Residue Analysis of Glyphosate in Blackberries by High-Performance Liquid Chromatography and Postcolumn Reaction Detection

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A procedure is described for determining residue levels of *N*-(phosphonomethyl)glycine (glyphosate) in blackberries. The sample is extracted in acid medium, cleaned up on a cation and anion resin column, and analyzed by HPLC with postcolumn derivatization with *o*-phthalaldehyde and a fluorescence detector at an excitation wavelength of 230 nm and with a 418-nm cutoff filter for emission. The method sensitivity is 0.05 ppm. The blackberries treated 81 days before harvest with 4 + 5 lb of a.i./acre plus a 33% wiper solution of glyphosate and another plot with 8 + 10 lb of a.i./acre plus a 33% wiper solution of glyphosate had residue levels of <0.05 ppm and recoveries ranged from 80.0 to 92.0% with a mean of 85.0%.

Glyphosate [*N*-(phosphonomethyl)glycine] is a broad-spectrum nonselective postemergence herbicide with low mammalian toxicity and little or no residual effect in the soil (Baird et al., 1971). (Aminomethyl)phosphonic acid has been shown to be the major metabolite in plants, water, and soil (Sprankle et al., 1978), but significant residues of this metabolite are very infrequently encountered when formulations of glyphosate are used at prescribed rates.

Residue procedures for the analysis of glyphosate have been reported employing gas chromatography (Guinivin et al., 1982; "Pesticide Analytical Manual", 1977; Rueppel et al., 1976; Thompson et al., 1980), thin-layer chromatography (Putnam, 1976; Nomura and Hilton, 1977; Ragab, 1978; Rueppel et al., 1977; Sprankle et al., 1978; Young et al., 1977; Zandstra and Nishimoto, 1977), high-performance liquid chromatogrphy (Moye et al., 1983), and differential pulse chromatography (Bronstad and Friestad, 1976).

Most of these methods have deficiencies in the extraction procedures of not extracting potential conjugates of glyphosate with plant constituents, lengthy derivatization steps, inadequate or tedious cleanup procedures, and/or poor recovery of the compounds under investigation. The thin-layer chromatographic procedures are not adequately quantitative.

The quantitative high-performance liquid chromatographic method presently described is relatively rapid, easy, and precise and provides reproducible recoveries with a mean of 85.0% (range 80.0–92.0%) when fortifications of blackberry controls of 0.05, 0.10, and 0.25 ppm were analyzed.

MATERIALS AND METHODS

Apparatus and Reagents. The following reagents were used: deionized water, purified with a Norganic water purification system (Millipore, Bedford, MA); hydrochloric acid, AR; Hydrochloric acid, purified, 6 M (GFS Chemicals, Columbus, OH), diluted to 0.02 N for use; phosphoric acid, 85% AR; sulfuric acid, concentrated; cation-exchange resin, AG 50W-X8, 100–120-mesh hydrogen form (Bio-Rad, Richmond, CA); anion-exchange resin, AG 1-X4, 50–100-mesh chloride form (Bio-Rad, Richmond, CA); sodium hydroxide, pellets, AR; potassium phosphate, monobasic, AR; calcium hypochlorite, crystals; boric acid, granular; potassium hydroxide, pellets; *o*-phthalaldehyde (Aldrich, Milwaukee, WI); mercaptoethanol (Pierce Chemical Co., Rockford, IL). The HPLC mobile phase consisted of 1.5 g of phosphoric acid plus 0.15 g of sulfuric acid dissolved in 500 mL of purified water and filtered by suction through a Whatman GF/F glass microfiber filter. The cleavage reagent was 0.5 g of calcium hypochlorite dissolved in 500 mL of purified water. Five milliliters of this solution was added to 1 L of degassed buffer containing 1.36 g of monobasic potassium phosphate, 0.4 g of sodium hydroxide, and 11.6 g of sodium chloride. The mixture was filtered through a Whatman GF/F glass microfiber filter. The OPA reagent consisted of 0.2 g of *o*-phthalaldehyde dissolved in 2 mL of methanol and 0.25 mL of mercaptoethanol added to 250 mL of degassed buffer containing 3.1 g of boric acid and 3.1 g of sodium hydroxide. The solution was vacuum filtered through a Whatman GF/F filter.

