Table I. Percent Recovery of Flucythrinate from Fortified Control Samples

sample	% recovery[a] 0.05 ppm	0.10 ppm	0.25 ppm	0.50 ppm	1.00 ppm	mean
red cabbage	75	93	90	90	89	87
white cabbage	90	85	89	83	88	87
savoy cabbage	80	98	92	85	94	90
broccoli	92	88	83	82	92	87
apples	85	85	90	107	90	91
wheat grain	85	75	93	74	77	81
wheat ears	85	100	96	99	98	96
wheat foliage	70	83	83	94	78	82
wheat straw	105	100	96	89	86	95
barley grain	95	98	90	101	93	95
barley ears	90	73	80	83	95	84
barley straw	110	73	79	83	95	88
green hops	73	83	104	76	85	84
dried hops	94	74	118	90	97	95

[a] Each value is the result of a single experiment.

at 30 mL/min. The operating parameters were as follows: detector temperature, 300 °C; injection port temperature 250 °C; column temperature, 240 °C. Under these conditions, flucythrinate gives two peaks at retention times of approximately 13 and 14 min, due to the two diastereoisomeric pairs (*RR*, *SS*, *RS*, *SR*). A linear response (the summed height for each peak) was observed over the range 0.06–1.25 ng of flucythrinate, using 5-μL injections of standard solutions. Quantitiation of apparent residues found was achieved by comparing the total peak height from the sample injection (5 μL) with the total peak height of a 0.50- or 0.625-ng standard injection. [Samples were reconstituted in a volume of toluene such that when injected (5 μL) the response observed was within the linear range tested.]

RESULTS AND DISCUSSION

The percent recoveries of flucythrinate from crops, fortified with standard material in the range 0.05–1.00 ppm prior to extraction, are given in Table I. The recoveries given are the results of single experiments. Typical chromatograms of standards and untreated and fortified samples are shown in Figures 1 and 2. Different sample weights were used for different crops in order to aid solvent extraction. Petroleum ether (60–80 °C) was used in the extraction of hops in order to reduce the amount of crop-related coextractives. Changes in the Florisil column chromatography were found to be necessary with different crop types in order to achieve adequate sample cleanup. For example, fruit and vegetable crops need no hexane/toluene column wash prior to elution with toluene whereas hops required two Florisil columns. For hops, overnight storage of the sample, at −6 °C before the second column, was found to be essential to remove interference.

The methods have been used successfully for the analysis of flucythrinate residues in treated crops. The basic method (cabbage, broccoli, apples) allows a throughput of approximately six samples per analyst per day whereas the modified methods allow analysis of four samples per analyst per day. The methods listed should be readily applicable to other crops.

ACKNOWLEDGMENT

I thank all members, past and present, of the Plant Health Residues Laboratory, Product Research and Development Laboratories, Cyanamid of Great Britain, Ltd., Gosport, England, for their technical assistance.

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Validation of an Analytical Residue Method for Analysis of Glyphosate and Metabolite: An Interlaboratory Study

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A new residue method for the analysis of glyphosate and (aminomethyl)phosphonic acid has been validated with an interlaboratory study. Five different analysts from Monsanto Co. and other laboratories participated in testing of five different matrixes: alfalfa forage, cabbage, grapes, soybean grain, environmental water. These were chosen to represent the wide variety of matrixes analyzed for glyphosate-related residues. The cornerstone of the method is concentration and isolation via chelation ion exchange, with subsequent quantitation by HPLC with postcolumn reaction detection. The method was validated over the concentration range from 0.05 to 5.00 ppm with overall analytical recoveries of 80.9 ± 13.8% for glyphosate and 79.2 ± 13.8% for (aminomethyl)phosphonic acid. The coefficient of variation for both analytes was 17%, which fits well with that predicted for the analysis of compounds in this concentration range.

