Determination of Glyphosate and (Aminomethyl)phosphonic Acid in Soil, Plant and Animal Matrices, and Water by Capillary Gas Chromatography with Mass-Selective Detection

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A residue method for the determination of glyphosate [*N*-(phosphonomethyl)glycine, PMG] and its principal metabolite, (aminomethyl)phosphonic acid (AMPA), in soil, crops, animal products, and water was developed. PMG and AMPA were extracted from soil using a mixture of 0.25 M NH_4OH and 0.1 M KH_2PO_4, from crops with water, and from animal products with either 0.1 N HCl or 0.6% acetic acid. Extracts of crops and animal products were cleaned up on a cation exchange column. Soil extracts, purified extracts of crops and animal products, and water samples were directly reacted with a mixture of heptafluorobutanol and trifluoroacetic anhydride. Derivatized analytes were quantified by using capillary gas chromatography and a mass-selective detector operated in the selected-ion-monitoring mode. The limit of quantitation was demonstrated to be as low as 0.01 mg/kg for each analyte. For PMG, the mean recoveries from soil, crops, animal matrices, and water ranged from 90 to 100% with coefficients of variation (CV) ranging from 6 to 14%. For AMPA, the mean recoveries ranged from 89 to 103% with CVs ranging from 8 to 13%.

Keywords: *Glyphosate; (aminomethyl)phosphonic acid; mass-selective detection; capillary gas chromatography; derivatization; heptafluorobutanol; trifluoroacetic anhydride*

INTRODUCTION

Glyphosate [*N*-(phosphonomethyl)glycine, PMG] and glyphosate-trimesium [trimethylsulfonium *N*-(phosphonomethyl)glycine(1−)] are, respectively, the active ingredients in the commercial herbicides Roundup, marketed by Monsanto, and Touchdown, marketed by Zeneca Ag Products. They are nonselective, postemergence herbicides used for control of many grasses and broad-leafed weeds. Glyphosate is registered for a number of preplant and postharvest uses and a number of noncrop uses. Glyphosate has become increasingly popular among homeowners. Glyphosate-trimesium is currently registered for weed control in noncrop areas around the farm and nonbearing trees and vines. PMG's principal degradation product in crops, soil, and water has been identified as (aminomethyl)phosphonic acid (AMPA) (Rueppel et al., 1975, 1977; Sprankle et al., 1978).

Chemical properties of PMG that contribute to its effectiveness as a herbicide also make its analysis, as well as that of AMPA, difficult, especially at residue levels in the variety of environmentally significant matrices where they might be found. Their polar nature and high water solubility make extraction difficult and limit the options for using the various standard derivatization techniques often employed for gas chromatographic (GC) analysis. In spite of numerous published methods, it has been noted that "the analysis of glyphosate at residue levels has tested the patience of many experienced analysts" (FAO, 1986).

The similarity of PMG and AMPA to naturally occurring amino acids and amino sugars further contributes to the difficulty in determining residues of these compounds in crops and animal products. This has usually required the use of lengthy cleanup procedures that sometimes involve both anion and cation exchange columns. The lack of a chromophore or fluorophore also necessitates derivatization techniques for the determination of AMPA and PMG residues by liquid chromatography (LC). Methods published for the determination of PMG and AMPA in crops by LC include postcolumn derivatization with *o*-phthalaldehyde (Wigfield and Lanouette, 1991) or 9-fluorenylmethylchloroformate (Roseboom and Berkhoff, 1982).

Many investigators have taken a GC approach to the residue analysis of PMG and AMPA. Extensive derivatization of the analytes is required. Derivatization has involved the use of trifluoroacetic anhydride and diazomethane (Pesticide Analytical Manual, 1977), heptafluorobutyric anhydride and 2-chloroethanol (Guivivan et al., 1982), *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide (Moye and Deyrup, 1984), and trifluoroacetic anhydride and trifluoroethanol (Roy and Konar, 1989) to take advantage of the resolution and selectivity provided by GC analysis. Recently, Tsunoda (1993) reported on the determination of *tert*-butyldimethylsilylated derivatives of PMG using GC-ion trap mass spectroscopy, although trace level determinations were not described. The analysis of PMG and AMPA in soil and plant tissue has been reviewed (Bardalaye et al., 1985).

Mixtures of fluorinated anhydrides and perfluorinated alcohols have been reported to form derivatives of PMG and AMPA suitable for gas chromatographic analysis (Deyrup et al., 1985). In this paper, we present a derivatization method that involves the direct addition of an aqueous extract or water sample to a mixture of trifluoroacetic anhydride (TFAA) and 2,2,3,3,4,4,4-heptafluoro-1-butanol (HFB) to derivatize the analytes. The derivatives are then analyzed by using capillary gas chromatography with a mass-selective detector (GC-MSD) operated in the selected-ion-monitoring mode. The method was developed to determine low-level residues in a variety of environmental matrices in support of tolerance petitions for new registered uses of glyphosate-trimesium, the trimethylsulfonium salt of glyphosate. This method has been applied to the determination of PMG and AMPA in a variety of soils (silty clay loam

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and sandy loam), crops (corn, soya, wheat, nuts, apples, and plums), crop commodities (pomace, oil, and soapstock), and animal products (beef muscle, kidney, liver, fat, cows' milk, and chicken eggs). The method provides advantages over previous residue methods in that GC-MSD analysis provides an extremely sensitive and selective detection technique that requires a minimum of sample cleanup. Although once found in only the best equipped laboratories, the mass-selective detector has been found to be a cost-effective alternative to other GC detection systems that require more intensive sample preparation.

EXPERIMENTAL PROCEDURES

Instrumentation. The analytical system consisted of a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a HP 5970A MSD, HP 7673 automatic sampler/injector, and HP UNIX ChemStation for data acquisition. The chromatograph was installed with a split-splitless inlet system operated in the splitless mode. The analytical column was a 30 m by 0.25 mm i.d., fused-silica column bonded with a 0.25-μm film thickness of Durabond 5.625 (J&W Scientific, Folsom, CA). The injection port liner was a 4 mm i.d., single-piece, double-restrictor type (Restek Corp.) that was packed with fused-silica wool. Structure confirmation of the derivatives was conducted on a Finnigan MAT Model TSQ 70 mass spectrometer. A Haake-Buchler (HBI) Rotary Evapo-Mix was used to evaporate purified extracts, although a Zymark Turbo Vap evaporator may be substituted.