The following apparatus were used: a Haake Model D3-L constant temperature circulating water bath (Haake, Saddle Brook, NJ); a high-performance liquid chromatograph–Waters 6000A chromatographic pump (Waters Associates, Milford, MA), a Rheodyne Model 7120 sample injector valve equipped with a 100-μL loop (Rheodyne, Berkeley, CA); a 250 mm × 4 mm i.d. stainless steel column packed with Aminex A-27 (Bio-Rad) encased in a HPLC column water jacket (Alltech Associates, Deerfield, IL); a model URS-051 postcolumn reactor with two 1-mL reaction chambers (Kratos Analytical Instruments, Westwood, NJ); and a Kratos Model 970 fluorescence detector, excitation set at 230 nm and a 418-nm cutoff filter for emission; a strip chart recorder, 10-mV input, Model 261 (Linear Instrument Corp., Irvine, CA), with a chart speed of 20 cm/h; a polar planimeter, Planix Model 2 (Tamaya, Tokyo, Japan).

Procedure. Fifteen grams of blackberries was weighed into a 500-mL round-bottom flask. The berries were mashed with a glass rod. Forty-five milliliters of 0.25 N hydrochloric acid was added. The flask was attached to a reflux condenser and placed in a 100 °C water bath and refluxed for 15 min, allowed to cool, and filtered through a 7-cm Whatman No. 4 filter paper with suction into a 500-mL vacuum flask. The solids, including the filter paper, were transferred to the 500-mL round-bottom flask. Another 45 mL of 0.25 N hydrochloric acid was added, the sample was refluxed 15 min, cooled, and filtered, and the extraction was repeated for a total of three refluxes. The extracts were pooled into a 250-mL gradualted cylinder, the volume was adjusted to 150 mL with purified water, and the sample was stored at 4 °C in an amber glass bottle until analyzed. The acid reflux has the advantage of hydrolyzing possible conjugates of glyphosate, as has been shown in the case of 1-naphthaleneacetic acid (Archer and Stokes, 1983), and also plant constituents, which improves

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the efficiency of cleanup of sample extracts on the resin columns.

Four hundred fifty-four grams of cation-exchange resin was prepared by rinsing several times with 100-mL aliquots of 0.25 N hydrochloric acid until the rinses were free of fines. The resin was stored in 0.25 N hydrochloric acid until used.

A glass column (1 × 30 cm) equipped with a Teflon stopcock was plugged with glass wool. Twelve milliliters of the acid-washed cation-exchange resin was added to the column, and it was washed with 100 mL of purified water, which was discarded. Ten milliliters of the sample extract, equivalent to 1 g of sample, was added to the top of the column, and a 150-mL beaker was placed under the column as a receiving vessel. Glyphosate was eluted from the column with 70 mL of purified water. The eluate was adjusted to pH 10 by adding 50% potassium hydroxide solution dropwise to pH 2 and then adding 0.2 N sodium hydroxide to pH 10. Sample transfer was to a 200-mL round-bottom flask with three 1-mL beaker rinses using purified water followed by sample concentration on a rotary evaporator at 40 °C and 10–20 mmHg to 1–2 mL of volume. Sample cleanup was not adequate on the cation column alone. Efficient cleanup was accomplished only by a further treatment on the anion column.

Four hundred fifty-four grams of anion-exchange resin was prepared by rinsing it with 100-mL aliquots of purified water until the washes were colorless and free of fines. The resin was stored in purified water until used.

A glass column (1 × 30 cm) equipped with a Teflon stopcock was plugged with glass wool; 15 mL of the washed anion-exchange resin was added to the column; it was rinsed with 50 mL of 1 N hydrochloric acid; the column was washed with 300 mL of purified water; the sample from the cation column was added to the anion column; the column was rinsed with 200 mL of the purified water, which was discarded. a 150-mL beaker was placed under the column, and the glyphosate was eluted with 75 mL of 0.02 N hydrochloric acid. The pH of the eluate was adjusted to 7 with 0.2 N sodium hydroxide. The sample was transferred to a 200-mL round-bottom flask and evaporated in vacuo at 40 °C and 10–20 mmHg to approximately 1 mL. The solution was transferred with three 1-mL water washes to a 15-mL graduated centrifuge tube, and the final volume was adjusted to 4 mL for analysis by HPLC.