INTRODUCTION

There has been significant interest in recent years in analytical residue methodology for glyphosate [*N*-(phosphonomethyl)glycine] and its metabolite [(aminomethyl)phosphonic acid, AMPA] as a result of the increasing use of the herbicides Roundup, Rodeo, and Bronco. Numerous methods have been developed that determine the two compounds from specific matrices [Moye and St. John (1980); Guinivan et al., (1982); Roseboom and Berkoff (1982); Friestad and Bronstad (1982); Moye et al., (1983)], but many of these methods have been applied primarily to matrices that contain a high percentage of water and, by experience, have proven to be

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easier cleanup challenges. To date only the *Pesticide Analytical Manual* method (PAM, 1977) has been applied to a large number of widely different matrices (i.e., approximately 100 different matrices from avocados to walnuts). This residue method, first developed in 1972, was state of the art at that time, but by today's standards is long and time consuming to perform. Developments in methodology have progressed, initially with HPLC chromatography conditions (Burns and Tompkins, 1979) and with specific detection using HPLC–postcolumn reaction (PCR) with ninhydrin reagent (Rogers and Daniels, 1977, referenced but unpublished work). Additionally, fluorimetric HPLC–PCR quantitation was developed with air-segmentation PCR equipment that utilized hydrogen peroxide to oxidize glyphosate to a primary amine prior to *o*-phthalaldehyde (OPA) derivatization (Cowell, 1980, referenced but unpublished work). A similar procedure was developed with continuous flow HPLC–PCR–OPA quantitation using calcium hypochlorite as the oxidant (Moye and St. John, 1980). This method was further modified to solve the problem of $Ca(OCl)_2$ dissolution (Muth, 1980, unpublished work). Advances in cleanup technology, involving isolation and concentration of glyphosate and AMPA by chelation and ion-exchange chromatography (Nord, 1986, manuscript in preparation) and commercially available HPLC–PCR instrumentation, have allowed the evolution of a new simplified method that has been successfully tested on a variety of different matrices in an interlaboratory study. This interlaboratory method validation is the subject of this document.

The new methodology employs a biphasic aqueous–organic extraction of the matrix followed by cleanup of the supernatant aqueous extract utilizing first iron-loaded Chelex 100 resin (ligand-exchange) and then AG-1-X8 (anion-exchange) resin columns. Quantitation of the extract is then performed by HPLC coupled to a postcolumn reactor (PCR) specific for primary amines or compounds that can be converted to primary amines (OPA). The method is simple and is routinely used to perform 12 concurrent analyses in one 8-h day provided chromatography can proceed unattended until the next working day.

The five matrices chosen for this study were intended to represent major crop types: soybeans (grains, legumes), grapes (fruits), cabbage (vegetables), alfalfa (forage), environmental water. Five separate analysts from different laboratories all used the same methodology in performing analyses of blind fortified samples. The means utilized in evaluating this study were simple statistical tests designed to measure variability in a number of comparisons. Thus, the procedure does not conform to the definition of "ruggedness" established in the *AOAC Statistical Manual* (Youden and Steiner, 1975) but is perhaps closer to their definition for an interlaboratory collaborative study. However, the authors feel that the successful exhibition of performance of the method in other hands is the true practical test of reliability.

MATERIALS AND METHODS

Instrumentation. HPLC equipment from various manufacturers was employed in the HPLC–PCR systems utilized by the laboratories in this study. A generic schematic of the instrument configuration is shown in Figure 1. One of the systems was composed of a Perkin-Elmer Series 10 HPLC pump, a Varian 8000 autosampler, a Kratos postcolumn derivatization system (Model URS 051), and a Perkin-Elmer LS-4 fluorescence spectrophotometer set at 340-nm excitation and 455-nm emission wavelengths. The analytical column was a 30 cm × 4.6 mm i.d. Bio-Rad Aminex A-9 thermostated at 50 °C

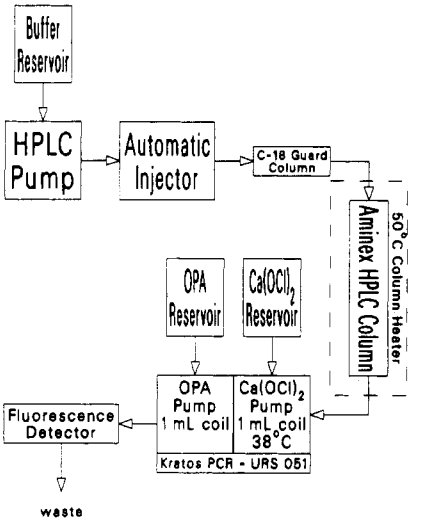

Figure 1. HPLC–OPA postcolumn reactor system for glyphosate and (aminomethyl)phosphonic acid residue analysis.

with a Rainin column heater. The oxidation reaction coil was also thermostated at 38 °C with a Kratos URA 200 temperature controller. Two other postcolumn reactor systems were evaluated and found equivalent to the Kratos postcolumn reaction system. These were the Perkin-Elmer and the Waters amino acid analysis systems.

