Journal of Chromatography A, 1589 (2019) 116–121

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A method for determining glyphosate and its metabolite aminomethyl phosphonic acid by gas chromatography-flame photometric detection

Weidong Zhang[a], Yanru Feng[a], Li Ma[a], Jing An[a], Huayin Zhang[a], Mengsi Cao[a], Huaijiao Zhu[a], Weijun Kang[a,*], Kaoqi Lian[a,b,*]

[a] Department of Sanitary Inspection, School of Public Health, Hebei Medical University, Shijiazhuang, 050017, China
[b] Hebei Key Laboratory of Environment and Human Health, Shijiazhuang 050017, China

ARTICLE INFO

Article history:
Received 24 November 2018
Accepted 19 December 2018
Available online 20 December 2018

Keywords:
Gas chromatography
Glyphosate
AMPA
Degradation
Migration characteristics

ABSTRACT

As a globally popular herbicide, glyphosate (GLY) and its metabolite aminomethylphosphonic acid (AMPA) pose potential hazards to the ecological environment. In this study, a sensitive and reliable method for detecting GLY and AMPA was utilized to facilitate exposure risk assessment of the analytes in environmental systems such as water and soil. GLY and AMPA were extracted from the sample using a solid-phase extraction (SPE) procedure, derivatized by heptafluorobutyric anhydride and heptafluorobutanol, and detected by gas chromatography-flame photometric detection (GC-FPD). The linearities of GLY and AMPA in the range of 10–1000 ng/mL were good (r=0.9998, r=0.9991), and the limits of quantitation (LOQ) for GLY and AMPA were 0.37 and 0.81 ng/mL, respectively. The method has been successfully applied for detecting GLY and AMPA in water, soil and monitoring the degradation of GLY under different environmental conditions. Simulated migration characteristics of GLY and AMPA in soil were investigated for evaluating the potential hazards of GLY and AMPA to the ecological environment.

1. Introduction

Glyphosate (*N*-phosphonomethyl glycine, GLY) is a popular, highly efficient, low toxicity and broad-spectrum herbicide. Excessive use of GLY causes environmental pollution. Subsequently, GLY and its aminomethylphosphonic acid (AMPA) metabolite residues in the soil may enter the human body through food and drinking water and exert deleterious effects. It has been reported in the literature that GLY causes reproductive toxicity. GLY can mediate the transcription and expression of estrogen receptors, and the toxic effects of GLY on human placental cells are concentration and time-dependent [1,2]. Several investigations among Canadian farmers showed that GLY exposure before or during pregnancy may cause late spontaneous abortion, and damage the DNA in Raji cells [3,4]. At the same time, GLY also presents neurotoxicity. GLY can pass through the blood-brain barrier, damage the human nervous system, and exacerbate autism and Alzheimer's disease [5–7]. In addition, a meta-analysis predicted a positive correlation between occupational GLY exposure and non-Hodgkin lymphoma [8].

Because of the high similarities between zebrafish and human genes, zebrafish is often employed in *in vivo* toxicity studies of GLY and AMPA. GLY inhibits the cholinesterase activity in zebrafish and interferes with steroid synthesis and oxidative stress mechanisms [9,10]. GLY also presents cardiovascular toxicity and neurotoxicity to zebrafish [11,12]. On the other hand, GLY can deteriorate the fertility of zebrafish by reducing the sperm quality and affect the swimming behavior of the juvenile and adult fish [13,14].

Different reports from 2001 to 2015 have acknowledged that GLY and AMPA were detected in the urine of 31.8% and 40.1% of the German population, respectively [15]. China is a large manufacturer and consumer of GLY. GLY and AMPA may easily migrate into food and drinking water and cause serious health hazards. Due to the toxic effects and widespread existence of GLY and AMPA, the migration of these chemicals in soil and water should be closely monitored. The qualitative and quantitative determination of GLY and AMPA are extremely difficult due to the absence of fluorophores or chromophores in their structures. Chromophores/fluorophores can be inserted in the structure of GLY and AMPA using different derivatization reagents so that the derivatized products can be detected by the HPLC-UV/FD method [16–19]. However, substantial by products of the derivatization process may interfere with the HPLC procedure by diminishing the selectivity and efficiency. Although LC–MS/MS procedures can easily detect and quantify GLY and AMPA [20–22], the high capital

* Corresponding authors.
E-mail addresses: kangwj158@163.com (W. Kang), liankq@hebmu.edu.cn (K. Lian).

https://doi.org/10.1016/j.chroma.2018.12.039
0021-9673/

and operational costs of LC–MS/MS often discourage the practical applications of this instrument.