High-Performance Liquid Chromatography. By use of a lower concentration of hypochlorite than other published procedures (Moye et al., 1983), the flow rate could be increased and the pulse background was eliminated. The mobile phase (pH 2.2) flow rate was 0.6 mL/min and the column temperature was 65 °C. The cleavage reagent flow rate was 1 mL/min and the pH was 4.2 in the reaction chamber. The derivatizing reagent flow rate was 0.5 mL/min and the reaction pH was 9.8. All solutions were maintained under a helium atmosphere. These were the conditions that produced the optimum results. The fluorescence detector sensitivity setting was 540 and the range setting was 0.1 μA full-scale deflection. Quantitation was accomplished by measurement of chromatogram peak areas with a polar planimeter relative to a primary standard. The detector response was linear over the range of 0–25 ng (Figure 1). The retention time of glyphosate using the above described conditions was 17.0 min (Figure 2).

Blackberry Plot Design. Glyphosate herbicide was used to control or suppress perennial weeds during the establishment of planting. Three random blocks with four plots per treatment with five plants per plot with row

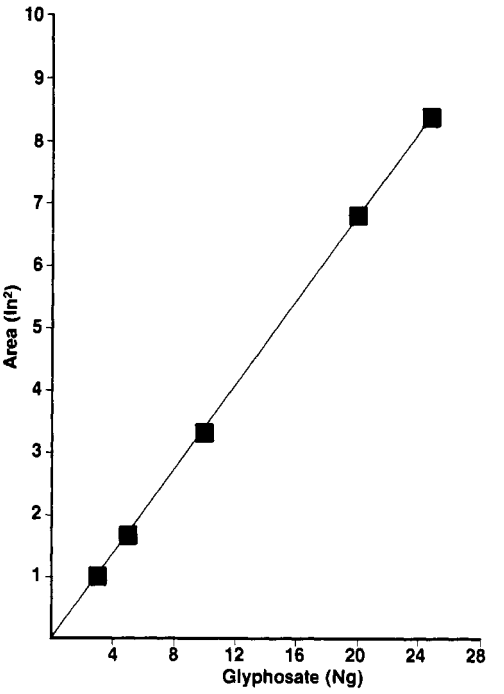

Figure 1. Standard curve for glyphospate from 0 to 25 ng vs. response in area as in.^{2}.

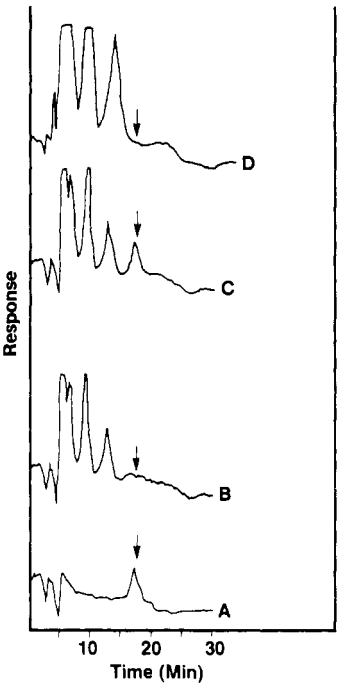

Figure 2. (A) 2.5 ng of glyphosate standard; (B) 50 mg of control blackberries; (C) 50 mg of control blackberries plus 0.05 ppm of glyphosate; (D) 50 mg of blackberries treated with 8 + 10 lbs/acre a.i. plus a 33% wipe.

spacing of 9 ft and plant spacing of 6 ft within the rows were established for treatments. One block was for a no-treatment control; a second block was for 4 lb/acre active ingredient (a.i.) postplant directed-spray treatment followed by a 5 lb/acre a.i. application and one wiper application of a 33% solution of glyphosate in water, and a third block was for an 8 lb/acre a.i. treatment followed by a 10 lb/acre a.i. application and one wiper application of a 33% solution of glyphosate. The spray application equipment was a high-volume boom sprayer with flat fan nozzles at 40 psi and an application of 40 gal/acre. The berries were sampled 81 days after the postplant treat-

ment.

