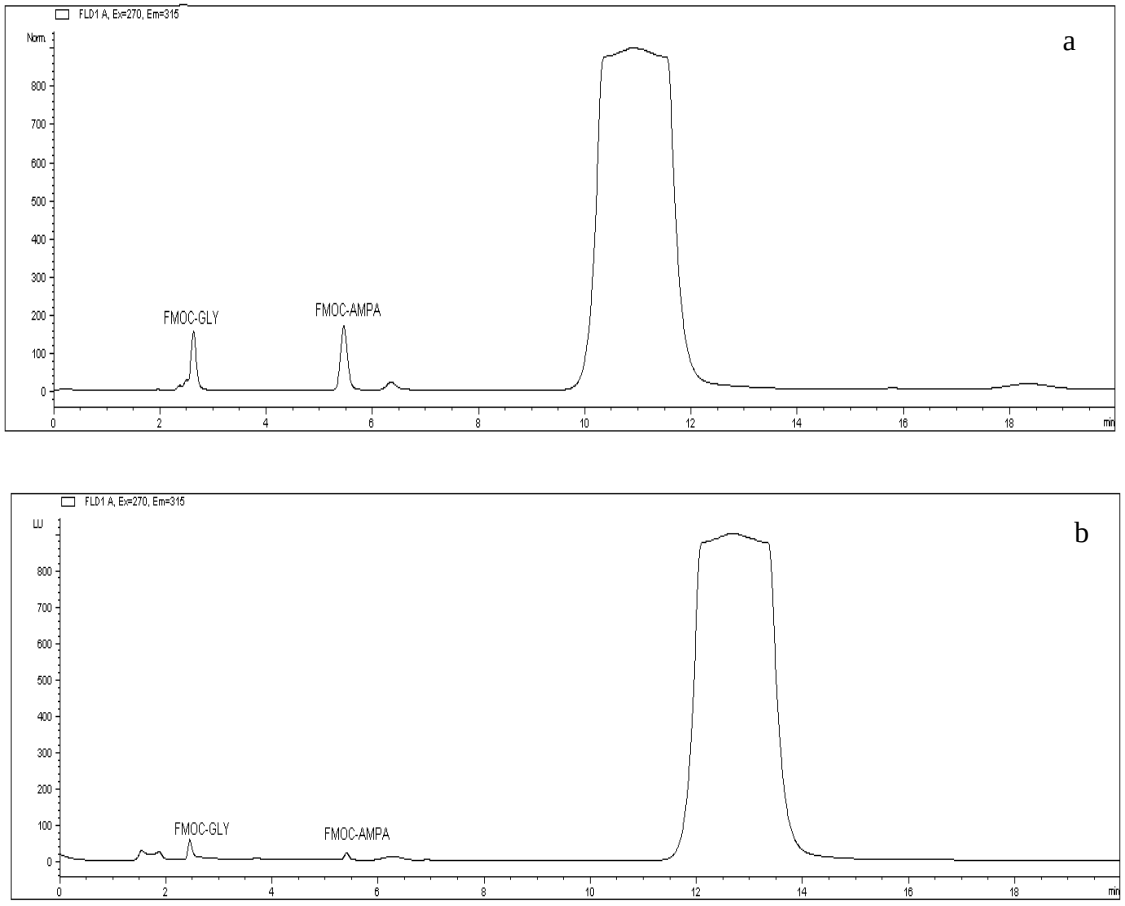

Fig 3. Chromatograms of 1 mg L-1 glyphosate and AMPA in water (a) and extracted residues (b) from spiked clay soil by LC-FLD

Table 2. Validation parameters for method of glyphosate and AMPA analysis by LC-FLD

		Parameters			
Compound	Matrix	Recovery (%)	RSD (%)	LOD	LOQ
GLY	Water	80-110	0.2-1	0.008 mg L^{-1}	0.028 mg L^{-1}
	Clay soil	34-74	12-24	0.132 mg kg^{-1}	0.399 mg kg^{-1}
	Sandy soil	49-105	6-30	0.021 mg kg^{-1}	0.064 mg kg^{-1}
AMPA	Water	73-103	0.1-0.7	0.004 mg L^{-1}	0.015 mg L^{-1}
	Clay soil	35-51	10-26	0.224 mg kg^{-1}	0.678 mg kg^{-1}
	Sandy soil	32-70	10-28	0.074 mg kg^{-1}	0.331 mg kg^{-1}

