Liquid Chromatographic Determination of Glyphosate in Fortified Soil and Water Samples

Robert L. Glass

A high-performance liquid chromatographic (HPLC) method which uses fluorescence detection and an amine phase column is described for the determination of glyphosate in fortified soil and water samples after the derivatization of the herbicide. Glyphosate was extracted and subsequently separated with Bio-Rad AG 1-X8 anion-exchange resin (HCO_3^- form) prior to derivatizing with the fluorogenic labeling reagent, 9-fluorenylmethyl chloroformate (FMOCCl). The rate of conversion of glyphosate to the glyphosate–FMOCCL derivative was ca. 60%. The minimum levels of detection for glyphosate were 10 ppb in fortified water, 5 ppm in a sandy loam soil, and 50 ppm in a clay loam soil. Recoveries of glyphosate were greater than 90% in water fortified at 20 ppb–4 ppm, 55% in the sandy loam soil, and ca. 20% in the clay loam soil fortified at 200 ppm. In comparison to other reported methods, this HPLC method is simpler, faster, and specific for glyphosate.

The herbicide glyphosate, *N*-(phosphonomethyl)glycine, is extensively used in agriculture for the control of many annual and perennial weeds. Although several analytical methods have been reported for glyphosate, as discussed by Glass (1981), most of these methods suffer from such technical difficulties as being too lengthy and complicated, lack specificity for glyphosate, or require expensive, specialized equipment.

However, a method developed by Moye and Boning (1979) in which glyphosate was derivatized with 9-fluorenylmethyl chloroformate (FMOCCl) and measured by high-performance liquid chromatography (HPLC) using fluorescence detection appears to be very promising for the analysis of glyphosate in field soil and water samples. This method has been referred to also as the precolumn derivatization of glyphosate with FMOCCl. The glyphosate–FMOCCl derivative, whose fluorescence maximum occurs at 315 nm, is readily formed under alkaline conditions.

Recently, Moye and St. John (1980) compared this precolumn labeling HPLC arrangement with a postcolumn labeling arrangement, reported earlier by Moye et al. (1977), in the determination of glyphosate in various crops. Recoveries of glyphosate at 0.1 ppm in cantaloupe were 100% by using the precolumn labeling arrangement and 70–96% with the latter arrangement.

In the present investigation, the objective was to determine if the new HPLC method using the precolumn derivatization of glyphosate could be successfully used for the analysis of glyphosate in fortified soil and water samples.

EXPERIMENTAL SECTION

Liquid Chromatograph. A Spectra-Physics Model 8000B pumping system was used for solvent delivery. An Aminco fluoromonitor (70 μL flow cell) was used for detecting the fluorescent derivative of glyphosate, whose excitation and emission energies have been reported at 270 and 313 nm, respectively (Moye and Boning, 1979). A Corion 253.7-nm interference filter (15% transmission at 253.7 nm) was installed as the primary filter in order to isolate the 254-nm line from the 313 nm, 365 nm, and other undesirable lines emitted by the 4-W germicidal lamp as described by Argauer (1980) in an earlier paper. The chromatographic column was an amine bonded phase (Chromosorb LC-9) column, 15 cm × 4.6 mm (i.d.). Similar results (not shown) were obtained with other amine bonded phase columns such as LiChrosorb NH_2 and Alltech NH_2. The mobile phase was 85% acetonitrile–15% 0.1 M KH_2PO_4 buffer (pH 5.4) with a flow rate of 1.5 mL/min. Samples were injected with a 5-μL Valco sample loop.

Chemicals. Analytical-grade glyphosate (94% purity) was obtained from Monsanto Chemical Co., St. Louis, MO, and used without further purification. The fluorescent derivatizing reagent, 9-fluoroenylmethyl chloroformate (FMOCCl), was obtained from Aldrich Chemical Co. Bio-Rad AG 1-X8 (Cl^-) (100–200-mesh) anion-exchange resin was received from Bio-Rad Laboratories but was converted to HCO_3^- in the laboratory. All other chemicals were reagent grade and were used without further purification.