GLY and its metabolite AMPA have high boiling points, low volatility, high aqueous solubility, and low solubility in organic solvents (methanol, ethanol, acetone, and ether). Previous GC methods for detecting and quantifying GLY and AMPA utilized complicated, less sensitive and less efficient processes involving liquid-liquid extraction (LLE) with dichloromethane, solid-phase extraction (SPE) and derivatization of GLY and AMPA with trifluoroacetic anhydride-trifluoroethanol/heptafluorobutanol [23–28]. Recently, micro solid phase extraction (MSPE) and ultrasound-enhanced air-assisted liquid-liquid microextraction (USE-AALLME) are becoming new methods for preconcentration, which presents the advantages of effectiveness, simplicity and low cost [29,30]. In this study, a Cleanert® PEP-2 SPE column was used for preconcentration. The PEP-2 SPE column introduces modification and rebonding techniques on the surface of the parent substrate, polyethylene divinylbenzene, to which was bound pyrrolidone and trace amounts of urea-based functional groups. Due to the introduction of urea groups, PEP-2 has a charge-rich surface structure. It can adsorb highly polar compounds and tolerate extreme acid-base solutions of pH 0-14. Neutral, acidic or alkaline materials can be adsorbed on the surface of materials without strips. The pH value of the sample inhibits the ionization of the compound and maintains a proper wetting state throughout the long-term drying process, thus avoiding the low recovery and instability of the experimental results caused by the drying out of the traditional silica gel matrix C18 material. The sample extraction is completed in one step by the PEP-2 SPE column, greatly saving sample preparation time and increasing the sample recovery rate.

In early studies, isobutylchloroformate, isopropylchloroformate, trifluoroacetic acid, trifluoroacetic anhydride and trimethyl ortho-acetate were used as derivatizing agents for GLY and AMPA analysis by GC methods [31–33]. In this study, many derivatizing reagents were investigated. Finally, heptafluorobutyric anhydride and heptafluorobutanol were selected for derivatizing GLY and AMPA. The reaction temperature, time and the ratio of the derivatizing reagents were optimized through single-factor analysis and orthogonal tests to achieve the maximum yield.

The combination of a flame photometry detector (FPD) with gas chromatography (GC) can easily detect and quantify GLY and AMPA after derivatization owing to the high selectivity of the former technique towards the organic compounds containing sulfur and phosphorus, the high resolution, rapid analysis and low injection volume. In this study, a reliable and sensitive GC-FPD method has been developed for detecting and monitoring GLY and AMPA migration in soil, degradation in water and for further assessing the potential hazards in the ecological environment.

2. Experiments

2.1. Reagents and materials

Glyphosate (GLY) was purchased from J&K Chemical Ltd. (Beijing, China, purity 96%, Lot: LIC0O64). Aminomethylphosphonic acid (AMPA) was purchased from Sigma (St. Louis, MO, USA, purity 99%, Lot: MKBX8824 V). Each standard stock solution was prepared at 1 mg/mL in 18 MΩ cm^{-1} water and stored at 4 °C, which were diluted with water to obtain calibration standard solutions.

Trifluoroacetic anhydride (TFAA, for GC derivatization, purity ≥ 99.0%), pentafluoropropionic anhydride (PFPA, for GC derivatization, purity 99%), 2,2,3,3,3-pentafluoro-1-propanol (PFP, for GC derivatization, purity ≥ 98.5%), heptafluorobutyric anhydride (HFBA, for GC derivatization, purity ≥ 99.0%), 2,2,3,3,4,4,4-heptaflouro-1-butanol (HFB, purity 98%), hydrochloric acid (HCl) solution (32%), and HPLC grade ethyl acetate were purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd. (China). 2,2,2-trifluoroethanol (TFA, for GC derivatization, purity 99.0%) was purchased from J&K Chemical Ltd. (Beijing, China). HPLC grade methanol was purchased from Fisher Scientific (Fair Lawn, NJ, USA). A Dragon Lab Centrifuge D3024R (Dragon Laboratory Instruments Limited, Beijing, China) and SPE cartridges were used for sample preparation. Ultra-pure water was obtained from a Heal Force SMART-N system (Heal Force Bio-Meditech Holdings Limited, Shanghai, China, 18.2 ΩM cm^{-1}).

2.2. GC-FPD analysis

The GC-FPD system included an Agilent 7890B series GC equipped with a split/splitless injection port and an FPD. An Agilent HP-5 capillary column (30 m × 0.32 mm I.D, 0.25 μm film thickness) was used for separation of analytes. Nitrogen (purity ≥99.9999%) was used as a carrier gas at a constant flow rate of 15 mL/min. A split injection (split ratio, 5: 1) and injector temperature of 250 °C were employed. The GC oven temperature was programmed to increase from an initial temperature of 120 °C (held for 3 min) to 250 °C (held for 20 min) at a rate of 10 °C /min. The detector temperature was 280 °C A 1 μL extract was injected for analysis.

2.3. Sample preparation

Water samples (including Minxin River, Hutuo River and two topwater) and yellow-brown soil samples (six cultivated soil samples at the depth layer of 0–20 cm) were collected from Shijiazhuang, Hebei Province, China. After drying, the soil samples were mixed, powdered and sieved through 60-mesh. For soil samples, 2 g soil samples were mixed with 10 mL water into 50 mL centrifuge tubes by vigorously vortexing and 20 °C ultrasonic treatment for 10 min. Thereafter, extracts and water samples were centrifuged at 15,000 rpm for 10 min. Finally, 2 mL water samples were used for analysis. After extraction, the eluents were collected and dried with a slow stream of nitrogen at 70 °C. The derivatizing reagents were added and vortexed for 1 min. After vortexing, the extracts were derivatized following the optimized derivatization process. The derivatized samples were dried by blowing with nitrogen at room temperature, and redissolved in 100 μL of ethyl acetate, vortexed for 5-min, and then 1 μL of each sample was injected into the GC for analysis.

