Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Glyphosate (Touchdown, PMG)

MRID #: 443265-06

Matrix: Soil

Analysis: GC/MS

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443265-06

ZENECA Ag Products
1800 Concord Pike
Wilmington, DE 19850

Volume 8

Study Title

TOUCHDOWN®: Determination of GLYPHOSATE and AMINOMETHYLPHOSPHONIC ACID in Soil by Gas Chromatography and Mass-Selective Detection

Data Requirement

GUIDELINE SERIES 171-4(c)

Author

P.L. Alfemess

Study Completed On

January 21, 1994

Performing Laboratory

ZENECA Ag Products
Western Research Center
1200 S. 47th St.
Richmond, CA 94804

Laboratory Project ID

GLYP-92-AM-01

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Company Zeneca Ag Products

Submitter M. E. Rhodes Date 1-20-94

Regulatory Product Manager (Title)

M E Rhodes (Signature)

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Study Number: GLYP-92-AM-01

Report Title: TOUCHDOWN®: Determination of Glyphosate and Aminomethylphosphonic Acid in Soil by Gas Chromatography and Mass-Selective Detection

GOOD LABORATORY PRACTICE (GLP) COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR Part 160.

Submitter M. E. Rhodes — Date 1-20-94
Regulatory Product Manager
Registrations and Regulatory Affairs
Zeneca Ag Products
Telephone: (615) 982-9076

Sponsor M. G. Kleinschmidt, Ph.D. — Date January 20-1994
Manager,
Environmental Chemistry Section
Zeneca Ag Products

Study Director Philip L. Alferness — Date 1-21-94
Research Chemist
Zeneca Ag Products

Study No.: GLYP-92-AM-01

Study Title: TOUCHDOWN: Determination of Glyphosate and Aminomethylphosphonic Acid in Soil by Gas Chromatography and Mass-Selective Detection

Report No.: RR 92-010B

QUALITY ASSURANCE STATEMENT

In accordance with Zeneca's policy and procedures for complying with the provisions of the EPA's FIFRA Good Laboratory Practice Standards (Final Rule, 40 CFR Part 160, August 17, 1989), the conduct of this study has been inspected/audited by the Quality Assurance Unit at the Western Research Center, Richmond, California, United States of America.

Date (MM-DD-YY)	Inspection/Audit	Report Date
03-04-92	Protocol	03-04-92
03-03-92	Study Conduct	03-06-92
03-12-93	Final Report	07-20-93

In addition, the following facility and/or procedural inspections associated with this type of study were made.

04-21-92	Analytical Lab Facility	06-26-92
10-06-92	Analytical Lab Facility	10-06-92

To far as can be reasonably established, the methods described and results incorporated in this report accurately reflect the raw data produced during the study.

Kenneth D. Walburn
Chemist - QA

January 20, 1994
Date

tudy Number: GLYP-92-AM-01

eport Title: TOUCHDOWN®: Determination of Glyphosate and Aminomethylphosphonic Acid in Soil by Gas Chromatography and Mass-Selective Detection

CERTIFICATION OF AUTHENTICITY

, the undersigned, hereby declare that this study was performed nder my direction and that this report represents a true and ccurate record of the results obtained.

tudy Director _____ 1-24-94
Philip L. Alferness
Research Chemist
Date

ne following Zeneca personnel performed work on this study:

Names	Title
Philip L. Alferness	Research Chemist
Diana M. Gonzalez	Regulatory Studies Coordinator
Sharon Patterson	Technical Assistant
Rae L. McCann	Research Technician III
Lauryl Van Neste	Chemist
Deborah J. Elvira	Secretary

pproved By:

_____ 1/21/94
G. Graham, Ph.D.
roup Leader
nvironmental Chemistry Section
Date

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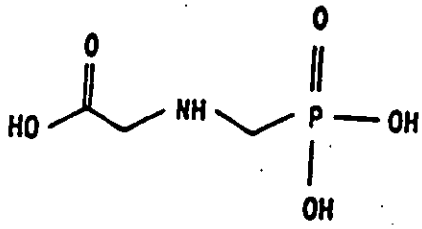
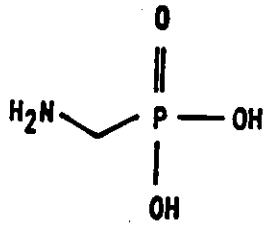
1 SUMMARY/INTRODUCTION

This method is intended for the determination of residues of glyphosate [N-(phosphonomethyl)glycine, PMG] and aminomethylphosphonic acid (AMPA) in soil. PMG is an active herbicidal ingredient in the formulated product marketed by Zeneca Ag Products (formerly ICI Americas Inc.) under the trade name "TOUCHDOWN". AMPA is the principal degradate/metabolite of PMG. The chemical structures and molecular weights (MW) are shown below.