Glyphosate–FMOCCl Derivative. The fluorescent derivative of glyphosate was made by labeling the secondary amine group of glyphosate with FMOCCl according to the procedure described by Moye and Boning (1979). Macroquantities of the derivative were prepared as follows: 101 mg of glyphosate (0.6×10^3 mol) dissolved in 6.5 mL of 0.1 N NaOH was combined with 161 mL of FMOCCl (0.6×10^3 mol) dissolved in 15 mL of acetone. To the mixture was added 8.5 mL of distilled water. The mixture was initially adjusted to pH 9.0 with 0.1 N NaOH and maintained near that pH during 30 min of stirring. Afterward, the mixture was shaken with 25 mL of ethyl ether in order to remove unreacted organic substances. The aqueous phase was acidified with 0.1 N HCl to pH 1.5 and then extracted with two portions of ethyl acetate. The extract that contained the glyphosate–FMOCCl derivative was roto-evaporated to dryness. The derivative was precipitated from acetone with hexane and isolated on glass on glass fiber filter disks. Then the derivative was redissolved in acetone and subsequently roto-evaporated to dryness. The glyphosate–FMOCCl derivative, which existed as a noncrystalline substance, was dissolved in ethyl acetate and then chromatographed on silicic acid (10 g) with a hexane–ethanol (1:1) mixture. The derivative was monitored by UV absorbance at 265 nm. Yield of 43% was found for the isolated eerivative.

Sample Preparation. The soil and water samples were prepared as follows: (1) Both distilled and river water samples were fortified with glyphosate ranging in con-

Weed Science Laboratory, Agricultural Environmental Quality Institute, Beltsville Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.

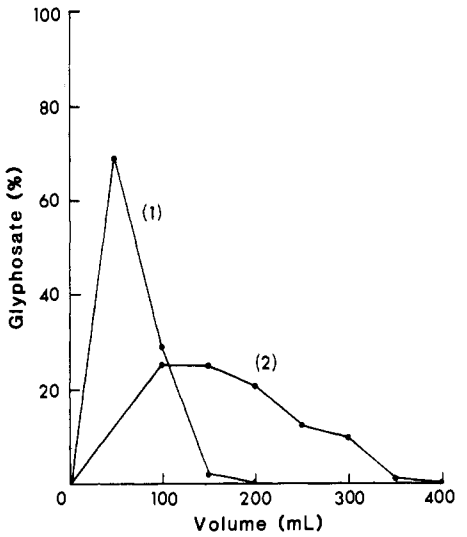

Figure 1. Comparison of elution profiles of glyphosate (1000 mg) from 8 g of Bio-Rad AG 1-X8 anion-exchange resin (HCO_3^- form) with 0.2 N NaCl (1) and 0.1 N NaCl (2). Column: 30 cm × 25 mm (i.d.).

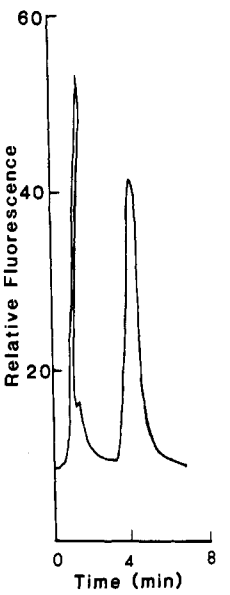

Figure 2. Chromatogram of 5 ng of the glyphosate–FMOCCl derivative.

centrations from 10 ppb to 4 ppm. Potomac River water was collected near Fort Washington, MD. (2) Iuka soil (silt loam, 1.5% 0.M., pH 6.1, from Beltsville, MD), Sassafras soil (sandy loam 1.2% 0.M, pH 5.6, from Beltsville, MD), and Houston soil (clay loam, 2.93% 0.M., pH 7.4, from College Station, TX) were fortified with glyphosate ranging from 5 to 200 ppm. Water was added to the soil samples to give about 10% moisture before applying glyphosate.

Sample Analyses. *Water Samples.* Glyphosate in fortified river and distilled water samples (pH 5–7) was initially isolated by anion-exchange chromatography using Bio-Rad AG 1-X8 anion-exchange resin (HCO_3^-). Borosilicate glass columns (30 cm × 25 mm) filled with 8 g of resin (2% moisture) were used for the isolation. Glyphosate that was dissolved in 10–1000 mL of water was applied to the column and then eluted with a 150-mL volume of 1.2 N NaCl. A comparison of the elution profiles of glyphosate, which was subsequently derivatized with FMOCCl, was made with 0.1 and 0.2 N NaCl (Figure 1).