2.4. Optimization of derivatization process

2.4.1. Optimization of derivatization reagents

GLY and AMPA were to obtain volatile derivatives by acylation and esterification, which can be directly detected by GC. The derivatizing reagents were optimized by a three-three cross derivatization process with 50 μL of TEA, PFP, and HFB and 100 μL of TFAA, PFPA, and HFBA, along with GLY and AMPA. After derivatization, the samples were dried by blowing with nitrogen at room temperature. Finally, the samples were redissolved in 100 μL of ethyl acetate, vortexed for 5-min and 1 μL of each sample was injected for analysis. The derivatization reagents were determined based on the retention time, peak area, and resolution of the targets.

2.4.2. Optimization and selection of derivatization conditions by single factor experiments

The influences of reaction temperature (60–110 °C), time (30–80 min), and the ratio of derivatizing reagents on the derivatization process were investigated separately by a single factor experiment.

Table 1
Factors and levels.

Level	Factor		
	A Temperature (°)	B Time (min)	C Proportion of derivatizing reagents (*v*/*v*, μL)
1	60	10	10:90
2	70	20	20:80
3	80	30	30:70
4	90	40	40:60
5	100	50	50:50

2.4.3. Orthogonal experimental design

Based on the single factor experiment, five levels were selected for each of three factors affecting the derivatization process (temperature, time, and the ratio of derivatizing reagents) and SPSS 16 was adopted to design a three-factor and five-level orthogonal test table. The test was performed according to the L_{25} (5^3) orthogonal table. Factor levels were listed in Table 1.

2.5. Optimization of SPE conditions

A one-step SPE method was utilized to study the extraction efficiency of GLY and AMPA from the samples by SPE columns, which included C18E (200 mg/6 mL), PEP-2 (200 mg/6 mL), PEP-2 (500 mg/6 mL), C18 (200 mg/6 mL) and SIM (200 mg/6 mL). The variety and amounts of washing solvent and eluent highly influence the extraction process. With GLY and AMPA being insoluble in methanol, 0.5, 1, 2, and 3 mL of methanol were used as washing solvent to evaluate the effects of different volumes of methanol for removing impurities. Acidified methanol (HCl in methanol solution), water and methanol solution were selected as the eluents.

2.6. Method validation

Eight mixed concentrations of GLY and AMPA (5, 10, 20, 50, 100, 200, 500, and 1000 ng/mL) in water were used for the calibration curve. The linear equations and correlation coefficients were calculated. The limit of detection (LOD) and limit of quantification (LOQ) were determined at signal-to-noise (*S*/*N*) ratios of 3 and 10, respectively. The intraday and interday accuracy and precision of the method were assessed at low, medium and high levels (20, 200, and 800 ng/mL, n = 6) within one day and different days (n = 3). The extraction recoveries were evaluated by comparing the peak areas of postextracted GLY and AMPA, spiking in known amounts of standards in real samples with the corresponding standard solution at three levels (20, 200, and 800 ng/mL, n = 3).

3. Results

3.1. Optimization of derivatization process

3.1.1. Optimization of derivatization reagents

The results of the three-three cross derivatization process showed that derivatives of GLY and AMPA with HFBA and HFB showed better separation, more symmetrical peak shapes and lower detection limits in GC analysis. Based on the results, HFB and HFBA were selected as derivatizing agents for esterification and acylation of GLY and AMPA.

3.1.2. Optimization and selection of derivatization conditions by single factor experiments

The optimum conditions for derivatization by single factor experiments were determined as the reaction temperature of 90 °C, reaction time of 60 min, and the ratio of derivatizing reagents, HFB: HFBA of 30 μL: 70 μL, *v*/*v*.

Table 2
Design and results of orthogonal test.

Number		A	B	C	Peak area	
					AMPA	GLY
1		1	3	3	705.6	198.5
2		1	2	2	854.9	203.2
3		2	3	1	1416.8	550.7
4		3	1	2	144.0	65.5
5		4	3	2	1846.7	1299.9
6		3	5	1	1900.0	1445.9
7		3	3	4	1799.4	1263.9
8		4	4	3	1715.1	2778.2
9		5	4	1	1453.2	980.6
10		2	4	2	1239.1	543.0
11		3	4	5	483.6	308.7
12		4	1	5	1650.6	664.0
13		5	2	4	2080.8	1744.4
14		5	3	5	1307.6	1172.3
15		2	5	3	1686.6	901.8
16		5	5	2	1490.2	1176.1
17		1	4	4	1605.0	466.9
18		1	5	5	1428.3	476.7
19		1	1	1	330.2	75.6
20		4	5	4	2228.4	1940.1
21		5	1	3	1549.6	943.0
22		2	2	5	1837.3	535.7
23		3	2	3	738.2	375.6
24		4	2	1	1820.7	1053.6
25		2	1	4	319.0	69.1
AMPA	K_1	4923.9	3993.3	6920.9		
	K_2	6498.8	7331.9	5574.7		
	K_3	5065.2	7076.0	6395.0		
	K_4	9261.5	6496.0	8032.6		
	K_5	7881.3	8733.4	6707.4		
	R	4337.6	4740.2	2457.9		
GLY	K_1	1420.8	1817.1	4106.3		
	K_2	2600.1	3912.4	3287.6		
	K_3	3459.5	4485.2	5197.1		
	K_4	7735.7	5077.3	5484.3		
	K_5	6016.4	5940.5	3157.2		
	R	6314.9	4123.5	2327.1		