The present method involved simple steps and direct determination of GLY and AMPA without further clean up prior to derivatization hence very cost effective. The high linear regression and specificity of both compounds indicated that, the method can be reproducible and has very good reliability. The result

of the method validation showed high recovery of both compounds with low RSD from water samples compared to the soils. Glyphosate was reported to strongly adsorbed to Fe and Al oxides minerals, polyvalent cations and soil organic matter [29]. Table 3 indicated that both soils had substantial content of sesquioxides and soil organic matter with clay soils having high content than sandy soil. Therefore, this low recovery of both compounds from soils was attributed to adsorption by oxide minerals and soil organic colloids. Similarly, the greater content of sesquioxides and organic matter in clay were suggested to cause low recoveries of these compounds as well as their high LOD and LOQ compared to the sandy soil. Glyphosate and AMPA rapidly formed non-extractable residues in soils due to their strong adsorption [30] therefore, most studies experienced low recovery from many soils [31]. The KH_2PO_4 solution extracts both soluble and weekly adsorbed form of these compounds hence their recovery depends on soil clays, organic matter, pH, inorganic phosphorus and exchangeable actions [29–32]. The KH_2PO_4 is the best extracting solution of GLY and AMPA from soil and extraction by agitation was shown to provide best efficient recovery [18, 30]. This evidently shows that, the low recovery of these compounds from soils compared to the water samples was attributed to their adsorption by these soils. However, this extraction procedure can still be applied with soils where adsorption of these compounds was less. The average GLY recovery obtained from sandy (77%) and clay (54%) from the

present study was still high than what was reported for clay loam, sandy loam and silty clay loam soil extracted with 0.1 M KH_2PO_4 solution [30]. The LOD of GLY from both sandy and clay soil were less than that reported by Glass [17] from sandy- loam soils (5 mg kg^{-1}) and clay-loam soils (50 mg kg^{-1}). Miles and Moye [33] also extracted GLY from two soils and reported the LOD of 0.5 mg kg^{-1} for sandy soils and 1 mg kg-1 for clay soils. However, a recent study conducted by Druart and co-workers [18] on clay-loam soils reported LOD for GLY and AMPA as 0.1 mg kg^{-1} and 0.016 mg kg^{-1} respectively, the values which are similar to the present study. The LOD of both compounds from water samples were very low than the maximum GLY residue level in drinking water set by US environmental protection agency and health Canada [16]

Table 3. Selected properties of the soils used for method validation

Property	Sandy soil	Clay soil
Free FeO (%)	0.473	2.297
Amorphous FeO (%)	0.218	0.655
Free AlO (%)	0.476	1.815
Amorphous AlO (%)	0.262	0.550
SOM (%)	5.233	10.567
CEC (coml(+) kg^{-1})	12.667	11.905

3.3. HPLC-DAD Method Performance and Validation

As shown in Fig 4, there was only large peak of FMOC-OH from the control chromatograms which evidently showed a high sensitivity of FMOC-OH to UV light compared to fluorescent. The retention time of GLY and AMPA for LC-DAD as shown by analysis of 5 mg L^{-1} standard solution (Fig 5) were 2.38 and 5.30 minutes respectively, the times which are similar to LC-FLD. The presence of FMOC-OH peaks only from blank and elution of GLY and AMPA peaks from standard solutions indicated the specificity of this method even with DAD. Table 4 shows regression equations and coefficient of determination of nine concentration of GLY and AMPA standard solution (0 – 40 mg L^{-1}) with greater linear regression from both compounds (R^2 > 99%) thus, indicating the linearity of LC-DAD. Fig 6 is the chromatograms of GLY and AMPA in water and extracted residues from the spiked soils. The retention time of GLY and AMPA from clay soils was 2.57 and 5.38 minutes respectively.