Glassware and Reagents. The glass column dimensions for the Chelex 100 resin were 2.2 cm i.d. × 22 cm with a Teflon stopcock and a 250-mL reservoir. The anion-exchange column dimensions were 1.7 cm i.d. × 22 cm with a Teflon stopcock and a 30-mL reservoir.

The Chelex 100 (100–200-mesh sodium form) and AG-1X8 (200–400-mesh chloride form) resins were obtained from Biorad Laboratories, Richmond, CA. The Chelex 100 resin was converted to the Fe(III) form by magnetically mixing 0.9 kg of resin in a total aqueous volume of 3 L and adding 50 mL of 6 M HCl followed by 1 L of 0.1 M $FeCl_3$ solution. After the resin was allowed to settle, the aqueous phase was decanted. Deionized water (2 L) and 500 mL of 0.1 M $FeCl_3$ were then added to the resin with mixing, and the aqueous phase was decanted. This wash was repeated a second time, and the resin was then transferred to a large glass column with a fritted-disk support and rinsed with 4 L of 0.02 M HCl. The resin was stored at room temperature in amber glass bottles under deionized water until used.

The mobile phase for the Aminex A-9 column was 4% methanol in 0.005 M potassium dihydrogen phosphate solution adjusted to pH 1.9 with concentrated phosphoric acid. After normal filtering (0.22 μm) and degassing procedures, the mobile phase was delivered to the column at a rate of 0.5 mL/min.

The oxidative solution was prepared by dissolving 1.36 g of KH_2PO_4, 11.6 g of NaCl, and 0.4 g of NaOH in 0.5 L of deionized water. Then, 15 mg of $Ca(OCl)_2$ dissolved in 50 mL of deionized water was added and the solution diluted to a total volume of 1 L with deionized water. This solution was filtered and mixed with the column eluent stream at a rate of 0.2 mL/min.

Fluoraldehyde, the commercially (Pierce Chemical Co.) available *o*-phthalaldehyde reagent, was mixed with the oxidized effluent stream at a rate of 0.3 mL/min.

Residue Analysis Procedure. (*a*) *Crops.* A homogeneous sample (30.0 g) was blended with 50 mL of chloroform and 150 mL of 0.1 M HCl for 1 min, transferred into a 250-mL polypropylene centrifuge bottle, and centrifuged at 11 000 rpm for 20 min in a refrigerated cen-

trifuge (4 °C). Exactly 125 mL of the aqueous supernatant extract (equivalent to 25 g of plant tissue) was decanted into a graduated cylinder and diluted to 400 mL with deionized water prior to application to the Chelex column.

(*b*) *Environmental Water.* A 100-mL sample was acidified to pH 2.0 ± 0.4 with 6 M HCl and then filtered through glass fiber filter paper prior to application to the Chelex column.

(*c*) *Chelex Column Cleanup.* Chelex 100 resin, 15 mL in Fe(III) form, was transferred to a column containing 7–8 mL of deionized water. The prepared sample was applied to the column and eluted at a rate of 6–8 mL/min. After sample elution, the walls of the column and resin bed were rinsed with approximately 50 mL of deionized water. The column was rinsed additionally with 100 mL of 0.2 M HCl with a wide open stopcock. All eluates were discarded.

The column was then eluted at a rate of 4 mL/min (or less) with 22 mL of 6 M HCl solution, the last 15 mL of which was combined with 10 mL of concentrated HCl and retained for anion-exchange cleanup.

(*d*) *Anion-Exchange Column Cleanup.* The column was prepared by adding 7–8 mL of deionized water and approximately 7 mL of AG-1X8 anion-exchange resin. The resin bed was adjusted to 5 cm, and the column was rinsed with 15 mL of 6 M HCl solution shortly before applying the sample. The eluate from the Chelex 100 column was applied with the stopcock wide open, and the sample container (25 mL graduated cylinder) was completely rinsed with 2 mL of 6 M HCl solution onto the column. Just as the last of the sample and rinse entered the column, 8 mL of 6 M HCl solution was applied and the eluate collected in a 250-mL recovery flask. The eluate was concentrated to dryness on a rotary film evaporator by slowly increasing the temperature of the water bath from 20 to 60 °C. The final traces of moisture were removed with a stream of dry nitrogen. The residue was dissolved in 2.0 mL of deionized water and filtered through a 0.45-μm pore size membrane filter prior to quantification of glyphosate and AMPA by injecting 60 μL into the HPLC–PCR–OPA system. A guard column (Brownlee ODS or equivalent) was installed in the system to protect the analytical column. Sample quantitation was based on the relative peak heights or areas of the sample to standard peak heights or areas across the range of expected sample concentrations.