3.1.3. Orthogonal experimental design

The test was performed according to the L_{25} (5^3) orthogonal table, and the results are listed in Table 2. The influence of reaction temperature (A) was the highest on the derivatization process, followed by reaction time (B) and the ratio of derivatizing reagents (C) (Table 2).

The factors affecting the derivatization were optimized through single-factor and orthogonal tests. Combining the results of the single factor optimization and orthogonal test, the optimal condition solution for derivatization was selected as A4B5C4, i.e., a reaction temperature of 90 °C, derivatization time of 50 min and the ratio of HFB: HFBA of 40 μL: 60 μL, *v*/*v*.

3.2. Optimization of the solid phase extraction conditions

Three SPE columns (C18E, C18 and SIM) showed lower retention values for GLY and AMPA, whereas the PEP-2 SPE column exhibited the best retention values for those compounds. In addition, as PEP-2 (500 mg, 6 mL) showed better extraction efficiency than PEP-2 (200 mg, 6 mL) for GLY and AMPA and satisfied the experimental requirements, PEP-2 (500 mg, 6 mL) was selected as the SPE column.

When the volume of methanol exceeded 1 mL, the recovery rates of GLY and AMPA were reduced; therefore, the optimum volume of methanol was 1 mL as the washing solvent. Acidified methanol (HCl in methanol solution) was selected as the eluent, based on

Table 3
Results of precision and accuracy ($\bar{x} \pm s$, n=6).

Compound	Linearity range (ng/mL)	r	Spiked concentration (ng/ml)			LOD (ng/mL)	LOQ (ng/mL)
			Low[a]	Middle[b]	High[c]		
GLY	10-1000	0.9998	105.2(9.5)	100.8(2.8)	101.2(1.8)	0.10	0.37
AMPA	10-1000	0.9991	102.9(7.3)	102.0(3.1)	101.7(3.3)	0.22	0.81

[a] GLY and AMPA spiked at 20 ng/mL level.
[b] GLY and AMPA spiked at 200 ng/mL level.
[c] GLY and AMPA spiked at 800 ng/mL level.

the properties of GLY and AMPA. When 2 mL of acidified methanol solution (methanol: HCl 9:1, *v*/*v*) was employed as the eluent, the recoveries of GLY and AMPA were the highest.

3.3. Methodological validation

GLY and AMPA showed a favorable linear relationship over the ranges of 10–1000 ng/ml, and the linear equations were $y = 9.3969x - 20.237$ ($r = 0.9998$) and $y = 4.2455x + 9.4607$ ($r = 0.9991$). The LOD valus of GLY and AMPA ($S/N = 3$) were 0.10 and 0.22 ng/mL, while the LOQ values ($S/N = 10$) were 0.37 and 0.81 ng/mL, respectively. The intraday and interday precisions (RSD, %) were between 1.3–5.5% and the accuracies were between 93.5–109.7%, whereas the recovery rates were between 75.7–86.9%. The results are exhibited in Table 3. The chromatographic run time was 6 min, which was suitable for high-throughput sample analysis.

3.4. Methodological applications

3.4.1. Studies on degradation of GLY in water

The 100 ng/mL GLY solutions at different pH values (pH = 5, 7, 9) were divided into two groups. One group was placed outdoors, while the other was placed indoors without light. The concentration of GLY was determined periodically to evaluate the effects of acid and light on the degradation of GLY over 100 days (October 13, 2017– January 22, 2018). Under the outdoor conditions after 100 days, the degradation rates of GLY were 9.6%, 2.8%, and 1.4% at pH 5, 7, and 9, respectively. Under the dark indoor conditions after 100 days, the degradation rates were 1.6%, 0.7%, and 0.4% at pH 5, 7, and 9, respectively. At the same pH, the degradation rates of GLY in outdoor samples were greater than those indoors without light, and the degradation rate of GLY decreased with the increase in pH.

3.4.2. Study on the migration of GLY and AMPA in soil

A 20 cm-height soil column in a 30 cm plastic column with a diameter of 1 cm was prepared. Accurately, 100 μL of a mixed solution of GLY and AMPA (concentration: 100 μg/mL) was added to the column. The column was kept under 200 mm simulated rainfall at an intensity of 1 mm/min for 200 min. After raining, the soil column was stratified with a 0.5 cm intercept. The soil sample was placed in a 10 mL beaker, ultrasonically extracted with 5 mL of water for 30 min, and the sample was prepared and treated as described in Section 2.3. The retention times for GLY and AMPA were 5.41 min and 3.30 min, respectively. The typical chromatograms of 100 ng/mL GLY, 100 ng/mL AMPA and soil samples are shown in Fig. 1. GLY and AMPA were distributed from the surface to the 6.5 cm deep soil layer and the maximum concentration of GLY and AMPA appeared in the 4.0 cm deep soil layer. The results are shown in Fig. 2.