Table 4. Linearity AMPA and Glyphosate standard solutions by LC-DAD

Compounds	Regression equation (n=9)	R^2
AMPA	y= 73.201x+6.1748	0.996
Glyphosate	y= 59.956x+51.172	0.996

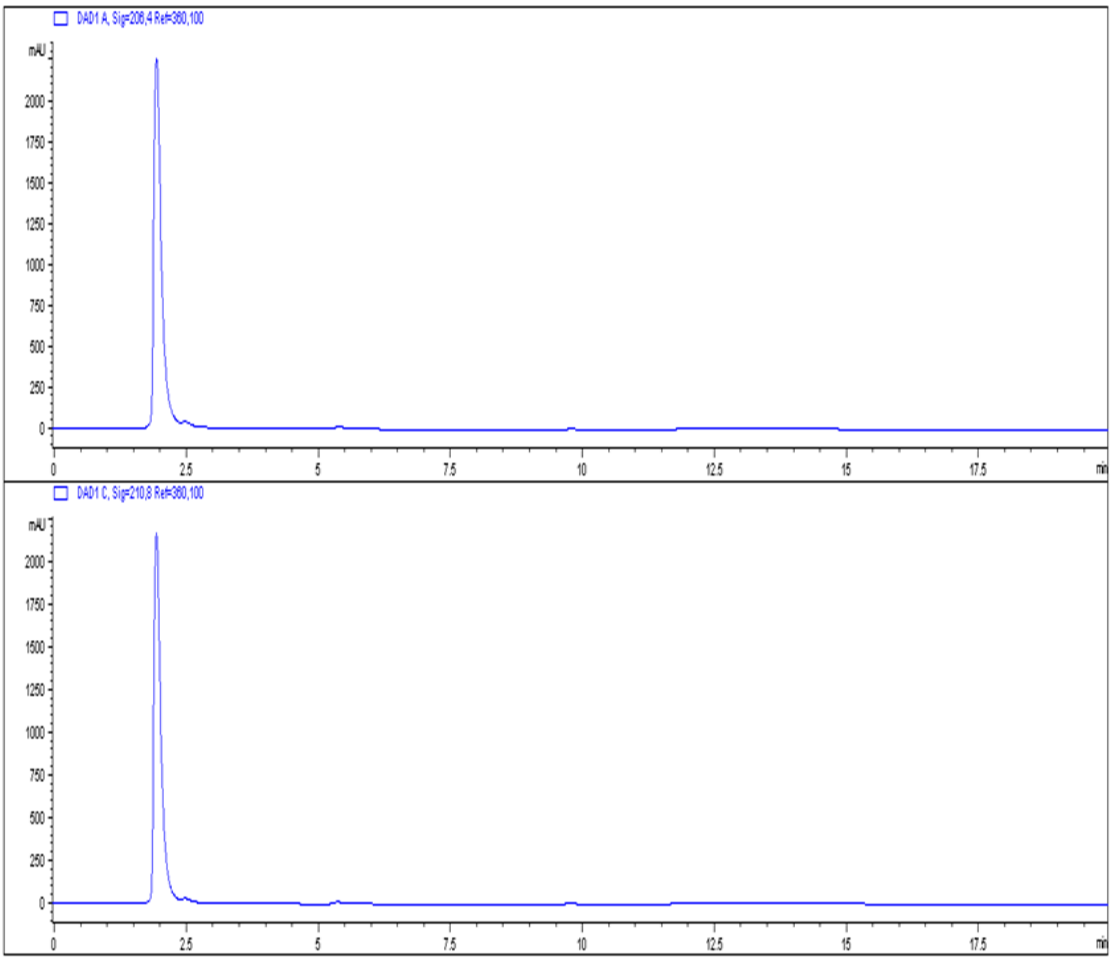