Study Details. The five laboratories involved were Analytical Bio-Chemistry Laboratories, Inc. (Columbia, MO); Craven Laboratories, Inc. (Austin, TX); and three Monsanto Co. laboratories. Samples analyzed consisted of the five matrices each with control samples (n = 5), analyst-fortified samples (n = 10), and samples blind fortified with the two analytes at various residue levels (n = 5) and coded by the study administrator. Five fortification levels were distributed in the 0.05–5.00 ppm range for both glyphosate and AMPA. The ruggedness of the method was judged by comparing the actual recovery and the variation in recovery as a function of five variables: (1) compound, (2) analyst, (3) matrix, (4) fortification level, (5) analyst's knowledge of level. The results were calculated by a common method of applying a power function curve fit of the external standards response data for quantitation of sample responses. This eliminated any variation due to the method of calculation (such as using a linear curve fit, etc.). In addition, any background present in each respective control matrix was averaged and subtracted from the fortified sample results. This background subtraction helps avoid "inflated" recovery values at the lower fortification levels. The actual data (ppm) for each sample were then converted to percent recovery so that all data could be compared regardless of fortification level. These data are presented in Tables I and II.

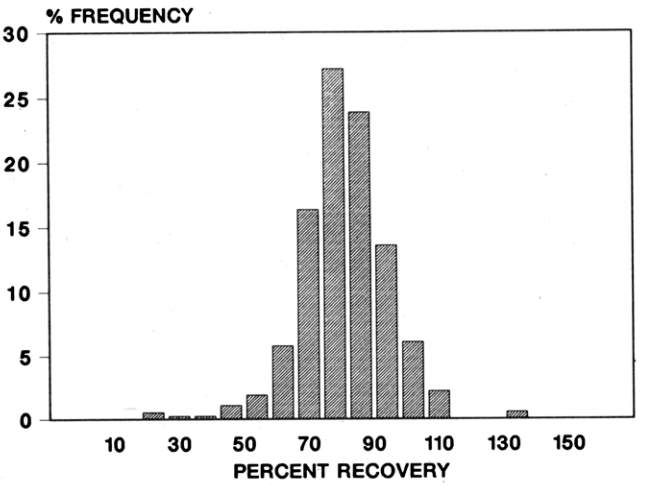

Figure 2. Distribution of glyphosate recovery data.

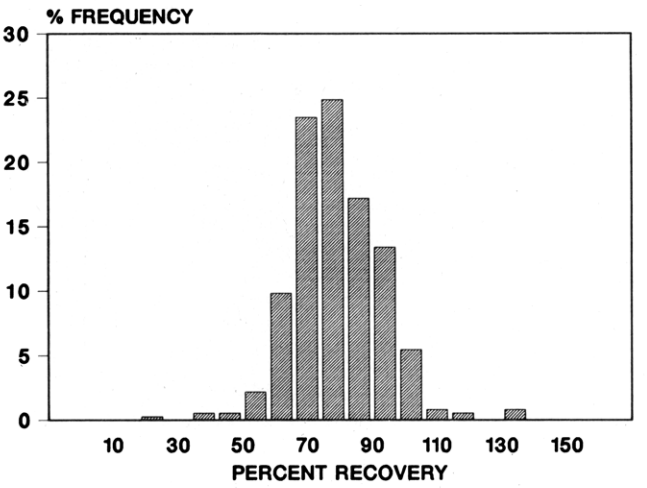

Figure 3. Distribution of AMPA recovery data.

RESULTS AND DISCUSSION

The overall analytical recovery for the study was 80.9 ± 13.8 for glyphosate and 79.2 ± 13.8 for AMPA. The range of recoveries was from 21.4 to 135.5 and 20.0 to 136.0 for glyphosate and AMPA, respectively. This clearly shows that the method works equally well for glyphosate and AMPA. The coefficient of variation for both analytes is 17%, which fits well with what is predicted for this concentration range (Horwitz, 1980). According to Horwitz's findings, determination of analytes in the concentration range of 0.05–5.00 ppm should result in a coefficient of variation between 25 and 12%. Figures 2 and 3 represent the overall distribution of the recovery data. In these figures the distribution data were calculated over 8% intervals and plotted at the midpoint of the interval. The corresponding distributions are normally distributed about their respective averages.