Reagents and Glassware. PMG of 99% purity was obtained from Zeneca Ag Products, Western Research Center, Richmond, CA. AMPA of 99% purity was purchased from Aldrich Chemical Co. TFAA was purchased from Spectrum Chemical Manufacturing Corp. HFB and citral (3,7-dimethyl-2,6-octadienal) were purchased from Aldrich. All solvents were of a purity that was suitable for trace analysis. All other reagents were reagent grade or better. Reagent solutions included (1) a solution containing 0.25 M NH_4OH and 0.1 M KH_2PO_4 for extraction of soils, (2) a 0.1 N HCl solution for extraction of animal tissues and eggs, (3) a 0.6% (v:v) acetic acid solution for extraction of milk samples, (4) an acidic modifying solution containing 16 g of KH_2PO_4, 40 mL of methanol, 13.4 mL of concentrated HCl, and 160 mL of water, and (5) a cation exchange (CAX) mobile-phase solution containing 160 mL of water, 40 mL of methanol, and 2.7 mL of concentrated HCl. Disposable Poly-Prep CAX cleanup columns containing AG 50W-X8 resin, H+ form (2-mL bed volume, 200–400 mesh) were obtained from Bio-Rad Laboratories, Hercules, CA. Derivatizations were performed in standard 2-mL autosampler vials fitted with phenolic plastic open-top caps and double-thickness polymeric fluorocarbon (PTFE) septa obtained from Sunbrokers, Inc., Wilmington, NC.

Calibration Solutions. Individual AMPA and PMG stock calibration solutions of 1000 μg/mL were prepared by dissolving a known amount of each analyte in water. Aliquots of each stock solution were combined to produce a high-level stock calibration solution containing both analytes at 100 μg/mL. Working calibration solutions of 10, 1, and 0.1 μg/mL were made by serially diluting this solution as needed. Three drops of concentrated HCl were added to each solution as a biocidal preservative.

A requirement of the derivatization procedure is that the amount of water added to the derivatizing reagent mixture remains constant. As a result, a series of daily-use standards is prepared that, when derivatized, span the calibration range required for the GC analysis. These standards are prepared by diluting each of a series of working calibration solutions. An aliquot (20–100 μL) of working standard is diluted to 1.0 mL using the cation exchange mobile-phase solution, or soil extracting solution (see below) as the diluent. All stock solutions were assigned a shelf life of 1 year based upon prior laboratory experience. Daily-use standards were derivatized within 4 h of preparation.

Fortification. Portions (25–50 g) of homogeneous crop and tissue samples were weighed into Waring blender jars and 16-oz, wide-mouth jars, respectively. Soil samples (20 g) were weighed into an 8-oz, wide-mouth jar. Aliquots (25–50 μL) of a fortification solution containing both PMG and AMPA in water were added to the samples to produce fortification levels of 0.01–1.0 mg/kg of each analyte. Fortification of fat samples required a fortification solution prepared in methanol. Unfortified controls were prepared and analyzed to demonstrate freedom from interferences.

Extraction. Homogeneous soil samples (20 g) were extracted with 80 mL of an aqueous solution containing 0.25 M NH_4OH and 0.1 M KH_2PO_4. Samples were shaken on a mechanical, reciprocating shaker for 90 min at 100–200 strokes/min. Samples were then either centrifuged for 20 min at approximately 2000 rpm, or a 2-mL aliquot of the supernatant liquid was removed and filtered using a 0.45-μm syringe filter unit.

Homogeneous crop samples (25 or 50 g) were extracted with water at a water to crop ratio of 5:1. Dry matrices such as dry apple pomace, prunes, or corn fodder tend to absorb more water and make maceration difficult. For these matrices the crop to water ratio is increased to 10:1. After accounting for the amount of water in the sample (Watt and Merrill, 1963), an amount of water was added to bring the total aqueous volume to 125 or 250 mL. Samples were macerated in a Waring blender at high speed for 3 min. After the extracts were allowed to settle, a 30-mL aliquot was transferred to a 4-oz, wide-mouth jar and centrifuged for 10 min at about 2000 rpm. A 15-mL aliquot was transferred to a 2-oz wide-mouth jar and partitioned with 15 mL of chloroform. The chloroform was discarded, and a 10-mL aliquot of the remaining aqueous extract was transferred to a clean, 2-oz narrow-mouth jar. One milliliter of the acidic modifying solution was added to the extract. If needed, a 2-mL aliquot of the extract was filtered using a 0.45-μm syringe filter unit. Samples with high oil content such as nutmeats, soybean oil, or soapstock required two chloroform partitions. Soybean soapstock was extracted using the tissue method described below, with additional concentrated HCl being added during the extraction to maintain a pH <2.

Homogenized egg and tissue samples (25 g of beef muscle, kidney, liver, fat; or 50 g of eggs) were extracted with 0.1 N HCl and 25 mL of chloroform. After accounting for the amount of water in the sample (Watt and Merrill, 1963), an amount of 0.1 N HCl was added to bring the total aqueous volume to 137.5 mL. Samples were macerated for 5 min, using a Tekmar Tissumizer. Sodium sulfate (1.0 g) was added, and the samples were macerated for an additional 2 min. The extracts were centrifuged for 10 min at about 2000 rpm. Fat samples required an additional chloroform extraction in which 15 mL of aqueous extract was shaken with 15 mL of chloroform. A 2-mL aliquot of the aqueous extract was filtered using a 0.45-μm syringe filter unit. Milk samples (50 g) were extracted with a 0.6% (v:v) solution of acetic acid. After accounting for the amount of water in the sample (Watt and Merrill, 1963), an amount of the 0.6% acetic acid solution was added to bring the total aqueous volume to 137.5 mL. Samples were shaken on a mechanical shaker for 10 min. Chloroform and sodium sulfate were not added to the extraction mixture. After centrifuging the extraction mixture, a 15-mL aliquot of the aqueous layer was removed and extracted with 15 mL of chloroform. A 2-mL aliquot of the aqueous layer was filtered using a 0.45-μm syringe filter unit.

Extract Cleanup. A cation exchange (CAX) cleanup was performed on crop, egg, milk, and tissue extracts using disposable Bio-Rad Poly-Prep columns containing AG 50W-X8 resin (H+ form). All elutions were performed using gravity. The column was prepared for use by washing with 10 mL of deionized water. A 0.55-mL aliquot of extract was added to the column and eluted until the liquid level reached the top of the resin. Sugars and other coextractives were then eluted with 2.0 mL of the CAX mobile-phase solution. Analytes were eluted with 12.5 mL of the CAX mobile-phase solution. The eluate was collected in a 50-mL centrifuge tube. The collected fractions were evaporated to dryness using a vortex-type

evaporator with the water bath at 40 °C. The residuum was dissolved in 1.2 mL of the CAX mobile-phase solution.

Analysis of Water Samples. Drinking water samples were analyzed by directly derivatizing an aliquot as described in the following section. To determine levels less than 10 μg/L, 50-mL aliquots were evaporated to dryness, and the residuum was dissolved in 1.25 mL of the CAX mobile phase solution described in the preceding section.