PMG
MW=169 daltons



AMPA
MW=111 daltons

PMG and AMPA residues are extracted from soil using a mixture of dilute ammonium hydroxide and potassium phosphate. The analytes in the aqueous extract are derivatized directly using a mixture of trifluoroacetic anhydride and heptafluorobutanol. The carboxylic and phosphoric acid functional groups are derivatized to form the corresponding heptafluorobutyl esters. The amine functional groups are derivatized to form the corresponding trifluoroacetyl derivatives. The water reacts with the excess trifluoroacetic anhydride to produce trifluoroacetic acid (TFA). Proposed structures for the derivatives of AMPA and PMG are shown in Appendix B. The mass spectra shown in Appendix B are consistent with the formation of the expected derivatives. After derivatization, the excess TFA and reagents are evaporated, and the residuum is dissolved in ethyl acetate. The extract is analyzed using capillary gas chromatography (GC) with mass-selective detection (MSD).

2. MATERIAL/METHODS

The equipment and reagents described below were used to generate the data and chromatograms presented in this report. Equipment with equivalent performance specifications and reagents of comparable purity can be used.

2.1 Apparatus

2.1.1 Gas chromatograph. Hewlett-Packard (HP) model 5880, or model 5890 Series II, designed for use with capillary columns, split-splitless injection, and temperature programming of the column oven. The gas chromatograph is equipped with a Hewlett-Packard model 7673 automatic, high speed sampler/injector.

2.1.2 Mass-selective detector. Hewlett-Packard model 5970A mass-selective detector with Pascal, MS-DOS, or UNIX ChemStation software. The detector is manually tuned using perfluorotributylamine as the calibration standard.

2.1.3 Gas-chromatographic column. 30 m by 0.25 mm i.d., fused-silica, capillary column bonded with a 0.25-μm film thickness of cross-linked, 95% methyl - 5% phenyl silicone (Durabond 5.625, J&W Scientific, #122-5631).

2.1.4 Inlet liners. Double-restrictor, single-piece type (Restek #20784).

2.1.5 Fused-silica wool. Fused-silica wool for packing inlet liner (Restek #20790; inserter tool, Restek #20114).

2.1.6 Syringes, GC injection. 10-μL capacity (Hamilton 701N) for HP 7673 autosampler.

2.1.7 Syringes, sample handling. 10-, 25-, 100-, 250-, 1000-μL capacity Gas-Tight syringes (Hamilton 1700 series) for derivatized extract and standard handling.

2.1.8 Syringe, crude extract handling. 3-mL plastic, disposable syringe with Luer-Lok fittings (Becton/Dickenson #9585).

2.1.9 Eppendorf automatic pipet. Eppendorf Variable Volume pipet, 10-100 µL (Brinkman #2233351; Baxter #P5063-2). Eppendorf Variable Volume pipet, 100-1000 µL (Brinkman #22333607; Baxter #P5063-3).

2.1.10 Filters. Disposable syringe filter units, 0.45-µm pore size with Luer hub. (Zetapor brand from Alltech #5-8072; or Gelman Nylon Acrodisc from Baxter #F3057-47A).

2.1.11 Glass pipets. 2-, 5-, and 10-mL disposable, graduated glass pipets for general use.

2.1.12 Glass bottles. 4- or 8-oz, wide-mouthed bottles equipped with Poly-Seal caps. 4-oz narrow-mouthed bottle equipped with Poly-Seal caps.

2.1.13 Auto-sampler vials. Standard 2.0-mL (12 mm x 32 mm) crimp-top vial (Sunbrokers #200-000) with standard crimp top (Sunbrokers #200-100) and 250-µL limited volume insert (Sunbrokers #200-228).

2.1.14 Derivatization vials. Standard 2.0-mL (12 mm x 32 mm) screw-top, autosampler vial (Sunbrokers #200-250) with phenolic plastic, open-top cap (Wheaton #240506), and double-thickness PTFE septum (Sunbrokers #200-338).