All analysts had overall average recoveries greater than 70%. To visually demonstrate the variability of the method due to analyst variation, an analysis of variance was performed with respect to the individual analysts. The results of this are shown in Table III. This was performed with the aid of a computer program called MINITAB (Pennsylvania State University, 1982) run on a VAX 11/780 computer. Variation due to the different analysts represented by individual 95% confidence levels was

Table I. Round-Robin Analyses Recoveries for Glyphosate[a]

		analyst					
		1	2	3	4	5	overall
alfalfa	T	90.8 ± 16.0		79.7 ± 16.2	82.2 ± 17.4	85.4 ± 10.9	84.5 ± 15.5
	K	84.0 ± 9.2	NC	84.0 ± 7.3	80.7 ± 20.1	79.8 ± 6.4	82.1 ± 11.7
	U	104.3 ± 19.2		71.0 ± 25.6	85.1 ± 11.8	96.6 ± 9.5	89.2 ± 20.8
cabbage	T	69.3 ± 10.0	86.6 ± 10.7	75.7 ± 5.4	75.9 ± 5.8	73.7 ± 3.8	76.2 ± 9.4
	K	71.2 ± 10.7	86.6 ± 9.4	78.2 ± 4.7	76.0 ± 3.6	73.3 ± 3.8	77.0 ± 8.7
	U	65.5 ± 8.2	86.5 ± 14.4	70.9 ± 2.9	75.6 ± 9.5	74.5 ± 4.2	74.6 ± 10.7
grapes	T	79.2 ± 12.0	89.6 ± 8.5	72.9 ± 8.7	73.0 ± 10.9	73.8 ± 8.7	77.7 ± 11.5
	K	81.6 ± 13.3	89.6 ± 9.6	73.4 ± 6.5	75.4 ± 7.2	71.5 ± 5.2	78.3 ± 10.8
	U	74.3 ± 8.0	89.6 ± 6.7	72.0 ± 13.1	68.2 ± 15.9	78.3 ± 12.8	76.5 ± 13.1
soybeans	T	81.8 ± 33.2	89.2 ± 5.0	80.5 ± 10.9	74.7 ± 7.7	74.9 ± 17.5	80.2 ± 18.3
	K	97.7 ± 22.5	90.1 ± 5.8	82.1 ± 5.8	73.7 ± 8.6	71.6 ± 19.0	83.0 ± 16.9
	U	50.1 ± 29.0	87.4 ± 2.6	77.2 ± 17.8	76.9 ± 5.6	81.4 ± 13.5	74.6 ± 20.0
water	T	84.2 ± 14.2	97.6 ± 2.7	85.2 ± 4.4	79.4 ± 5.1	86.6 ± 9.0	86.6 ± 10.0
	K	81.0 ± 11.3	97.5 ± 2.0	83.8 ± 4.3	78.8 ± 5.4	90.0 ± 7.2	86.2 ± 9.5
	U	90.6 ± 18.4	97.9 ± 4.1	88.2 ± 3.3	80.7 ± 4.6	79.8 ± 8.8	87.4 ± 11.1
overall	T	81.1 ± 19.8	90.7 ± 8.3	78.8 ± 10.7	77.1 ± 10.7	78.9 ± 12.2	80.9 ± 13.8
	K	83.1 ± 16.1	90.9 ± 8.2	80.3 ± 6.9	76.9 ± 10.6	77.2 ± 11.9	81.3 ± 12.2
	U	77.0 ± 25.6	90.4 ± 8.9	75.8 ± 15.5	77.3 ± 11.1	82.1 ± 12.2	80.1 ± 16.5

[a] Recovery = [(found (ppm) − background)/applied (ppm)] × 100. Key: K = known Fortifications (n = 10, per matrix per analyst); U = unknown (n = 5); T = known + unknown (n = 15); NC = not completed. 0.0 level fortifications are not included in recovery calculations.