Derivatization. The derivatizing reagent mixture was freshly prepared each day by adding 1 volume of HFB to 2 volumes of TFAA. Aliquots (1.6 mL) of the reagent mixture were added to 2-mL autosampler vials. Vials were sealed using phenolic-plastic screw caps with double-thickness PTFE septa or liners. Vials were placed in a aluminum heating block having 13-mm-diameter holes, and the block was placed on, and surrounded by, slabs of dry ice. The block was cooled to a temperature of −50 to −60 °C before proceeding. A variable volume Eppendorf pipet was used to add a 36-μL aliquot of sample extract or daily-use standard solution to the derivatizing reagent. The aliquot was drawn into the disposable pipet tip and then dispensed under the surface of the chilled reagent. The tip was rinsed several times with the contents of the vial. The volume of standard or sample added to the chilled reagent mixture remained fixed at 36 μL for all derivatizations within a sample set.

Analyte derivatization was performed by transferring the reaction vial to a second aluminum block with 13-mm holes. Vials were heated at 92−97 °C for 1 h. After allowing vials to cool to room temperature, the excess derivatization reagents were evaporated under a gentle stream of nitrogen. To ensure complete evaporation of the acidic residues, the evaporation process was continued for an additional 30 min after apparent dryness was achieved. The residuum was dissolved in 200 μL of ethyl acetate containing 2.0 μL of citral/mL of solvent. All samples were derivatized in duplicate. Derivatized solutions were found to be stable for at least 1 month.

Gas Chromatographic Analysis. Helium carrier gas velocity measured at 180 °C was adjusted to 30 cm/s. Injection port temperature was 200 °C, although some work was carried out at 270 °C. MSD interface temperature was 275 °C. Initial oven temperature was 90 °C and was held for 2.0 min. The oven was then heated at 30 °C/min to a final temperature of 290 °C and held for 3 min. Injections were conducted in the splitless mode with the inlet liner purged 1.25 min after injection. Injection volume ranged from 2 to 5 μL. The approximate retention times of the AMPA and PMG derivatives were 5.3 and 6.2 min, respectively. The mass-selective detector was manually tuned using perfluorotributylamine (PFTBA) as the calibration compound. Manual tune masses were m/z 414, 502, and 614. Tuning was conducted over the range of m/z 300−650. After tuning under these parameters, the MSD sensitivity was improved by increasing the peak width (width at half-height) of the calibration ions to approximately 2.6 amu. This was accomplished by manually lowering the AMU gain control. Tuning ion masses were then recalibrated by manually adjusting the mass gain and mass offset controls.

The MSD was operated in the selective-ion-mode (SIM). Up to two ions (m/z 584 and 611) were monitored simultaneously for the detection of the PMG derivative, and up to three ions (m/z 372, 446, and 502) were monitored for the detection of the AMPA derivative. Most work was conducted using m/z 611 and 446 for detection of PMG and AMPA, respectively. When one ion was monitored for each analyte the dwell time was set at 135 ms. This value was reduced when multiple ions were monitored. Chromatographic resolution of the AMPA and PMG derivatives allowed sufficient time for the monitored ion(s) to be switched between peaks. Samples were quantitated using the external standard method with calculations based on peak heights.

RESULTS AND DISCUSSION

Recovery of PMG and AMPA from Soil. Extraction of PMG from soil has been facilitated under alkaline conditions using 0.5 M NH_4OH (*Pesticide Analytical Manual*, 1977) and in the presence of phosphate using 0.5% phosphoric acid (Roy and Konar, 1989). Table 2 shows data obtained during the method development process in which the molarity of the two components of the extraction solvent was varied, as was the fortification-to-extraction interval. Selection of an extraction solvent containing 0.25 M NH_4OH was primarily based on chromatographic concerns, including the production of fewer coextractives and an improvement in chromatographic ruggedness. Adsorption of PMG in soil has been related to the phosphonic acid function of PMG and the competition with inorganic phosphate for binding sites within the soil (Sprankle, 1975). In development of this method, there was slight improvement in recoveries with the addition of phosphate. Also reported are reduced recoveries for soils with high clay content (Glass, 1983; Roy and Konar, 1989). Recently, Aubin and Smith (1992) reported on the extraction of glyphosate from Saskatchewan soils.

Soil with a clay content of 39% (Table 1) and fortified at 0.05 and 0.50 mg/kg was analyzed with this method with recoveries averaging 93% (CV = 11) and 99% (CV = 8) for PMG and AMPA, respectively. Values reported in Table 3 were obtained to determine procedural recoveries during routine analysis of samples for soil dissipation studies. In these studies soil samples were fortified 1 h prior to extraction. Similar recoveries from two sandy loam soils are also reported in Table 3. Determination of PMG residues from these field incurred, aged samples was consistent with the amount of chemical applied, and known degradation rates. Table 2 also reports recovery data on fortified, aged soil samples. After fortification soil samples were allowed to dry and then stored at 25 °C prior to analysis. Adequate recovery was obtained after storage intervals of 18 h, 7 days, and 44 days.

Table 1. Characteristics of Tested Soils

characteristic	soil source		
	Othello, WA	Visalia, CA	Champaign, IL
classification	sandy loam	sandy loam	silty clay loam
cation exchange, mequiv/100 g	7.8	8.7	21.5
organic matter, %	0.6	0.9	1.5
pH	8.0	8.2	6.7
sand, %	66	59	15
silt, %	29	25	46
clay, %	6	16	39

Table 2. Effect of Variations in Extraction Solution Molarity and Fortification-to-Extraction Interval on Recovery of PMG and AMPA from Washington Soil

fortification-to-extraction interval[a]	extraction solution ratio[b]	fortification level,[c] mg/kg	PMG recovery, %	AMPA recovery, %
1 h	0.50/0.05	5.0	92	105
1 h	0.25/0.05	5.0	92	93
1 h	0.13/0.05	5.0	88	94
1 h	0.15/0.05	1.0	87	102
1 h	0.10/0.05	1.0	74	90
1 h	0.08/0.03	1.0	75	85
18 h	0.25/0.05	0.20	70	87
18 h	0.15/0.05	0.20	63	81
18 h	0.25/0.10	1.0	94	95
18 h	0.50/0.05	1.0	90	96
7 days	0.25/0.10	0.10	81	97
7 days	0.25/0.10	0.10	72	94
44 days	0.25/0.10	0.10	71	80

[a] 18 h, 7 day, and 44 day samples were dried and then stored at 25 °C. [b] Molar concentration of extraction solutions (ammonium hydroxide/potassium phosphate). [c] Amount added, each analyte.