2.1.15 Evaporation manifold. 12-unit evaporation manifold with aluminum heating/cooling block, 13-mm tube size (Chemical Research Supplies #201188).

2.1.16 IEC Model K centrifuge.

2.1.17 Eberbach mechanical shaker (horizontal).

2.1.18 Thermometer. -100 °C to +50 °C (Baxter #T2120-2).

2.2 Reagents

2.2.1 Ethyl acetate. High purity for pesticide residue analysis.

2.2.2 Ammonium hydroxide, concentrated. 58% ACS grade.

2.2.3 Hydrochloric acid, concentrated. 37% ACS grade.

2.2.4 Water. Deionized or distilled.

2.2.5 2,2,3,3,4,4,4-Heptafluoro-1-butanol. (Aldrich #H160-4)

2.2.6 Trifluoroacetic anhydride. (Janssen Chimica #14.781.37, from Spectrum Chemical.)

2.2.7 Citral. 3,7-dimethyl-2,6-octandienal, 95% (Aldrich #C8,3007).

2.2.8 Potassium phosphate, monobasic. 99%, ACS grade.

2.2.9 Extracting solution. Aqueous solution consisting of 0.25 M ammonium hydroxide (17 mL concentrated ammonium hydroxide/L) and 0.10 M monobasic potassium phosphate (13.6 g monobasic potassium phosphate/L).

2.3 Reference Materials

2.3.1 AMPA and PMG analytical reference standards. Available from Zeneca Ag Products, 1200 South 47th Street, Box Number 4023, Richmond, CA 94804-0023; Attention: Manager, Environmental Chemistry Section.

The PMG and AMPA were both 99% purity, and had reference numbers ASW-838-C and ASW-1168-C, respectively.

2.3.2 Stock calibration and fortification solutions. Two stock PMG solutions are prepared in water. Each solution is prepared independently from a separate weighing of PMG. One is designated as a stock fortification solution. The other is

designated as a stock calibration solution. Two stock AMPA solutions are prepared and designated in a similar manner. Fortification solutions are prepared to fortify untreated (control) samples and demonstrate procedural recovery. Calibration solutions are used to calibrate the instrument. To prepare each of these stock solutions, at a nominal concentration of 1.0 mg/mL, place a known quantity (± 0.1 mg) of approximately 50 mg of active ingredient into a 4-oz narrow-mouthed bottle. Add to the bottle a known amount of water, to produce a solution of approximately 1.0 mg active ingredient/mL. Add 2 to 3 drops of concentrated hydrochloric acid as a preservative biocide. Close the bottle with a Poly-Seal cap, and mix the contents thoroughly to dissolve the analyte. Calculate the amount of water needed to produce a 1.0-mg/mL solution as follows:

$$A = \frac{(W \times P \times D)}{C(ss)}$$

Where

C(ss) = concentration of the analyte in the final solution (mg/mL)

W = weight of primary standard (mg)

P = purity of primary standard (100% = 1.00)

D = density of solvent (g/mL)

A = weight of solvent (g)

PMG and AMPA may not dissolve readily in water. Visually inspect the stock solutions carefully to ensure dissolution of the analytes.

2.3.3 Working fortification solutions. If both PMG and AMPA are to be determined, prepare working solutions by combining aliquots of the PMG and AMPA stock solutions, and diluting the combined aliquots with water. For example, to prepare a 100 µg/mL combined PMG/AMPA working fortification solution, combine 5.0 g of PMG stock fortification solution (1.0 mg/mL) and 5.0 g of AMPA stock fortification solution (1.0 mg/mL) in

a 4-oz narrow-mouthed bottle. Add 2 to 3 drops of concentrated HCL as a preservative. Dilute with water to a total weight of 50 g. Although dilution by weight is described here, dilution by volume is not precluded. The concentration of each analyte in this solution is calculated as follows:

$$C(ws) = \frac{C(ss) \times W(ss)}{W(ws)}$$

Where

C(ws) = concentration of analyte in the working standard (µg/mL)

C(ss) = concentration of analyte in the stock standard (µg/mL)

W(ss) = weight of stock solution added (g)

W(ws) = final weight of working standard (stock solutions plus diluent, g)

Serially dilute, by weight or volume, the above working solution to obtain other working fortification solutions as needed.