Fig 4. Chromatograms of the blank sample by LC-DAD

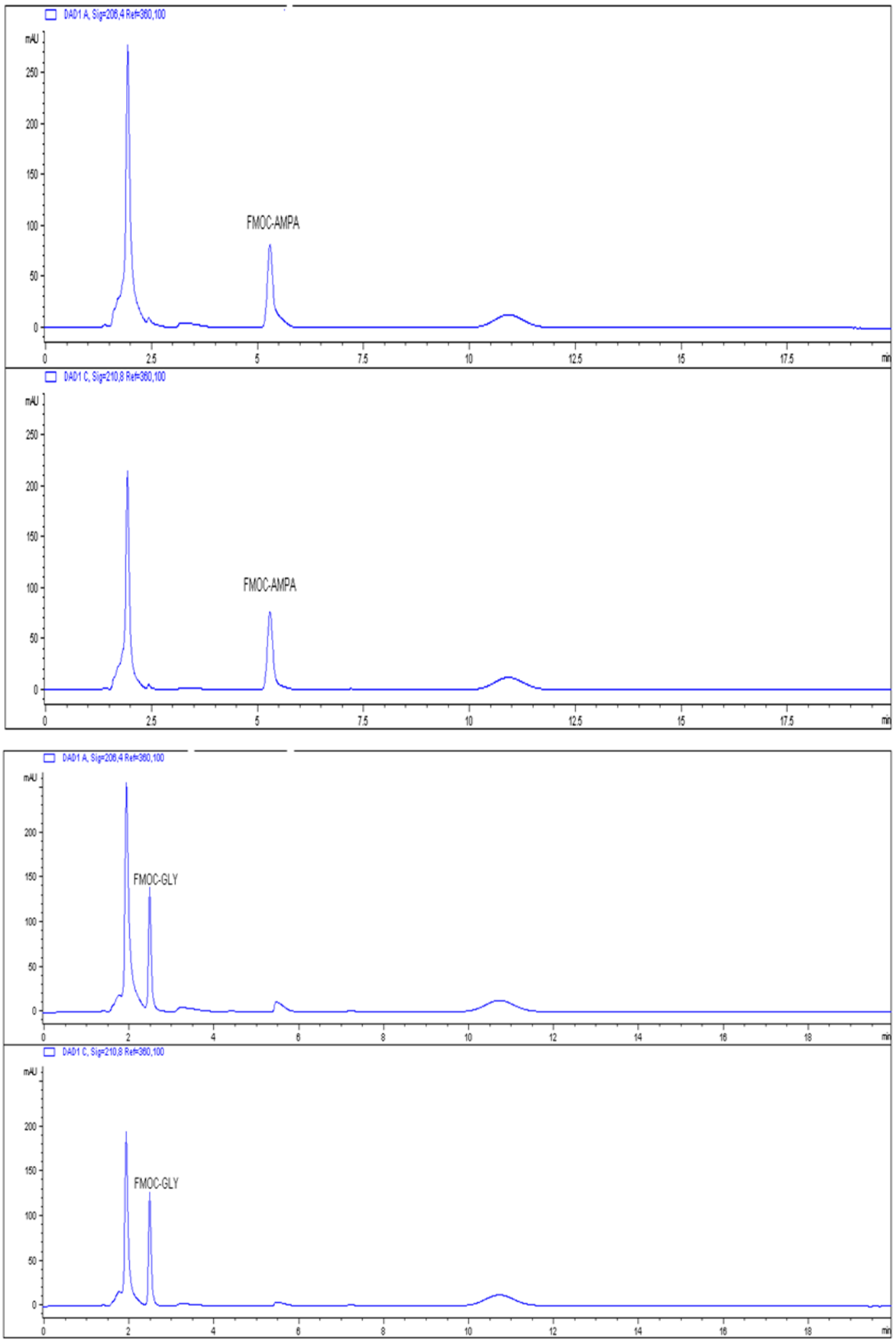

Fig 5. Chromatograms of 5 mg L^{-1} of AMPA or glyphosate standard solution by LC-DAD