Table 3. Recoveries of PMG and AMPA from Soil

soil source	soil type	N^a	fortification level,[b] mg/kg	PMG recovery, % mean	PMG recovery, % CV[c]	AMPA recovery, % mean	AMPA recovery, % CV
Othello, WA	sandy loam	7	0.05–0.50	97	14	88	15
Visalia, CA	sandy loam	37[d]	0.05–0.50	84	14	85	15
Champaign, IL	silty clay loam	36	0.05–0.50	93	11	99	8
all	PMG:	80	0.05–0.50	90	13	93	13
	AMPA:	66					

[a] Number of replicates. [b] Amount added, each analyte. [c] Coefficient of variation. [d] AMPA fortified in only 23 replicates. Fortification-to-extraction interval = 1 h.

Table 4. Recoveries of PMG and AMPA from Crops

matrix	commodity	N^a	fortification level,[b] mg/kg	PMG recovery, % mean	PMG recovery, % CV[c]	AMPA recovery, % mean	AMPA recovery, % CV
corn	grain	9	0.05–0.50	93	13	95	9
	forage	9	0.05–0.50	99	7	103	6
	fodder	10	0.05–0.50	86	9	90	9
soy	seed	8	0.05–0.50	98	18	93[d]	
	forage	12	0.05–0.50	104	11	86[e]	22
	hay	2	0.05–0.25	96	36	80	25
	crude oil	5	0.05–0.50	86	17	na[f]	
	soapstock	5	0.05–0.50	104	5	na	
	grain dust	5	0.05–15.0	99	7		
wheat	grain	3	0.10	75	12	100	6
	misc commodities[g]	8	0.05–20.0	95	16	na	
almonds	nutmeat	2	0.05	115	0	na	
	hulls	6	0.05–0.50	101	20	na	
pecans	nutmeat	1	0.05	92		na	
walnuts	nutmeat	3	0.05–0.50	95	10	na	
apples	fruit	5	0.05–0.50	96	14	na	
	dry pomace	1	0.05	103		na	
	wet pomace	1	0.05	82		na	
	juice	1	0.05	99		na	
plums	fruit	4	0.02–0.50	100	19	94	14
	prunes	1	0.08	108		126	
pears	fruit	3	0.05–0.50	101	15	na	
all	PMG:	104	0.02–20.0	96	14	95	13
	AMPA:	41					

[a] Number of replicates. [b] Amount added, each analyte. [c] Coefficient of variation. [d] $N = 1$. [e] $N = 4$. [f] Not analyzed, AMPA not fortified into all samples. [g] Miscellaneous wheat commodities include bran, middlings, shorts, and flour.

Recovery from Crops, Animal Products, and Drinking Water. Matrices with a high oil content can provide a challenging analytical problem with respect to cleanup and analysis. In addition, the similarity of PMG and AMPA to naturally occurring amino acids can cause additional problems in the selectivity of the method. Grain, forage, and fodder of field corn were fortified at 0.05–1.0 mg/kg and analyzed to determine recovery. Recoveries from all corn commodities averaged 92% (CV = 11%) and 97% (CV = 10%) for PMG and AMPA, respectively. Other commodities tested included seed, forage, hay, crude oil, and soapstock of soybeans, wheat grain, apples, plums, pears, and various nutmeats. Recoveries from all crop commodities averaged 96% (CV = 14%) and 95% (CV = 13) for PMG and AMPA, respectively. Due to a change in U.S. EPA regulatory requirements for AMPA, AMPA recovery was not determined in all matrices. See Table 4 for recoveries from other crops. The extraction solutions for animal products were acidic in nature to allow for protein precipitation. As a result the addition of the acidic modifier was eliminated. Recovery of PMG and AMPA from beef muscle, liver, kidney, fat, cows' milk, and chicken eggs averaged 98% (CV = 8%) and 89% (CV = 11%) for PMG and AMPA, respectively. See Table 5 for recoveries from animal products and Table 6 for recoveries from drinking water.

Table 5. Recoveries of PMG and AMPA from Animal Products

matrix		N^a	fortification level,[b] mg/kg	PMG recovery, % mean	PMG recovery, % CV[c]	AMPA recovery, % mean	AMPA recovery, % CV
beef	muscle	6	0.03, 0.30	98	8	102	11
	kidney	6	0.10, 1.0	105	3	89	5
	liver	6	0.10, 1.0	100	5	84	9
	fat	6	0.01, 0.10	101	3	86	8
cow	milk	6	0.01, 0.10	95	4	85	10
chicken	eggs	6	0.01, 0.10	92	12	87	14
	all	36	0.01–1.0	98	8	89	11

[a] Number of replicates; three replicates fortified at each level. [b] Amount added, each analyte. [c] Coefficient of variation.

Table 6. Recoveries of PMG and AMPA from Drinking Water

fortification level,[a] μg/L	PMG recovery, %	AMPA recovery, %
0.2	96	93
	97	87
	97	112
	95	112
10	112	99
	105	99
	99	106
100	99	106
	91	106
	105	109
mean:	100	103
CV:[b]	6	8
N:[c]	10	10

[a] Amount added, each analyte. [b] Coefficient of variation. [c] Number of replicates.

Crop and Tissue Extraction and Cleanup. Use of water and chloroform as a single extraction/partition step has been reported by Wigfield and Lanouette (1991) and others. In this work, due to selectivity of the mass-selective detector, the need for the chloroform partition was questionable. For some of the crops and commodities tested, there was a small reduction in detected coextractives, while with animal tissues, the chloroform partition was required to remove fats and oils. Due to the varying necessity of this step, it was included as a separate step in the methodology for crops. Due to the fat content of the animal products, the chloroform partition was integrated into the initial aqueous extraction for all products except milk. To minimize the usage of toxic chlorinated solvents, the need for the chloroform partition step should be evaluated for each matrix requiring analysis.

The CAX cleanup provided consistent recovery without the need for modifying the elution parameters for any of the commodities tested. As described above, most of the sugars and other unwanted coextractives elute in the 2.0-mL wash fraction. PMG elutes in the first 8 mL of the collected fraction and AMPA elutes in the last 8 mL. During method development, it was determined that coextractives eluting late in the wash fraction can produce peaks that interfere with the GC quantification of the AMPA derivative. However, increasing the initial wash volume can reduce the recovery of PMG. It is recommended that, when analyzing a new matrix, the elution parameters be tested by collecting and analyzing various fractions of the eluate to determine the actual elution profile. Modifications can then be made to optimize for recovery and minimization of interferences.