2.3.4 Intermediate calibration solutions. An intermediate calibration solution containing both analytes at a concentration of 100 µg/mL is prepared in the same manner, as described in section 2.3.3 for working fortification solutions. Since the derivatization method requires that the volume of sample extract or standard solution added to the derivatizing reagent remain constant, a range of concentrations of intermediate calibration solutions are required. Prepare these dilute intermediate calibration solutions by serially diluting the 100 µg/mL calibration solution described above. To quantitate a sample which has an analyte concentration of 0.05 mg/kg, and a final extract concentration equivalent to extractives from 0.02 g of soil per mL of extract, a intermediate calibration solution with an analyte concentration of 0.50 µg/mL is needed (see

Appendix C). This intermediate calibration solution will be further diluted to give one of the daily-use standards, described in section 2.3.5.

2.3.5 Daily-use calibration standards. The intermediate calibration solutions described in 2.3.4 are further diluted to produce daily-use calibration standards. Prepare these standards by diluting an aliquot of an intermediate standard to a known volume using the extracting solution (section 2.2.9), not water, as the diluent. It has been shown that PMG and AMPA have a tendency to adsorb onto glass. It is important that glass syringes are not used to prepare these standards, as carry-over can occur. To quantitate a sample with an analyte concentration of 0.05 mg/kg, and an extract concentration equivalent to extractives from 0.02 g of soil/mL, a series of daily-use standards should be prepared by diluting 0.50 and 5.0 µg/mL intermediate standards to daily-use standards with a concentration ranging from 0.0125 to 0.125 µg/mL. For example, using an Eppendorf pipet, dilute 100 µL of an intermediate standard to a volume of 4.0 mL. After derivatizing an aliquot of these daily-use standards, the final calibration standards will have concentrations ranging from 1.0 to 10.0 µg/mL. See Appendix C for examples of recommended dilutions.

3 ANALYTICAL PROCEDURE

3.1 Analyte Extraction

3.1.1 Place a 20-g subsample of a well-mixed soil sample into an 8-oz wide-mouthed bottle. Add 80 mL of extracting solution (0.25 M ammonium hydroxide/0.10 M monobasic potassium phosphate), and swirl to ensure good mixing. Shake sample for about 90 min using a mechanical shaker (100-200 strokes/min).

3.1.2 Centrifuge the extract at about 2000 rpm for 20 min. Alternately, filter the extract by passing 2 to 3 mL of

extract through a 0.45-μm disposable syringe filter. Derivatize extracts within four hours of extraction.

3.2 Analyte Derivatization

3.2.1 Prepare the derivatizing reagent in a suitable sized glass container with a PTFE-lined cap by adding 1 volume of 2,2,3,3,4,4,4-heptafluoro-1-butanol to 2 volumes of trifluoroacetic anhydride. Cap the container and shake gently. Carefully loosen the cap to release any pressure. Due to the potential for pressure build-up, do not fill the container to more than 75% of capacity. This derivatizing reagent mixture should be prepared fresh daily. The use of latex gloves when preparing and handling the reagent mixture is strongly recommended.

3.2.2 Add 1.0-mL aliquots of derivatizing reagent to 2.0-mL screw-topped autosampler vials. Cap the vials using phenolic-plastic, open-top caps with a double-thickness, PTFE septa. Chill the capped vials by placing them in an aluminum heating/cooling block and placing the block on a slab of dry ice, or in a pan containing crushed dry ice. Cool the vials to a temperature of -50 to -70 °C, as measured by a thermometer placed in the aluminum block. Prepare enough vials to derivatize each standard and sample extract in duplicate.

3.2.3 Add a 20-μL aliquot of the daily-use calibration standard or sample extract to the prechilled derivatizing reagent in the following manner. Using an Eppendorf pipet, withdraw 20 μL of extract or standard into the disposable tip. Place the pipet tip under the surface of the reagent, and slowly release the contents. Immediately rinse the pipet tip by repeatedly withdrawing reagent into the disposable pipet tip and releasing it back into the vial; always keep the pipet tip under the surface of the reagent.

After the aliquot is added to the reagent mixture, cap and manually shake the vial, and return it to the chilled

aluminum block. After all samples and standards are processed, remove the vials from the chilled block, and allow them to equilibrate to room temperature. Proceed with the derivatization reaction by placing the vials for one hour in a heating block maintained at 92 to 97 °C.