3.4.3. Sample analysis

Different pH NaOH solutions, potassium phosphate buffer, ammonia solution, boric acid buffer and water were investigated for extraction of GLY and AMPA from soil samples. After the extraction solution dried, salts were precipitated and the derivatization

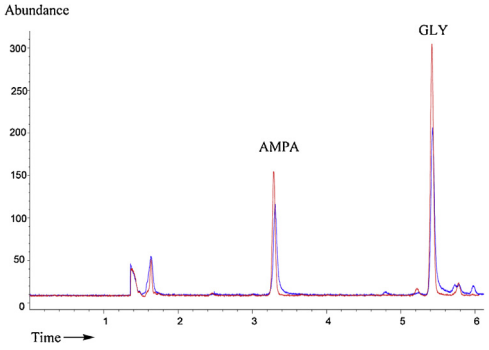

Fig. 1. 100 ng/mL GLY and 100 ng/mL AMPA typical chromatograms (red- reference, blue-soil sample) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

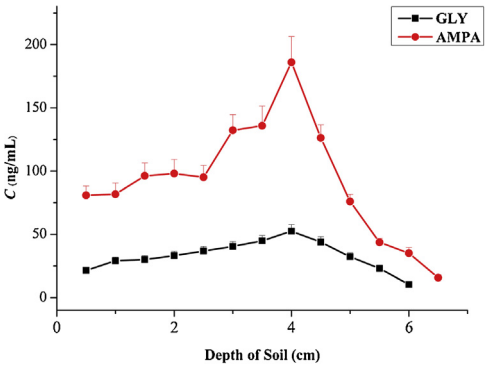

Fig. 2. The results of GLY and AMPA distribution in soil.

reaction was affected, which made it impossible to detect GLY and AMPA. The results indicated that water provided better extraction of GLY and AMPA in soil than others. Therefore, water was selected as the extraction solvent.

The method was used to analyze four water samples and six soil samples. The data showed that GLY and AMPA were not detected in the actual samples. The use of GLY is increasing with the widespread cultivation of certain transgenic crops in various countries. GLY and AMPA residues have become potential environmental hazards in many countries. China being a bulk producer and large consumer of GLY must monitor GLY and AMPA levels and investigate the long-term toxic effects of these compounds in human beings.

4. Discussion

GLY is a highly popular herbicide used globally. Accordingly, a simple, rapid, and reliable method was developed for detecting GLY, as well as its metabolite AMPA, which may help evaluate the potential role of GLY and AMPA as environmental hazards. In this study, an SPE GC-FPD method was successfully developed and validated for simultaneous measurement of GLY and AMPA in water and soil

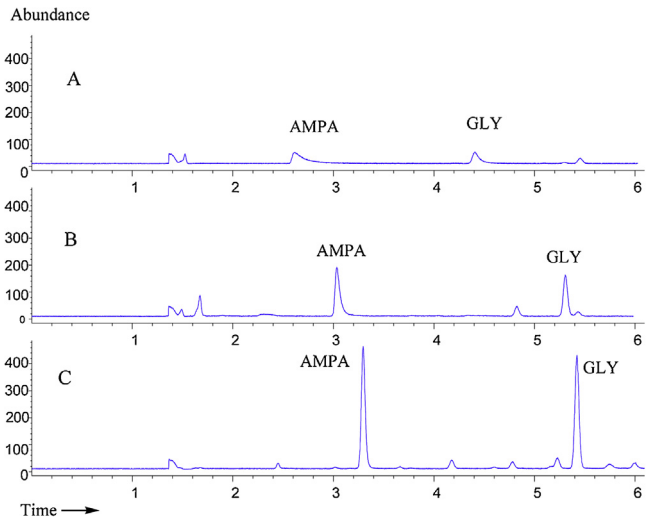

Fig. 3. Derivatives of GLY and AMPA with different derivatizing reagents (A TFA: TFAA, B HFB: TFAA, C HFB: HFBA).

samples. The LOQ of GLY in our study (0.37 ng/mL) is lower than in the study by Kataoka, H. et al. (1.2 ng/mL) [31]. The LODs of GLY and AMPA (0.10 and 0.22 ng/mL) in our study obtained by GC-FPD were lower than GC-FID (2.54 and 3.33 ng/mL) and GC-CI-MS (1.69 and 2.78 ng/mL) [33]. The method is better than "standard examination methods for drinking water-pesticides parameters" (the detection limits of GLY and AMPA were 25 ng/mL), which was promulgated by the National Standardization Administration Committee of China in 2006.

In this study, we tested several derivatizing reagents. Nevertheless, neither derivatizing reagents as TFA and TFAA nor TFA and HFB provides satisfactory results for the GC-FPD method. The data showed that derivatives of GLY and AMPA with HFBA and HFB showed better separation, symmetrical peak shapes and lower detection limits in GC analysis (Fig. 3), due to the higher boiling point and lower volatility of HFBA compared to TFAA. Furthermore, we reduced the amount of derivatizing reagents from 150 μL to 100 μL, which minimized the usage of organic solvents according to green analytical chemistry.