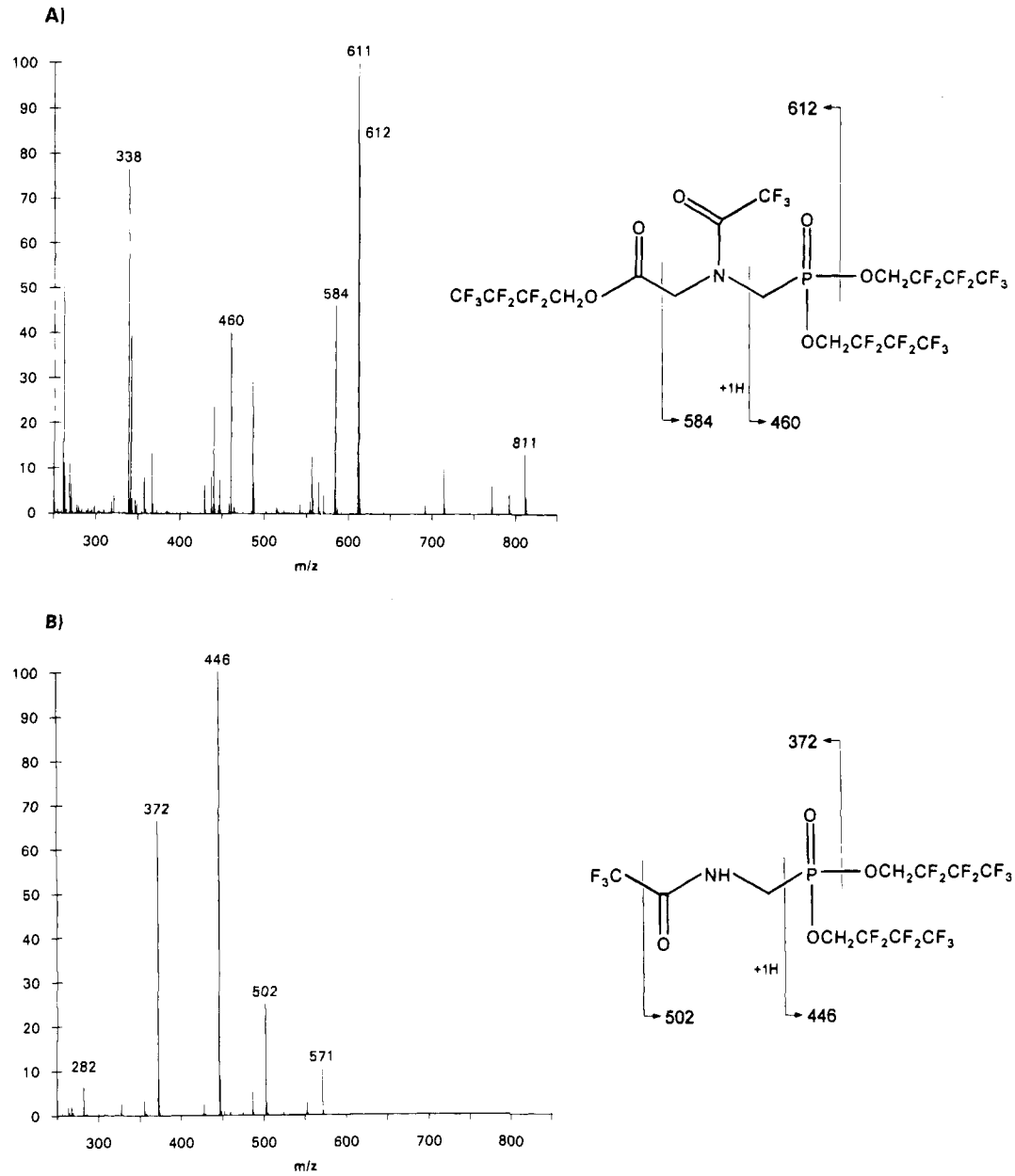

Figure 1. Electron impact mass spectra and structures of (A) PMG derivative (MW 811) and (B) AMPA derivative (MW 571).

Derivatization. Derivatization, involving the use TFAA and HFB, is based on the method of Deyrup et al. (1985), in which several other combinations of fluorinated anhydrides and perfluorinated alcohols were used to produce derivatives of PMG and AMPA. In this procedure the carboxylic and phosphonic acid functional groups were derivatized to form the corresponding heptafluorobutyl ester, while the amine functional group was derivatized to form the corresponding trifluoroacetyl derivative (Figure 1). HFB was selected for esterification to obtain derivatives that provided high mass fragments that could be selectively detected, with a minimum of interfering peaks. In the case of both PMG and AMPA, multiple high mass fragments (greater than m/z 370) were found to be suitable for detection purposes. Despite their high molecular weights, the derivatives were found (1) to be thermally stable to the point of withstanding injection port temperatures of 320 °C, (2) to be stable in solutions for several weeks, and (3) to exhibit rapid chromatographic elution that is typical of fluorinated compounds.

Standard procedures for derivatizations involving acetylation and/or esterification using trifluoroacetic anhydride have required the evaporation of aqueous extracts or water samples to dryness due to the reactivity of the anhydride with water. Low derivatization yields were obtained when aqueous solutions were evaporated to dryness in glass containers. This effect was enhanced when soil extracts were evaporated and then derivatized. The apparent, irreversible adsorption of PMG and AMPA onto glass or precipitated coextractives after evaporation is eliminated in this method by the direct addition of an aqueous extract to a premixed, chilled reagent. Water reacts with the excess trifluoroacetic anhydride to form trifluoroacetic acid, which is subsequently removed by evaporation.

Dangers normally associated with the addition of water to TFAA are mitigated by (1) the premixing of TFAA and HFB, (2) the prechilling of the reagent mixture prior to the addition of the aqueous sample, and (3) the high ratio of reagent mixture to sample (40:1). All derivatizations should be performed in a well-ventilated hood, and the analyst should wear protective gloves.