After heating, remove the vials from the heating block and allow them to cool to room temperature. Evaporate the excess derivatizing reagent and trifluoroacetic acid under a stream of nitrogen. Once apparent dryness has been achieved, the sample vials should remain under the stream of nitrogen for an additional 30 to 40 minutes. Residual derivatization reagents or trifluoroacetic acid can degrade the chromatography of the analytes.

3.2.4 Dissolve the residuum in 250 µL of ethyl acetate containing 2.0 µL citral/mL. Cap the vial, and shake to dissolve contents. Transfer contents to a crimp-top autosampler vial containing a limited volume (250 µL) insert. The volume of ethyl acetate may be increased if (1) allowed by the sensitivity of the detector; (2) the limit of quantitation required is higher than 0.05 ppm; or (3) soil residues are high. For increased ruggedness and reliability of the method, only inject dilute extracts (≤0.02 g soil/mL extract) when sensitivity requirements allow.

3.3 Fortification

If possible, analyze fortified and unfortified control samples with each sample set to demonstrate method recovery. For example, add 100 µL of working fortification solution (10 or 100 µg/mL) to separate control samples (20 g) to produce fortification levels of 0.05 or 0.50 ppm. Extract as detailed in section 3.1 above. It is recommended that one unfortified and 2 fortified control samples be analyzed with each set of 10 field samples. One of the two fortified control samples should be fortified at the method's limit of quantitation (LOQ, 0.05 ppm). Additional higher

fortification levels may be needed depending on the expected residue levels.

4 INSTRUMENTATION

Follow the manufacturer's instructions for operation of the gas chromatograph and mass-selective detector. The conditions listed below were used to generate the data and chromatograms presented in this report.

4.1 Operating Parameters-Outline

4.1.1 Gas chromatograph.

Model:	Hewlett-Packard 5880 (or 5890 Series II)
Column:	J&W DB-5.625, 30 meter, 0.25 mm i.d., 0.25-μm film-thickness
Carrier:	Helium, 7.5 lb/sq. in. at column head
Linear Velocity:	30 cm/sec, measured at 180 °C
Inlet Type:	Splitless with single piece, double-restrictor liner. Packed with fused silica wool (see section 11.5.2).
Inlet Temperature:	200 °C
MSD Interface Temp.:	270 °C

4.1.2 Oven temperature profile.

Initial Oven Temp.:	80 °C
Initial Time:	1.5 min
Program Rate:	30 °C/min
Final Oven Temp.:	260 °C
Final Time:	3 min

4.1.3 Other conditions.

Volume Injected:	5 μL
Split Valve Off:	1.0 min

Injection Solvent:	ethyl acetate (2.0 µL citral/mL added)
Total Run Time:	10.5 min

4.1.4 Mass-selective detector.

Model:	Hewlett-Packard 5970A
Software:	Pascal or UNIX ChemStation
Mode:	low resolution, selective ion monitoring (SIM)
Tuning:	manual tuned for m/z 414, 502 and 614 using perfluorotributylamine
Mass Monitored:	AMPA - m/z 446 for AMPA derivative PMG - m/z 611 for PMG derivative
Dwell:	135 msec

4.2 Mass-Selective Detector - Manual Tuning

4.2.1 Manual-tune procedure. To increase the sensitivity of the detector use an alternative to the normal "AutoTune" sequence. Using perfluorotributylamine (PFTBA) as the calibration standard, select the tuning masses m/z 414, 502 and 614 (with a scan range of m/z 300 to 650) in preparation for conducting a "manual tune". After the new masses are selected, perform an EXTENDED TUNE. Using these tuning masses usually results in a multiplier voltage 200 to 600 volts higher than would be obtained performing an AutoTune with the standard tuning masses of m/z 69, 219 and 502. After the tune is performed, manually adjust the following mass spectrometer parameters. Reduce the AMU gain to increase the bandwidth of the three tuning masses to between 2.4 and 3.0 AMU. Observe the bandwidths by turning the MSD on while in the "Edit Parameters" mode. Adjustments to the mass gain and mass offset may also be required, although the increased bandwidth helps to eliminate the need for precise adjustments in this area. Adjust the multiplier voltage to obtain an m/z 414 abundance of approximately 2,000,000. Refer to Appendix A for more details on this procedure, and the manual mass calibration procedure.