Although GLY degradation in water has been well studied and reported, the purpose of our study is investigating the degradation of GLY at different pH and storage conditions. The results show that degradation rates of GLY under different storage conditions were different during the 100-day degradation test. Light and acidic pH accelerated the degradation of GLY. In acidic solution, GLY was present in an alternate, easily degraded molecular. The residual stability of GLY in water (evaluated for 15 days) was consistent with earlier reports [34]. The degradation of GLY during 100 days was found to be insignificant, which agreed with the previous literature [35]. Therefore, GLY residues in water may cause long-term contamination, especially in deep water without light.

Soil column tests are often used to study the migration of fertilizers, pesticides, environmental pollutants and metal ions in soil [36–39]. However, soil absorption is a dynamic process which involves numerous steps, and the penetration into soil experiments may not be accurate under the current scenario. Under the existing conditions of the laboratory, we attempt to make the experimental conditions closer to the actual environment and study the migration of GLY and AMPA in the soil. A 20 cm-height soil column was prepared in a 30 cm plastic column, while a 10 cm-height column was used to accommodate 20 mL water. There was no drain or outlet system for the soil column.

Under the action of precipitation and surface water, GLY and AMPA migrate from surface soil to deep soil. GLY and AMPA were detected in 30 cm deep soils in the forest area of Galicia in northwestern Spain [40]. The contents of GLY and AMPA in 0–5 cm deep tillage soil in Argentina were 35–1502 and 299–2256 μg/kg, respectively [41]. GLY and AMPA were detected at a depth of 35 cm in the soil even after one month of GLY treatment on the fields [42]. The European Union estimated that the detection rates of GLY and AMPA in soil samples from different countries and regions were up to 45% [43]. GLY residues in the groundwater samples suggest that the residual GLY and AMPA can migrate from the soil surface to the deep soil layer. In this study, soil column leaching experiments were used to simulate the migration characteristics of GLY and AMPA in the soil under 200 mm rainfall. Both GLY and AMPA showed significant downward migration, and they were primarily distributed in the topsoil. GLY and AMPA migrated approximately 7 cm below the soil surface, and the concentrations of GLY and AMPA were highest at 4 cm below the surface. The results were similar to those obtained after GLY treatment to the cultivated soil in Argentina [42]. Frequent and heavy application of GLY may result in groundwater pollution, whereas the residual GLY and AMPA from the land surface would migrate and contaminate surface waters [44]. The presence of GLY and AMPA in groundwater indicated that GLY may exhibit a certain grade of mobility in soils [45].

The use of GLY is increasing with the widespread cultivation of certain transgenic crops in various countries. GLY and AMPA residues have become potential environmental hazards in many countries. China, being a bulk producer and large consumer of GLY, must monitor GLY and AMPA levels and investigate the long-term toxic effects of these compounds in human beings.

5. Conclusion

In this study, an accurate, sensitive and reliable GC-FPD method was suitable for simultaneous detection of GLY and AMPA in water and soil. The method showed good linearity, precision, accuracy and stability. A novel one-step SPE (Cleanert® PEP-2 SPE column) method was employed for extraction of GLY and AMPA from samples. HFB and HFBA were used as derivatization reagents, and the optimal derivatization parameters were obtained. This GC-FPD method may be helpful in monitoring the GLY and AMPA levels in soil or water and for assessing the impact of these compounds on the environment.

Acknowledgments

This work was supported by the Natural Science Foundation of Hebei Province (Nos. H 2018206122).

References

[1] S. Thongprakaisang, A. Thiantanawat, N. Rangkadilok, T. Suriyo, J. Satayavivad, Glyphosate induces human breast cancer cells growth via estrogen receptors, Food Chem. Toxicol. 59 (2013) 129–136.
[2] S. Richard, S. Moslemi, H. Sipahutar, N. Benachour, G.-E. Seralini, Differential effects of glyphosate and roundup on human placental cells and aromatase, Environ. Health Perspect. 113 (2005) 716–720.
[3] T.E. Arbuckle, Z. Lin, L.S. Mery, An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population, Environ. Health Perspect. 109 (2001) 851–857.
[4] M. Townsend, C. Peck, W. Meng, M. Heaton, R. Robison, K. O'Neill, Evaluation of various glyphosate concentrations on DNA damage in human Raji cells and its impact on cytotoxicity, Regul. Toxicol. Pharmacol. 85 (2017) 79–85.
[5] C.B. Chang, C.C. Chang, Refractory cardiopulmonary failure after glyphosate surfactant intoxication: a case report, J. Occup. Med. Toxicol. 4 (2009) 1–4.
[6] S.J. James, P. Cutler, S. Melnyk, S. Jernigan, L. Janak, D.W. Gaylor, J.A. Neubrander, Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism, Am. J. Clin. Nutr. 80 (2004) 1611–1617.