RESULTS AND DISCUSSION

When six repeated analyses using the HPLC system for each of 2.5, 5.0, and 10.0 ng of glyphosate were measured as areas (in.^{2}), the mean values, standard deviations, and percent deviation were 1.04 ± 0.06, 5.8%, 1.63 ± 0.05, 3.2%, and 3.3 ± 0.3, 7.7%, with an average of 5.6% deviation.

When five repeated recoveries at each level of 0.05, 0.10, and 0.25 ppm through the described procedure were analyzed, the mean ppm, standard deviation, coefficient of error, and percent recovery were as follows: 0.046 ± 0.004, 8.7%, and 92.0% ± 8.0; 0.083 ± 0.014, 16.9%, and 83.0% ± 14.0; 0.200 ± 0.034, 17.0%, and 80.0% ± 14.0. These data, calculated by the standard statistical procedure, indicate that the method has good precision and accuracy as shown by the recovery data.

Wauchope (1976) determined the acid dissociation of glyphosate to be $pK_1 = 2.32 \pm 0.03$, $pK_2 = 5.86 \pm 0.03$, $pK_3 = 10.86 \pm 0.03$. During the cleanup procedure it is very important that the pH of the eluate from the cation ion be maintained near the pK_1 value and that the pH of the sample be adjusted to near the pK_3 value before addition to the anion column for obtaining adequate recoveries from these resin columns.

Residues of glyphosate on blackberries harvested 81 days after application of the herbicide postplant resulted in less than 0.05 ppm on all treated samples as well as control samples.

This high-performance liquid chromatographic procedure including the rapid crop cleanup method is easy, accurate, and precise. Other crops could also be analyzed with little or no modification of the method.

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Determination of Extractable and Nonextractable Radioactivity from Small Field Plots 45 and 95 Weeks after Treatment with [^{14}C]Dicamba, (2,4-Dichloro[^{14}C]phenoxy)acetic Acid, [^{14}C]Triallate, and [^{14}C]Trifluralin

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The degradation of ring-labeled [^{14}C]dicamba (2-methoxy-3,6-dichlorobenzoic acid), ring-labeled [^{14}C]-2,4-D [(2,4-dichlorophenoxy)acetic acid], [2-^{14}C]triallate [*S*-(2,3,3-trichloroallyl) diisopropylthiocarbamate], and ring-labeled [^{14}C]trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) was studied under field conditions at rates of 1 kg/ha in small sandy loam plots. Duplicate plots were sampled to a depth of 10 cm after 45 and 95 weeks and extracted with aqueous acetonitrile to determine amounts of extractable radioactivity. The extracted soils were then oxidatively combusted to determine nonextractable, or bound, radioactivity. After 45 weeks, soluble radioactivity recovered from the dicamba-, 2,4-D-, triallate-, and trifluralin-treated plots was <1, 2, 50, and 77% of that applied, while the nonextractable activity accounted for 2, 10, 15, and 10% of that applied. After 95 weeks, <1, 1, 16, and 38% of the applied radioactivity were, on the average, extractable from the dicamba-, 2,4-D-, triallate-, and trifluralin-treated plots, while 3, 6, 30, and 22%, respectively, remained in a solvent nonextractable form.

The herbicides dicamba (Figure 1, **1**), 2,4-D (Figure 1, **2**), triallate (Figure 1, **3**) and trifluralin (Figure 1, **4**) are among the more commonly used herbicides in western Canada. In 1979, approximately 90% of land sown to cereal and oilseed crops in Saskatchewan received treatments with these chemicals for weed control (Smith, 1982).

Persistence of the four herbicides has been studied in prairie soils under labatory (Smith, 1973, 1974, 1978; Smith and Muir, 1980) and field (Smith and Hayden, 1976) conditions. It has been concluded (Smith, 1982) that whereas dicamba and 2,4-D are rapidly lost from treated soils, triallate and trifluralin are moderately persistent, so that residues can be carried over in the soil from one crop year to the next.

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