Table II. Round-Robin Analyses Recoveries for AMPA[a]

		analyst					
		1	2	3	4	5	overall
alfalfa	T	77.1 ± 14.7	94.4 ± 8.2	91.8 ± 5.6	93.2 ± 14.1	79.8 ± 12.6	86.8 ± 13.6
	K	71.0 ± 10.3	93.6 ± 8.2	94.6 ± 3.8	89.3 ± 8.7	76.1 ± 14.2	84.9 ± 13.4
	U	89.5 ± 15.3	102[b]	86.1 ± 4.4	100.9 ± 20.3	87.2 ± 2.4	91.4 ± 13.2
cabbage	T	65.6 ± 6.4	74.7 ± 4.2	74.2 ± 3.4	72.7 ± 5.6	70.6 ± 6.0	71.6 ± 6.1
	K	65.2 ± 6.7	76.4 ± 3.3	75.1 ± 2.5	74.5 ± 3.7	71.1 ± 5.7	72.5 ± 6.0
	U	66.5 ± 6.6	71.4 ± 4.0	72.4 ± 4.4	69.2 ± 7.5	69.6 ± 7.2	69.8 ± 5.9
grapes	T	66.2 ± 6.4	76.0 ± 16.2	72.4 ± 5.1	69.2 ± 14.9	71.9 ± 9.7	71.1 ± 11.5
	K	66.4 ± 5.7	83.1 ± 12.1	72.4 ± 5.1	73.3 ± 7.2	70.6 ± 5.0	73.2 ± 9.1
	U	65.7 ± 8.2	61.9 ± 14.7	72.4 ± 5.7	60.9 ± 23.1	74.7 ± 16.2	67.1 ± 14.7
soybeans	T	80.8 ± 22.3	98.7 ± 13.4	73.0 ± 11.1	83.6 ± 6.8	81.6 ± 11.9	83.5 ± 16.1
	K	90.8 ± 17.8	96.5 ± 14.8	74.2 ± 10.2	82.9 ± 6.1	79.2 ± 13.7	84.7 ± 15.0
	U	60.7 ± 16.5	103.0 ± 9.9	70.5 ± 13.7	84.9 ± 8.6	86.3 ± 5.2	81.1 ± 18.1
water	T	82.3 ± 13.0	97.0 ± 2.0	79.1 ± 6.9	72.8 ± 5.9	87.8 ± 6.2	83.8 ± 11.1
	K	78.2 ± 9.6	97.1 ± 2.1	79.6 ± 6.8	72.8 ± 6.7	89.9 ± 4.1	83.5 ± 10.8
	U	92.8 ± 15.7	96.7 ± 2.2	78.0 ± 8.1	73.0 ± 3.6	82.7 ± 8.2	84.7 ± 12.1
overall	T	74.3 ± 15.3	87.7 ± 14.9	78.1 ± 10.0	78.4 ± 13.5	78.2 ± 11.4	79.2 ± 13.8
	K	74.3 ± 14.1	89.4 ± 12.3	79.2 ± 10.1	78.6 ± 9.2	77.4 ± 11.6	79.8 ± 12.6
	U	74.3 ± 17.9	83.5 ± 19.7	75.8 ± 9.4	78.0 ± 19.9	80.0 ± 10.9	78.1 ± 16.1

[a] Recovery = [(found (ppm) − background)/applied (ppm)] × 100. Key: K = known fortifications (n = 10, per matrix per analyst); U = unknown (n = 5, except water–where n = 4); T = known + unknown (n = 15, except water–where n = 14). 0.0 level fortifications are not included in recovery calculations. [b] Only one sample analyzed.

surprisingly small except that analyst number 2 had significantly higher recoveries for both glyphosate and AMPA. Since this analyst's recoveries were consistently higher for all matrices and all fortification levels whether or not analyst number 2 knew the fortification level, this was not considered a cause for concern. Analyst number 2 did have a problem with laboratory contamination of alfalfa samples during workup with a glyphosate coincidental peak. Although the interferent could not be identified, the interference was confirmed at a Monsanto laboratory.

Variation due to matrix was slightly higher. Table IV presents the analysis of variance as a function of the matrix. While the overall recovery for all matrixes was greater than 70%, recoveries for grapes and cabbage were significantly lower for both glyphosate and AMPA than the other matrixes. Soybean grain, which is traditionally a difficult matrix to analyze, was the one with the greatest variation in recoveries having an overall mean coefficient of variation (CV) of 23%. This however was greatly influenced by a CV of 41% for this matrix by analyst number 1. None of these matrix variations are of serious concern and are to be expected of a universal method.

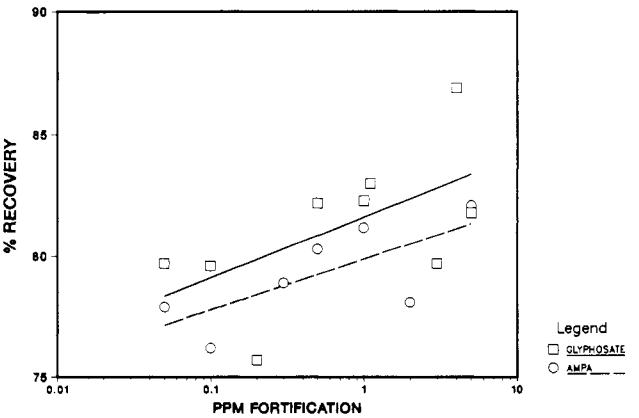

Figure 4. Dependence of analytical recovery on compound and fortification level.