[7] Lesley D. Morrison, David D. Smith, S.J. Kish, Brain S-adenosylmethionine levels are severely decreased in Alzheimer's disease, J. Neurochem. 67 (1996) 1328–1331.
[8] L. Schinasi, M.E. Leon, Non-hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis, Int. J. Environ. Res. Public Health 11 (2014) 4449–4527.
[9] J.Z. Sandrini, R.C. Rola, F.M. Lopes, H.F. Buffon, M.M. Freitas, Cd.M.G. Martins, C.E. da Rosa, Effects of glyphosate on cholinesterase activity of the mussel Perna perna and the fish *Danio rerio* and *Jenynsia multidentata*: in vitro studies, Aquat. Toxicol. 130-131 (2013) 171–173.
[10] T.M. Uren Webster, L.V. Laing, H. Florance, E.M. Santos, Effects of glyphosate and its formulation, roundup, on reproduction in zebrafish (*Danio rerio*), Environ. Sci. Technol. 48 (2014) 1271–1279.
[11] N.M. Roy, J. Ochs, E. Zambrzycka, A. Anderson, Glyphosate induces cardiovascular toxicity in Danio rerio, Environ. Toxicol. Pharmacol. 46 (2016) 292–300.
[12] N.M. Roy, B. Carneiro, J. Ochs, Glyphosate induces neurotoxicity in zebrafish, Environ. Toxicol. Pharmacol. 42 (2016) 45–54.
[13] F.M. Lopes, A.S. Varela Junior, C.D. Corcini, A.C. da Silva, V.G. Guazzelli, G. Tavares, C.E. da Rosa, Effect of glyphosate on the sperm quality of zebrafish Danio rerio, Aquat. Toxicol. 155 (2014) 322–326.
[14] D. Bridi, S. Altenhofen, J.B. Gonzalez, G.K. Reolon, C.D. Bonan, Glyphosate and Roundup® alter morphology and behavior in zebrafish, Toxicology 392 (2017) 32–39.
[15] A. Conrad, C. Schroter-Kermani, H.W. Hoppe, M. Ruther, S. Pieper, M. Kolossa-Gehring, Glyphosate in German adults - time trend (2001 to 2015) of human exposure to a widely used herbicide, Int. J. Hygiene Environ. Health 220 (2017) 8–16.
[16] M.V. Khrolenko, P.P. Wieczorek, Determination of glyphosate and its metabolite aminomethylphosphonic acid in fruit juices using supported-liquid membrane preconcentration method with high-performance liquid chromatography and UV detection after derivatization with *p*-toluenesulphonyl chloride, J. Chromatogr. A 1093 (2005) 111–117.
[17] F. Fang, R. Wei, X. Liu, Novel pre-column derivatisation reagent for glyphosate by high-performance liquid chromatography and ultraviolet detection, Int. J. Environ. Anal. Chem. 94 (2014) 661–667.
[18] S. Wang, B. Liu, D. Yuan, J. Ma, A simple method for the determination of glyphosate and aminomethylphosphonic acid in seawater matrix with high performance liquid chromatography and fluorescence detection, Talanta 161 (2016) 700–706.
[19] L. Sun, D. Kong, W. Gu, X. Guo, W. Tao, Z. Shan, Y. Wang, N. Wang, Determination of glyphosate in soil/sludge by high performance liquid chromatography, J. Chromatogr. A 1502 (2017) 8–13.
[20] M.X. Chen, Z.Y. Cao, Y. Jiang, Z.W. Zhu, Direct determination of glyphosate and its major metabolite, aminomethylphosphonic acid, in fruits and vegetables by mixed-mode hydrophilic interaction/weak anion-exchange liquid chromatography coupled with electrospray tandem mass spectrometry, J. Chromatogr. A 1272 (2013) 90–99.
[21] H. Guo, L.S. Riter, C.E. Wujcik, D.W. Armstrong, Direct and sensitive determination of glyphosate and aminomethylphosphonic acid in environmental water samples by high performance liquid chromatography coupled to electrospray tandem mass spectrometry, J. Chromatogr. A 1443 (2016) 93–100.
[22] L.C. Schrubbers, M. Masis-Mora, E.C. Rojas, B.E. Valverde, J.H. Christensen, N. Cedergreen, Analysis of glyphosate and aminomethylphosphonic acid in leaves from *Coffea arabica* using high performance liquid chromatography with quadrupole mass spectrometry detection, Talanta 146 (2016) 609–620.
[23] E. Borjesson, L. Torstensson, New methods for determination of glyphosate and (aminomethyl)phosphonic acid in water and soil, J. Chromatogr. A 886 (2000) 207–216.
[24] L. Pizzul, P. Castillo Mdel, J. Stenstrom, Degradation of glyphosate and other pesticides by ligninolytic enzymes, Biodegradation 20 (2009) 751–759.
[25] L. Bergström, E. Börjesson, J. Stenström, Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil, J. Environ. Q. 40 (2011) 98–108.
[26] K.N. Reddy, A.M. Rimando, S.O. Duke, Aminomethylphosphonic acid, a metabolite of glyphosate, causes injury in glyphosate-treated, glyphosate-resistant soybean, J. Agric. Food Chem. 52 (2004) 5139–5143.