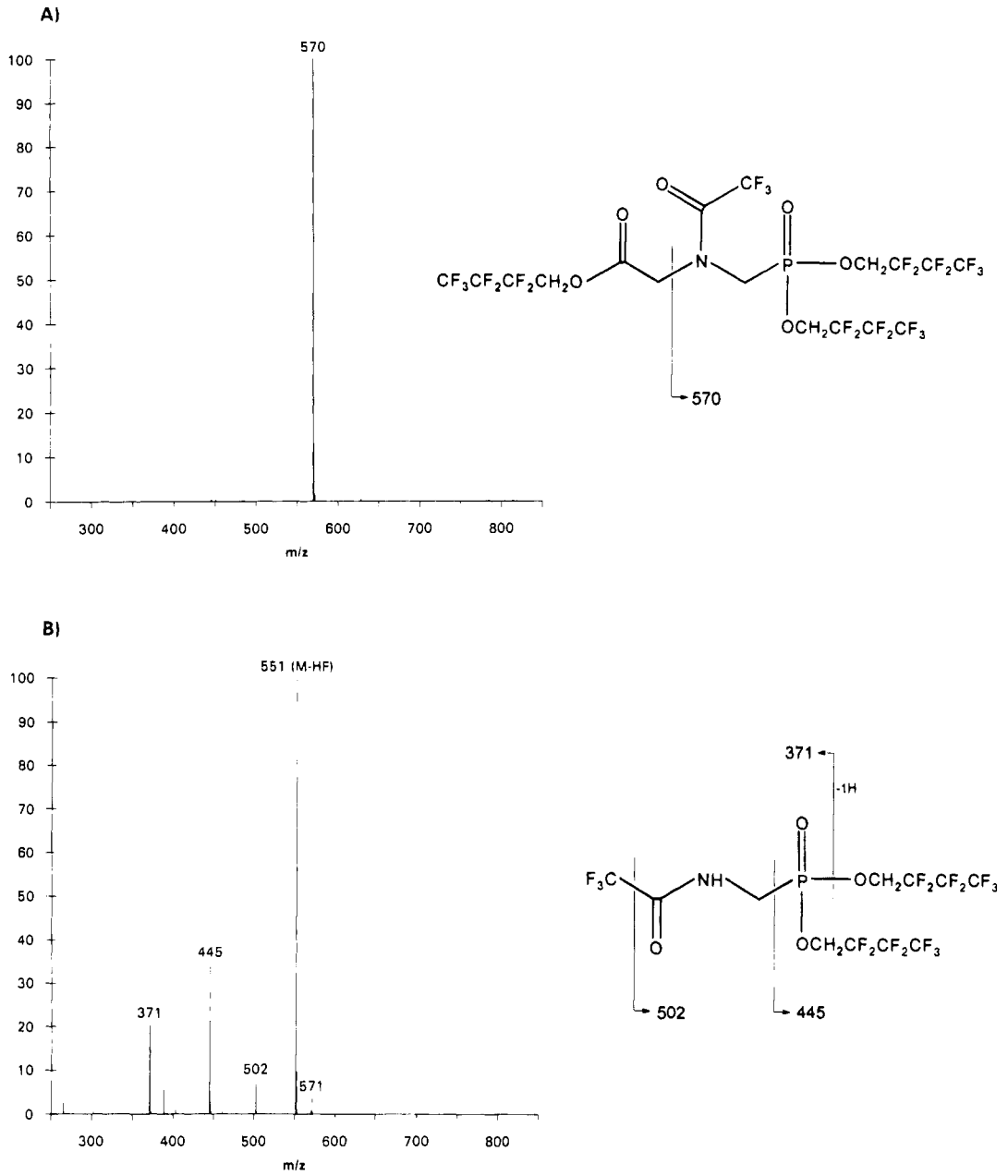

Figure 2. Negative ion chemical ionization mass spectra and structures of (A) PMG derivative (MW 811) and (B) AMPA derivative (MW 571).

Adsorption of the analytes onto glass was also a problem when using glass syringes for handling extracts and standards containing high analyte concentrations. This resulted in the carryover of trace amounts of analytes, and erroneous results. This problem was eliminated through the use of Eppendorf pipets with disposable, plastic tips.

The derivatization process was found to be highly reproducible. Replicate derivatizations (n = 8) were performed on a water sample fortified at 0.2 μg/L. Prior to derivatization, a 50-mL aliquot was concentrated to 1.25 mL using a vortex evaporator. One GC injection was made from each of the eight derivatized samples. The responses were averaged; coefficients of variation (CV) were 4.8 and 6.4% for PMG and AMPA, respectively. Similar precision has been obtained with soil, crop, and tissue extracts of fortified samples.

Mass Spectrometry. Expected structures of the derivatives were confirmed by obtaining electron impact (EI) mass spectra of the compounds using the mass-selective detector in the scan mode. Additional structural confirmation was made by obtaining both positive ion EI (Figure 1) and negative ion chemical ionization (CI) spectra (Figure 2) of the PMG and AMPA derivatives on the Finnigan mass spectrometer. The softer ionization provided by CI produced less fragmentation than EI especially with the PMG derivative (Figure 2). As a result, using CI, there are 30- and 3-fold increases in detectability for PMG and AMPA, respectively. With this increase in sensitivity, negative ion CI may eventually prove to be a more effective detection method. However, the simplicity of the negative ion CI spectra of the PMG derivative limits the potential for analyte confirmation due to the lack of other fragments.

Gas Chromatographic Analysis. This method relies on the accurate detection and quantitation of low picogram amounts of PMG and AMPA derivatives in order to offset limitations imposed by the direct derivatization of small amounts of aqueous extracts. Despite an injection volume of 5 μL, optimum chromatography

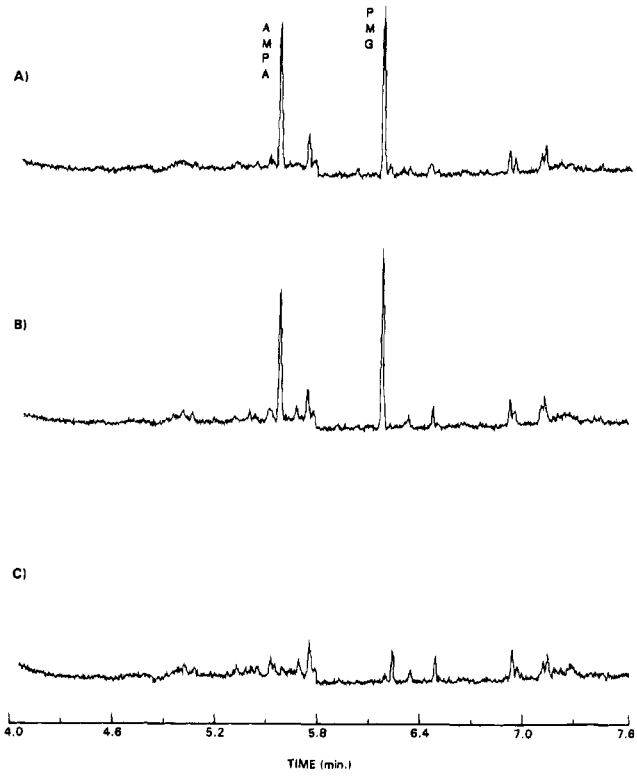

Figure 3. Gas chromatograms of (A) derivatized PMG and AMPA standard (0.6 ng/mL each prederivatized analyte), (B) extract of corn grain fortified at 0.05 mg/kg (0.012 g of crop/mL of extract), and (C) extract of unfortified corn grain (0.012 g crop/mL of extract). PMG and AMPA derivatives were monitored using m/z 611 and 502, respectively. Other conditions are described in the text.