The influence of fortification levels on analytical recovery was negligible for both glyphosate and AMPA over the fortification range studied. The effect of fortification

Table III. Analysis of Variance by Analyst[a]

GLYPHOSATE

SOURCE	DF	SS	MS	F
ANALYST	4	7563	1891	11
ERROR	355	60861	171	
TOTAL	359	68424		

INDIVIDUAL 95% CI'S FOR MEAN BASED ON POOLED S.D.

LEVEL	N	MEAN	S.D.
ANAL 1	75	81.06	19.80
ANAL 2	60	90.74	8.34
ANAL 3	75	78.81	10.66
ANAL 4	75	77.06	10.66
ANAL 5	75	78.86	12.15

POOLED S.D. = 13.09

78.0 84.0 90.0

Percent Recovery

AMPA

SOURCE	DF	SS	MS	F
ANALYST	4	7044	1761	10
ERROR	361	62366	173	
TOTAL	365	69410		

INDIVIDUAL 95% CI'S FOR MEAN BASED ON POOLED S.D.

LEVEL	N	MEAN	S.D.
ANAL 1	74	74.30	15.31
ANAL 2	70	87.70	14.86
ANAL 3	74	78.09	9.96
ANAL 4	74	78.36	13.49
ANAL 5	74	78.21	11.41

POOLED S.D. = 13.14

72.0 78.0 84.0 90.0

Percent Recovery

[a] CI = confidence interval.

Table IV. Analysis of Variance by Matrix[a]

GLYPHOSATE

SOURCE	DF	SS	MS	F
MATRIX	4	5682	1421	8
ERROR	355	62741	177	
TOTAL	359	68424		

INDIVIDUAL 95% CI'S FOR MEAN BASED ON POOLED S.D.

LEVEL	N	MEAN	S.D.
ALFALFA	60	84.51	15.53
CABBAGE	75	76.23	9.40
GRAPES	75	77.69	11.54
SOYBEANS	75	80.22	18.28
WATER	75	86.63	9.99

POOLED S.D. = 13.29

75.0 80.0 85.0 90.0

Percent Recovery

AMPA

SOURCE	DF	SS	MS	F
MATRIX	4	15987	3997	27
ERROR	360	52904	147	
TOTAL	364	68891		

INDIVIDUAL 95% CI'S FOR MEAN BASED ON POOLED S.D.

LEVEL	N	MEAN	S.D.
ALFALFA	70	86.63	13.56
CABBAGE	75	71.58	6.09
GRAPES	75	71.14	11.54
SOYBEANS	75	83.52	16.07
WATER	70	83.84	11.10

POOLED S.D. = 12.12

72.0 78.0 84.0

Percent Recovery

[a] CI = confidence interval.

level on analytical recovery is presented in Figure 4. In this figure the individual points represent the corresponding average recoveries at the different fortification levels (known and unknown) while the lines represent a simple linear regression of the data values. While there does seem to be a slight upward trend in the recovery as a function of fortification level, the correlation coefficient for each of these lines was less than 0.01, which would indicate a lack of statistical significance. The variation in recoveries was higher for the lower fortification levels as expected (Horwitz, 1980).

There was also a higher variation of recoveries in blind fortified samples than in samples where the analyst knew the fortification level. The actual recoveries for the two knowledge levels were similar overall, but some significant differences were noted for individual analysts and matrixes (e.g., analyst number 1 had a 98% recovery for known fortifications in soybeans but only achieved 50% recovery on samples for which he or she did not know the fortification level). On the other hand, analyst number 2 obtained nearly identical recoveries whether or not the fortification level was known for all matrices completed. A plot of the influence of fortification level and knowledge of that level on the variability of the recoveries is shown for glyphosate in Figure 5. Although overall known vs. unknown are very similar, this comparison does point to the advantage of using blind fortified samples to assess the reliability of an analyst's data.