Glyphosate in the NaCl eluant was derivatized by the following procedure: Aliquots (2–5 mL) of the 150-mL eluant were mixed with 2 mL of 0.025 M sodium borate in one vessel, and 1.5 mL of 1×10^{-2} M FMOCCl was mixed with 4 mL of acetone in another vessel. The two mixtures were then combined and allowed to sit without stirring for 15 min at room temperature. The pH of the medium was ca. 9.0. Unreacted FMOCCl was removed from the medium by shaking the mixture with 25 mL of ethyl acetate in a 125-mL separatory funnel. A portion of the water phase was removed with a syringe for chromatographic analysis.

Soil Samples. Glyphosate was extracted from fortified soils by shaking 20–30 g of soil for 1 h with 50 mL of 0.1 N NaOH in polypropylene tubes. The samples were then centrifuged for 15 min at 1900 rpm. Afterward, 10 mL of 0.1 M $CaCl_2$ was added in order to flocculate the suspended substances. The samples were centrifuged again for 15 min and then filtered to remove any particulate matter. The supernatant liquids (ca. pH 6) were subsequently passed through chromatographic columns (30 cm × 25 mm) containing 8 g of anion-exchange resin (HCO_3^- form). The columns were then rinsed with 50 mL of deionized water. Glyphosate was eluted from the resin with 150-mL aliquots of 0.2 N NaCl and subsequently derivatized in the same manner as described above.

Table I. Percent Conversions of Glyphosate to Glyphosate-FMOCCl Derivative in Distilled and River Water

	conversions, %, to glyphosate-FMOCCl[b]	
glyphosate,[a] μg	dist water	river water
50	64.2	47.0
20	66.5	67.5
10	60.0	70.0
means	63.5	61.5
SD	±2.7	±10.3

[a] Quantities of glyphosate dissolved in 100 mL of water. [b] Average values of at least two determinations.

RESULTS AND DISCUSSION

A typical chromatogram of the fluorescent glyphosate–FMOCCl derivative (5 ng) is shown in Figure 2. The derivative eluted from the amine bonded phase column in ca. 4 min with a flow rate of 1.5 mL/min. In the study by Moye and Boning (1979), it required ca. 18 min for the derivative to elute from a μ carbohydrate column using similar conditions. Calibration curves were linear from 1 to 25 ng in the present study.

The data in Table I show the percent conversions of glyphosate to the derivative in two water media. In distilled and river water, the average conversions were 63.6 and 61.5%, respectively. These results were lower than the percent conversion of 109% reported by Moye and Boning (1979). The use of larger quantities of certain reagents in the derivatization of glyphosate, such as acetone, may have inhibited the formation of the derivative in this study.

There were some other observations made during the course of the study which indicated that the glyphosate–FMOCCl derivative was unstable under certain conditions. The fluorescence responses of standard solutions of the derivative, which were stored in Pyrex vessels exposed to light at room temperature, began to decrease after ca. 30 days. Consequently, new standards had be prepared frequently. The changes in the fluorescence responses are attributed to the breakdown of the derivative, possibly due to chemical or microbial decomposition. It was also observed in the investigation that the fluorescence responses decreased after boiling aqueous fractions of the derivative.

Table II. Recoveries of Glyphosate from Fortified Water Samples after Cleanup and Derivatization

glyphosate, μg	concn	recoveries, %, of glyphosate[a]	
		dist water	river water
1000	4 ppm	89.5	92.6
400	80 ppb	80.0	
100	20 ppb	102.2	98.0
50	10 ppb	det[b]	det

[a] Average values of at least two determinations. [b] det signifies detectable.

Table III. Recoveries of Glyphosate from Three Fortified Soils

sample	glyphosate	
	added, ppm	recoveries, %
Houston clay loam	200 (2)[a]	18.99 ± 1.15[b]
	100 (2)	25.85 ± 0.60
	50 (2)	det[c]
Sassafras sandy loam	200 (4)	55.33 ± 1.35
	50 (4)	43.40 ± 1.10
	25 (4)	35.79 ± 3.25
	5 (4)	det
Iuka silt loam	200 (4)	52.70 ± 2.19
	50 (2)	39.55 ± 0.45
	25 (2)	30.69 ± 2.45
	5 (2)	det

[a] Number of determinations. [b] Averages and standard deviations. [c] det signifies detectable.