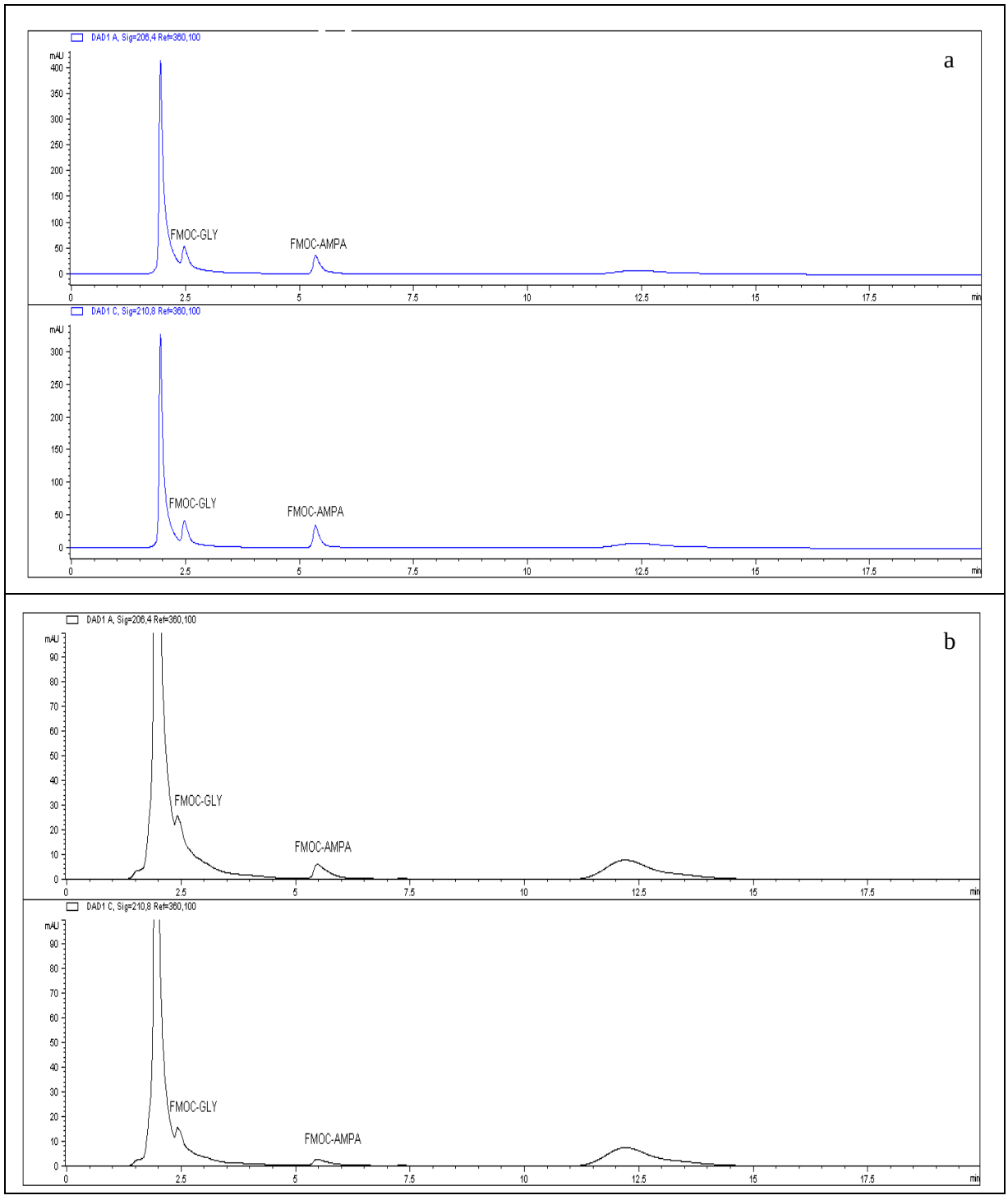

Fig 6. Chromatograms of 5mg L^{-1} glyphosate and AMPA in water (a) and extracted from clay soil (b) by LC-DAD

The percent recovery of GLY and AMPA in water from nine different concentrations (0-40 mg L^{-1}) as shown in Table 5 indicated a very good recovery ranging from 93% to 110% for GLY and 86% to 106% for AMPA hence reproducibility of the method even with DAD. The calculated LOD and LOQ for GLY from water sample was 0.024 mg L^{-1} and 0.083 mg L^{-1} while AMPA had 0.076 mg L^{-1} and 0.247 mg L^{-1} respectively. The RSD was 0.7-4% and 0.2-2% for GLY and AMPA respectively. This indicates that, the method is repeatable, efficient and has a very good precision. The percent recovery of GLY from the clay soil was 18-58% while the sandy soil had 27-64%. The LOD and LOQ of GLY from clay soil was 0.122 mg kg^{-1} and 0.360 mg kg^{-1} while the sandy soil had 0.731 mg kg^{-1} and 1.242 mg kg^{-1} respectively. The RSD was 1-5% for the clay soil and 6%-24% for sandy soil.