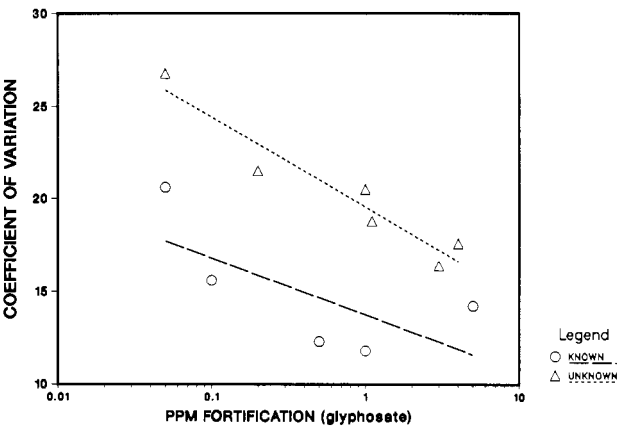

Figure 5. Dependence of coefficient of variation on fortification level and knowledge of level.

CONCLUSIONS

This study validates the reliability of a new analytical methodology for the analysis of glyphosate and AMPA in

a variety of matrixes when analyzed by a number of different analysts. The method has also been used on numerous other matrixes, albeit by a smaller number of analysts. It is presented as a universal method in that only minor modifications of extraction (especially soil) and cleanup steps may be required for unusual matrixes.

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Registry No. AMPA, 1066-51-9; H_2O, 7732-18-5; glyphosate, 1071-83-6.

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An Improved Procedure for the Isolation of Medicagenic Acid 3-*O*-β-D-Glucopyranoside from Alfalfa Roots and Its Antifungal Activity on Plant Pathogens

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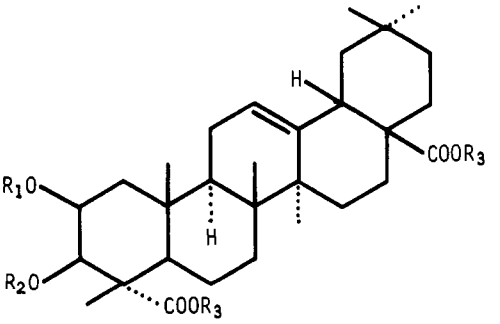
2β-Hydroxy-3β-*O*-(β-D-glucopyranosyl)-Δ^{12}-oleanene-23,28-dioic acid, known also as medicagenic acid 3-*O*-β-D-glucopyranoside, was isolated from alfalfa roots in pure form and was shown to possess potent fungistatic effects against *Trichoderma viride*, *Sclerotium rolfsii*, *Rhizopus mucco*, *Aspergillus niger*, *Phytophthora cinamommi*, and *Fusarium oxysporum* f. sp. *lycopersici*: i.e., mycelial growth inhibition of 95%, 86%, 68%, 53%, 51%, and 52%, respectively, for concentrations of 40 μg/mL and ID_{50} of 1.4, 2.3, 4.1, 1.7, 40, and 10.5 μg/mL, respectively.

INTRODUCTION

The antifungal activity, as well as other biological activities, of saponins was extensively reviewed in the literature (Kofler, 1929; Birk, 1969; Birk and Peri, 1979; Schlösser, 1983). Saponin extracts from alfalfa were shown to possess a fungistatic activity (Shani et al., 1970; Gestetner et al., 1971; Assa et al., 1972; Leath et al., 1972).

Medicagenic acid (2β,3β-dihydroxy-Δ^{12}-oleanene-23,28-dioic acid, I) was first identified by Djerassi and co-workers (Walter et al., 1955; Djerassi et al., 1957), and the corresponding 3-*O*-β-D-glucopyranoside II was first isolated from alfalfa roots and its structure determined by Morris et al. (1961) (Chart I). Although the significance of saponins derived from medicagenic acid in the antimycotic acitivity of saponin extracts from alfalfa roots was recognized in the past (Birk and Peri, 1979), a very limited study of such synthetic saponins was carried out (Gestetner et al., 1973) while none was carried out with pure native saponins presumably since alfalfa contains a large number of saponins that are difficult to separate.

Chart I



I: $R_1 = R_2 = R_3 = H$
II: $R_1 = H$, R_2 = β-D-Glc-*p*-, $R_3 = H$
III: $R_1 = R_2 = H$, $R_3 = Me$
IV: $R_1 = R_2 = Ac$, $R_3 = H$
V: $R_1 = R_2 = Ac$, $R_3 = Me$

Following screening of antimycotic compounds from alfalfa root extract, we identified a compound (G2) active against *Trichoderma viride* and *Sclerotium rolfsii*. In this work we report an improved method for the isolation of

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