These results (not shown) demonstrated that analytical samples containing the glyphosate-FMOCCl derivative cannot be concentrated simply by boiling off the water. No satisfactory procedure using heat was developed for concentrating samples containing derivatized glyphosate in this study.

The data in Table II show the recoveries (percent) of glyphosate from fortified water samples after a single column cleanup and HPLC analysis of derivatized glyphosate. The results were similar for the distilled and river water samples. The minimum level of detection was 10 ppb in these water samples. It required ca. 2 h for a complete water analysis.

The recoveries (percent) of glyphosate from fortified Sassafras, Iuka, and Houston soils are shown in Table III. The lowest recovery of glyphosate was found on the Houston, which was the soil that possessed the highest clay content. This low recovery is attributed to adsorption of glyphosate by soil components. In an earlier study, Sprankle et al. (1975) showed that glyphoshate readily adsorbed to various clay fractions and organic matter from soils. The higher recoveries of glyphosate from the Sassafras and Iuka soils presumably are due to the lower rates of adsorption by these soils. The minimum levels of detection were 5 ppm on the Sassafras and Iuka soils and 50 ppm on the Houston. A complete soil analysis required ca. 3 h. In conclusion, it has been demonstrated that this HPLC method is reproducible and sensitive for the determination of glyphosate in soil and water samples. In comparison to other reported methods, this method is simpler, faster, and specific for glyphosate.

ACKNOWLEDGMENT

I thank M. E. Vernon, R. M. Dews, and K. T. Adams for their excellent technical assistance and R. J. Argauer for his assistance in modifying the Aminco fluoromonitor.

Registry No. Glyphosate, 1071-83-6; water, 7732-18-5.

LITERATURE CITED

Argauer, R. J. "Pesticide Analytical Methodology"; American Chemical Society: Washington, DC, 1980; ACS Symp. Ser. No. 136, Chapter 7.

Glass, R. L. *Anal. Chem.* **1981**, *53*, 921.

Moye, H. A.; Boning, A. J. *Anal. Lett.* **1979**, *12*, 25.

Moye, H. A.; Scherer, S. J.; St. John, P. A. *Anal. Lett.* **1977**, *10*, 1940.

Moye, H. A.; St. John, P. A. "Pesticide Analytical Methodology"; American Chemical Society: Washington, DC, 1980; ACS Symp. Ser. No. 136, Chapter 6.

Sprankle, P.; Meggitt, W. F.; Penner, D. *Weed Sci.* **1975**, *23*, 230.

Received for review February 1, 1982. Revised manuscript received September 13, 1982. Accepted November 11, 1982. Mention of a trade name or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Determination of Reducing Sugars, Sucrose, and Inulin in Chicory Root by High-Performance Liquid Chromatography

Alistair W. Wight* and Pieter J. van Niekerk

A high-performance liquid chromatographic method for determination of reducing sugars, sucrose, and inulin in both freeze-dried and roasted chicory root is described. Reducing sugars are determined directly after postcolumn reaction with tetrazolium blue, and sucrose is determined indirectly as glucose after hydrolysis by β-fructosidase. Inulin is determined indirectly as fructose and glucose formed as a result of mild acid hydrolysis. The method is reasonably rapid and provides a more complete analysis than existing methods, and determinations can be made with a satisfactory degree of accuracy and precision.

Relatively little information on analysis of carbohydrates in chicory is available in the literature. Free fructose and glucose have been determined by enzymatic methods (Promayon et al., 1976; Blanc, 1978) while thin-layer chromatography (TLC) has been used to determine fructose, glucose, and sucrose (Bachman and Zegota, 1974). Chubey and Dorell (1978) determined fructose and glucose in two chicory cultivars harvested at different stages during two successive seasons.

Most of the methods described in the literature have limitations for routine analysis, however. TLC methods are rather time consuming and accurate quantitation is

National Food Research Institute, Pretoria, 0001, South Africa.

0021-8561/83/1431-0282$01.50/0