Table 5. Validation parameters for method of glyphosate and AMPA analysis by LC-DAD

		Parameters			
Compound	Matrix	Recovery (%)	RSD (%)	LOD	LOQ
GLY	Water	93-110	0.7-4	0.024 mg L^{-1}	0.083 mg L^{-1}
	Clay soil	18-58	1 -5	0.122 mg kg^{-1}	0.360 mg kg^{-1}
	Sandy soil	27-64	6-24	0.731 mg kg^{-1}	1.242 mg kg^{-1}
AMPA	Water	86-106	0.2-2	0.076 mg L^{-1}	0.247 mg L^{-1}

The FMOC- derivatives of GLY and AMPA are rarely analysed with LC with ultraviolet detector (UV) hence, we laid our hand only on Peruzzo and co-workers [34] despite the fact that Franz and co-workers [4] reported both derivatives to have ultraviolet properties. The common reagents used in pre-column derivatization and LC analysis of GLY and AMPA with UV detector includes 1-flouro-2,4-dinitrobenzene (FDNB) [3], p-toluenesulphonyl chloride (TsCl) [35], p-nitrobenzoyl chloride (PNBC) [36] , 4-chloro-3,5-dinitrobenzotriflouride (CNBF) [37] and 2,5-dimethoxy benzenesulfonyl chloride (DMOSC) [38]. The present work therefore agrees with Peruzzo and co-workers [34], which evidently shows that both FMOC- GLY and AMPA- derivatives can be analysed with LC-UV. Even though FLD has high sensitivity than DAD as shown by their respective LOD and LOQ. As an equipment, both detectors are bound to have technical problems and considering the high cost of FLD lamp compared to DAD, the latter can serve as alternative in case FLD got faulty especially when using a machine with dual detectors like the one in the present work. The present study therefore, re-explore the potential of DAD in detecting GLY and AMPA thus, serve as additional advantage of simple procedure which uses available analytical equipment. The LC-DAD shows high linear regression of standard solutions and high percent recovery of GLY and AMPA in water which indicated its reliability and repeatability. However, the percent recovery of GLY from both soils studied was poor but considering the LOD of GLY in both soils, the method can still be use in routine analysis of GLY extracted from matrixes other than soil . On the other hand, AMPA was poorly extracted which led to very low/no quantification hence resulted in rather similar values for all concentration range which makes it impossible to calculate LOD and LOQ from both soils. This was attributed to its strong adsorption to soil minerals and organic colloids. Therefore, it is possible to improve the AMPA quantification by more repeated extraction. This might likely help in extracting adsorbed compound [31] which will result in increased in quantification and recovery. The LOD and LOQ of GLY from the clay soil are similar to that reported by Peruzzo and co-workers [34] for soils and sediment (0.10 mg kg^{-1} and 0.25 mg kg^{-1}) but the sandy soil had greater values than what the authors have reported.

Table 6. Comparison of some LC-UV methods for glyphosate and AMPA analysis

Matrix	Enrichment/pre-treatment	Reagent for derivatization	LOD		Reference
			GLY	AMPA	
Soil	Ion exchange extraction, evaporation	FDNB	0.05 µg g^{-1}	0.1 µg g^{-1}	[3]
Fruits juice	Supported-liquid membrane	TsCl	0.01 mg L^{-1}	0.01 mg L^{-1}	[35]
Apple	Solid phase extraction	CNBF	0.01 µg g^{-1}	NA	[39]
water	evaporation	MOBSF	0.001 mg L^{-1}	0.001 mg L^{-1}	[16]
water	none	DMOSC	0.067 mg L^{-1}	NA	[35]
Water	none	FMOC-Cl	0.024 mg L^{-1}	0.076 mg L^{-1}	This work
Soil	none	FMOC-Cl	0.122 µg g^{-1}	NA	This work

NA no analysis

4. CONCLUSIONS

The present work demonstrate a simple pre-column derivatization of GLY and AMPA with FMOC-Cl and HPLC analysis of the derivatives with FLD or DAD. The derivatization reaction was completed in 1 hour at room temperature and diethyl ether was used in washing the excess FMOC for clear separation of the analyte. The FMOC- derivatives of GLY and AMPA eluted long before FMOC-OH in LC-FLD. The method recorded good recovery in both water and sandy soil especially for GLY hence, considered reliable for quantitative determination of GLY and AMPA. Similarly, these derivatives were quantified using LC-DAD also, a clear separation was obtained between FMOC-GLY, FMOC-AMPA and FMOC-OH. Even though, there was low GLY recovery from the soil matrixes but considering its low LOD, the LC-DAD can still be used in routine analysis of GLY especially in soil will less adsorption phenomena. We were unable to calculate LOD and LOQ for AMPA from soil matrixes which was attributed to its strong adsorption by soil minerals and organic colloids as well low sensitivity of DAD for AMPA. We therefore, suggested for more repeated extraction, which might increase quantification and recovery of the compound.

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