[27] A. Steinborn, L. Alder, B. Michalski, P. Zomer, P. Bendig, S.A. Martinez, H.G. Mol, T.J. Class, N.C. Pinheiro, Determination of glyphosate levels in breast milk samples from Germany by LC-MS/MS and GC-MS/MS, J. Agric. Food Chem. 64 (2016) 1414–1421.
[28] B.H. Zainudin, S. Salleh, R. Mohamed, K.C. Yap, H. Muhamad, Development, validation and determination of multiclass pesticide residues in cocoa beans using gas chromatography and liquid chromatography tandem mass spectrometry, Food Chem. 172 (2015) 585–595.
[29] B. Barfi, A. Asghari, M. Rajabi, A. Goochani Moghadam, N. Mirkhani, F. Ahmadi, Comparison of ultrasound-enhanced air-assisted liquid-liquid microextraction and low-density solvent-based dispersive liquid-liquid microextraction methods for determination of nonsteroidal anti-inflammatory drugs in human urine samples, J. Pharm. Biomed. Anal. 111 (2015) 297–305.
[30] F. Ahmadi, H. Asgharloo, S. Sadeghi, V. Gharehbagh-Aghababa, H. Adibi, Post-derivatization procedure for determination of hippuric acid after extraction by an automated micro solid phase extraction system and monitoring by gas chromatography, J. Chromatogr. B 877 (2009) 2945–2951.
[31] H. Kataoka, K. Hor, M. Makita, Determination of The Herbicide Glyphosate and Its Metabolite (Aminomethyl) phosphonic Acid by Gas Chromatography with Flame Photometric Detection, Agric. Biol. Chem. 55 (1991) 195–198.
[32] H. Kataoka, S. Ryu, N. Sakiyama, M. Makita, Simple and rapid determination of the herbicides glyphosate and glufosinate in river water, soil and carrot samples by gas chromatography with flame photometric detection, J. Chromatogr. A 726 (1996) 253–258.
[33] Z.H. Kudzin, D.K. Gralak, J. Drabowicz, J.L. uczak, Novel approach for the simultaneous analysis of glyphosate and its metabolites, J. Chromatogr. A 947 (2002) 129–141.
[34] C. Hao, D. Morse, F. Morra, X. Zhao, P. Yang, B. Nunn, Direct aqueous determination of glyphosate and related compounds by liquid chromatography/tandem mass spectrometry using reversed-phase and weak anion-exchange mixed-mode column, J. Chromatogr. A 1218 (2011) 5638–5643.
[35] R. Annett, H.R. Habibi, A. Hontela, Impact of glyphosate and glyphosate-based herbicides on the freshwater environment, J. Appl. Toxicol. 34 (2014) 458–479.
[36] R.M. Briones, A.K. Sarmah, Sorption and mobility of metformin and guanylurea in soils as affected by biosolid amendment: Batch and column tests, Environ. Pollut. 244 (2018) 19–27.
[37] B.K. Saha, M.T. Rose, V.N.L. Wong, T.R. Cavagnaro, A.F. Patti, Nitrogen dynamics in soil fertilized with slow release brown coal-urea fertilizers, Sci. Rep. 8 (2018) 1–10.
[38] Y. Sihn, S. Bae, W. Lee, Immobilization of uranium (VI) in a cementitious matrix with nanoscale zerovalent iron (NZVI), Chemosphere 215 (2018) 626–633.
[39] K. Ringering, Y. Kouhail, Y. Yecheskel, I. Dror, B. Berkowitz, Mobility and retention of indium and gallium in saturated porous media, J. Hazard. Mater. 363 (2019) 394–400.
[40] F. Veiga, J.M. Zapata, M.L. Fernandez Marcos, E. Alvarez, Dynamics of glyphosate and aminomethylphosphonic acid in a forest soil in Galicia, north-west Spain, Sci. Total Environ. 271 (2001) 135–144.
[41] V.C. Aparicio, E. De Geronimo, D. Marino, J. Primost, P. Carriquiriborde, J.L. Costa, Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins, Chemosphere 93 (2013) 1866–1873.
[42] L. Lupi, K.S. Miglioranza, V.C. Aparicio, D. Marino, F. Bedmar, D.A. Wunderlin, Occurrence of glyphosate and AMPA in an agricultural watershed from the southeastern region of Argentina, Sci. Total Environ. 536 (2015) 687–694.
[43] V. Silva, L. Montanarella, A. Jones, O. Fernandez-Ugalde, H.G.J. Mol, C.J. Ritsema, V. Geissen, Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils of the European Union, Sci. Total Environ. 621 (2018) 1352–1359.
[44] X. Yang, F. Wang, C.P.M. Bento, S. Xue, L. Gai, R. van Dam, H. Mol, C.J. Ritsema, V. Geissen, Short-term transport of glyphosate with erosion in Chinese loess soil–a flume experiment, Sci. Total Environ. 512-513 (2015) 406–414.
[45] J. Sanchis, L. Kantiani, M. Llorca, F. Rubio, A. Ginebreda, J. Fraile, T. Garrido, M. Farre, Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry, Anal. Bioanal. Chem. 402 (2012) 2335–2345.