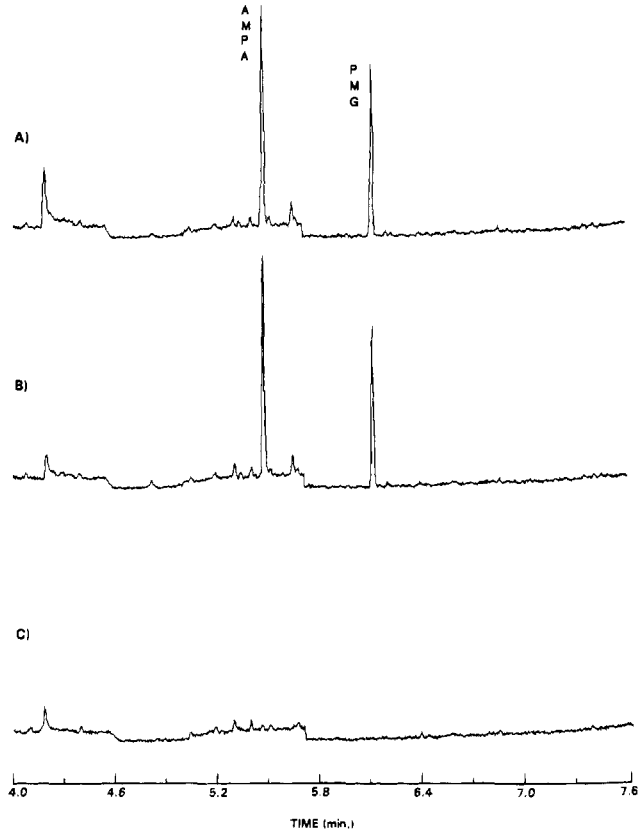

Figure 4. Gas chromatograms of (A) derivatized PMG and AMPA standard (1.0 ng/mL each prederivatized analyte), (B) extract of sandy loam soil fortified at 0.05 mg/kg (0.02 g of soil/mL of extract), and (C) extract of unfortified sandy loam soil (0.02 g soil/mL of extract). PMG and AMPA derivatives were monitored using m/z 611 and 446, respectively. Other conditions are described in the text.

could be maintained by carefully selecting the oven temperature profile, and taking advantage of cold trapping or solvent effects. It was found that an oven temperature program that included a lower initial temperature and an intermediate temperature ramp could, at times, enhance performance. For example, band broadening of the AMPA peak can be caused by the premature migration of the AMPA solute on the analytical column before deposition of the later-arriving PMG band. An alternate temperature program consists of an initial temperature of 60 °C held for 1.5 min, followed by ballistic heating to 90 °C with a 2.0 min hold. The analytes are then eluted by increasing the oven temperature to 290 °C at a rate of 30 °C/min. The exact nature of the chromatographic processes taking place upon injection were unclear, but it was found that small changes in the oven temperature profile could greatly affect peak shape and analyte response. The type of inlet liner used was also found to be critical. Chromatographic failure has been linked to the use of single-restrictor liners, although their use is not precluded. Double-restrictor liners without a fused-silica wool packing have also been used with success, although higher injection port temperatures (270 °C) were required.

Citral was added to the injection solvent at a rate of 2 μL/mL of solvent. The presence of citral appeared to help maintain a deactivated inlet and column, and thus reduce adsorption of the analytes, and thereby improving peak shape and low-level detectability (Hadjidemetriou et al., 1985). However, due to buildup of nonvolatiles it was found necessary to regularly replace (every 40–200 injections) the inlet liner and remove the first 1–2 ft of column to maintain optimum chromatography of the analytes. Coextractives from soil were found to play an important role; they affected both derivatization and chromatographic analysis. During method development for soil, analyte recoveries of greater than 150% were obtained and remain unexplained. Additionally, the analysis of some soils resulted in chromatographic matrix effects exhibited by changes in peak shape and/or response. Troubleshooting recovery or chromatographic problems was facilitated by the analysis of fortified extracts. Unusually high or low recoveries were investigated by fortifying a control extract just prior to derivatization, thereby making recovery primarily dependent on derivatization and independent of extraction efficiency. Chromatographic matrix effects were studied by fortifying a derivatized control extract with a high-level derivatized standard, and then comparing its chromatographic performance to a nonmatrix standard. As a general rule it was determined that preparation of dilute extracts containing the equivalent of extractives from 0.02 g of soil/mL of injection solvent helped maintain method ruggedness. With some soils containing high organic content, more dilute extracts (0.01 g of soil/mL) performed better. As with soil, the coextractives from crop and tissues could also effect chromatography. With some crops the dilution of the extracts with an equal portion of the ethyl acetate/citral solution can improve peak shape.

Considerations were also given to the components of the final extract other than those contributed by the sample matrix. The amount of water, HCl, methanol, phosphate and citral made significant, chromatographic-

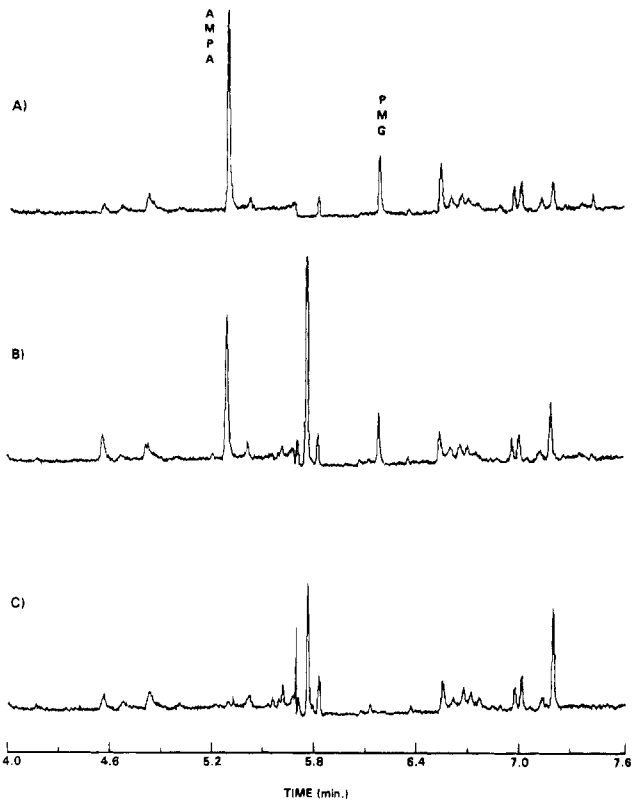

Figure 5. Gas chromatograms of (A) derivatized PMG and AMPA standard (0.3 ng/mL each pre-derivatized analyte), (B) extract of cows' milk fortified at 0.01 mg/kg (0.03 g of milk/mL of extract), and (C) extract of unfortified cows' milk (0.03 g milk/mL of extract). PMG and AMPA derivatives were monitored using m/z 611 and 446, respectively. Other conditions are described in the text.

related contributions to the final extract. To ensure that final concentrations of these components remained constant for all injected solutions, (1) daily-use standards were prepared by using the extracting solution (for soil extracts) or the CAX mobile phase (for crop or tissue extracts or water samples) as the diluent, (2) the concentration of the injected standards was determined by varying the concentration of the daily-use standard and not by varying the aliquot of the daily-use standard added to the derivatization mixture, and (3) the concentration of citral injected remained constant.

Detection Limits. Limit of detection (LOD) was defined as the amount of each analyte that would produce a signal-to-noise ratio of at least 10:1. This value would vary, based on the condition of the MSD ion source. An LOD value of 0.1 pg of each analyte/μL of injected solution was routinely obtained. Limit of quantitation (LOQ) was defined as the lowest fortification level used in recovery determination. LOQ values were based on proposed regulatory tolerances and were not necessarily based on limitations of the method. An LOQ value of 0.2 μg/L was set for water, 0.05 mg/kg for soil, 0.05 mg/kg for crops, and 0.01–0.10 mg/kg for various animal products. In most cases these LOQ values produced signal-to-noise values of greater than 30:1. On a routine basis the response of the MSD was found to be linear over the range of 0.3–40 pg/μL for both PMG and AMPA derivatives.

Mass-Selective Detection. Mass-selective detection provided a sensitive and selective method for the detection and quantitation of the derivatives of AMPA and PMG. The ability to monitor multiple ions provided the ability to confirm residues through the use of ion

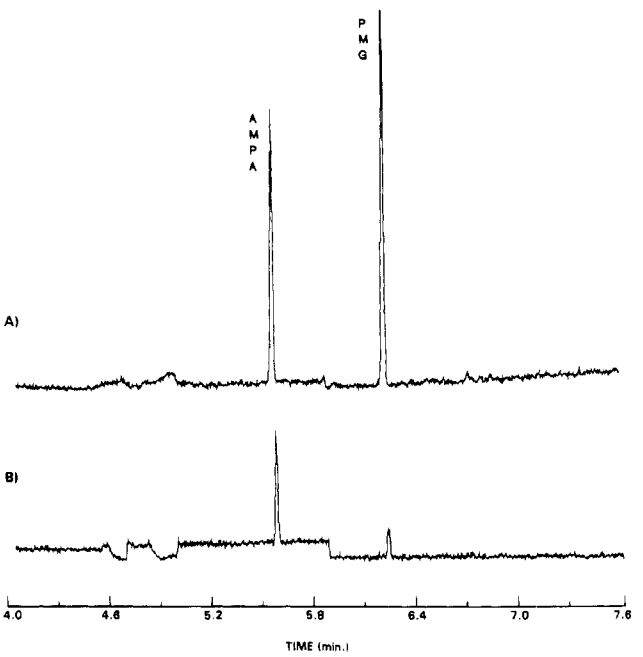

Figure 6. Gas chromatograms of derivatized PMG and AMPA standard (0.6 ng/mL each prederivatized analyte) obtained using (A) manual tune parameters as described in text, EI-MS, and (B) standard autotune parameters, EI-MS. PMG and AMPA derivatives were monitored using m/z 611 and 502, respectively. Other conditions are described in the text.

ratios. Although m/z 611 and 446 were the primary ions used, other ions, such as m/z 372 and 502 for the AMPA derivative; and 584 for the PMG derivative were sufficiently abundant to quantify residues at the LOQs established. The high masses of the ions that were monitored resulted in chromatographic profiles that were relatively free from interferences (Figures 3–5).

The MSD manual tune procedure reported above was found to be essential in obtaining the detection limits reported. Increasing the ion peakwidth to 2.6 amu is a continuation of the "Low Mass Resolution" option offered by the HP ChemStation software, and is a direct method for increasing analyte detectability. A potential problem with widening the ion peakwidth is the possibility of increasing background noise or detecting peaks from compounds with masses close to that of the target analyte. Due to the high masses of the ions monitored (greater than m/z 370), additional interferences did not pose a problem. Widening of the peakwidth was very effective for increasing the detectability of the PMG derivative. As shown in Figure 1, there exist both m/z 611 and 612 fragments of the PMG derivative. Monitoring at m/z 611.5 with a ion peakwidth of 2.6 amu detected both fragments simultaneously without the increase in noise, or loss of sampling points usually found when monitoring multiple ions. As a result of this tuning procedure the detectability of the AMPA derivative is increased by 2–4-fold and the PMG derivative by 8–10-fold (Figure 6).

Independent Laboratory Confirmation. Data presented in Tables 3–6 were collected during in-lab method validation studies and during studies conducted in support of residue tolerance petitions. In addition, two independent laboratory confirmation (ILC) studies were conducted in accordance with U.S. EPA's guideline requirement 88-5 to provide methods intended for use in tolerance monitoring of crop and animal products. These studies were conducted by laboratory personnel with no previous knowledge or experience with this

Table 7. Results of Independent Laboratory Confirmations

study no.	matrix	fortification level,[a] mg/kg	PMG recovery, %	AMPA recovery, %
1	corn grain	0.05	87, 75	na[d]
		0.25	112, 131	na
	corn forage	0.10	77, 76	na
		0.25	101, 85	na
	corn fodder	0.10	105, 99	na
		0.25	80, 92	na
		mean:	93	
		CV:[b]	18	
		N:[c]	12	
2	cows' milk	0.01	105, 90	98, 88
		0.10	114, 120	104, 110
	beef liver	0.10	102, 105	76, 84
		1.0	103, 80	99, 86
		mean:	102	93
		CV:	12	12
		N:	8	8

[a] Amount added, each analyte; two replicates fortified at each level. [b] Coefficient of variation. [c] Number of replicates. [d] Not analyzed; regulatory requirements no longer required AMPA analysis.

method. The first ILC was conducted on grain, forage and fodder of field corn. The second ILC was conducted on cows' milk and on beef liver. The results of these studies are given in Table 7.

Safety Considerations. One analyst has developed an allergic reaction to the derivatizing agent. The response consists of an initial itching followed by a skin rash. The postderivative evaporation step appears to cause the most problems. It is essential to exercise all precautions to avoid contact with the derivatizing agents.

Conclusions. A general residue method for the simultaneous determination of glyphosate and its primary metabolite, (aminomethyl)phosphonic acid, has been developed. The method uses simple, yet effective, extraction, cleanup, and derivatization techniques. Determination of the high mass, perfluorinated derivatives using capillary GC analysis with mass-selective detection provides a highly sensitive and selective detection system with excellent confirmatory capabilities. The method has been applied to a wide range of difficult environmental matrices on a routine basis. Previous work with this method indicates the ability to determine residues in new matrices with little or no modifications.

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