This manual tuning process has a marked effect on the response of the PMG derivative. The signal-to-noise ratio can be increased by a factor of 8 (see Appendix A, Figure A.5) over the AutoTune procedure. The effect on the AMPA derivative is less dramatic. The narrowing of the scan range causes an increase in response to larger fragments at the expense of the smaller fragments. An increase in response is also a direct result of the increase in mass bandwidth. This increase in response is a continuation of the effect that takes place when the "Low-Mass Resolution" option is selected in the SIM Acquisition parameter screen. In addition, evidence exists that there are both 611 and 612 m/z fragments resulting from the PMG derivative. The wider bandwidth obtained in this tuning procedure allows for both ions to be detected simultaneously.

4.2.2 Diagnostics. The manual-tune procedure must be done after each source cleaning or replacement. For diagnostic purposes, spectrum scans should be obtained using the current manual tune file with PFTBA. These scans should be compared to the original scan produced during the initial manual-tune procedure. The scan should be checked for adequate abundance and proper mass assignment. Spectrum scans may be done on a weekly basis, or when analyte signal-to-noise ratio is reduced. In the case of poor abundances or poor mass assignments, the manual-tune procedure should be repeated. If low abundances for the 3 tuning ions (<500,000) are obtained after manual tuning, a source cleaning may be required.

4.2.3 MSD source cleaning. Because this method relies on the detection of high mass fragments (up to 612 m/z) analyte detectability is very dependent on the condition of the MSD source unit. With heavy use, source cleaning may be required more frequently than required with other methods. During normal use, a slow reduction in the signal-to-noise ratio can be expected due to a deterioration in the condition of the source. However, abrupt changes in the signal-to-noise ratio may not be related to the condition of the source but to

chromatographic problems instead. Due to the sensitivity and time-consuming nature of source removal, all chromatographic-related problems should be eliminated prior to conducting a source cleaning.

4.3 Calibration and Analysis

Calibrate the gas chromatograph by using the daily-use calibration standards prepared in section 2.3.5 and derivatized in section 3.2. For a sample set where soil extracts are equivalent to extractives from 0.020 g of soil/mL, and where the controls have been fortified at 0.05 and 0.50 mg/kg, daily-use calibration standards at 1.0, 2.5, and 10.0 ng/mL are suggested. Prepare all standards and samples in duplicate. Make single injections from each prepared vial.

A suggested analytical scheme could include injections in the following order:

1. Replicate injections (3 - 5) of the extract of a fortified control sample in order to equilibrate the column.
2. Replicate low-level standards to assure constant response.
3. Ethyl acetate blank.
4. Control (injection of both controls not required).
5. Low-level standard.
6. Low-level fortified control sample extracts.
7. Low-level standard.
8. High-level standard.
9. High-level fortified control sample extracts.
10. High-level standard.
11. Low-level standard.
12. Sample extracts.
13. Low-level standard.
14. High-level standard.

Standards should be injected after analysis of every 3 to 5 samples (step 12). Quantitation is based on the response of the standard closest in concentration to the sample extracts. The average response of the standards bracketing the sample response of interest is used.

5 CALCULATIONS

The concentration of either analyte in the original sample is calculated by using the external standard method; i.e., the average response obtained for the analyte in the sample extract is compared to the response obtained for separate injections of a known amount of analyte (calibration solution). To use the calculations shown below, the injection volumes for both the calibration solution and sample extract must be fixed at the same volume. The standard with the average response (usually peak height) closest to that of the samples of interest is used for calculating the concentrations of the sample. The average response of the standard injections made before and after the sample injections of interest can be used to determine the calibration factor for the sample.

5.1 Linear Response Calculation Method

5.1.1 Calibration factor. Calculate the response factor, F, for injection of a calibration solution as follows:

$$F = \frac{C}{\bar{R}}$$

Where

F = response factor

C = concentration of calibration solution, µg/mL

R = average response units (e.g., peak height, peak area) from detector for calibration solution

5.1.2 Soil in extract. Calculate the concentration of the soil; i.e., the amount of soil the extract represents, as follows:

$$C = \frac{W\ (\text{sample})}{V\ (\text{solvent})} \times \frac{V\ (\text{aliquot})}{V\ (\text{final})}$$

Where

C = concentration of soil (g/mL)

W (sample) = weight of soil extracted (g)

V (solvent) = volume of extracting solvent used (mL)

V (aliquot) = volume of extract aliquot subjected to derivatization (section 3.2.3)

V (final) = final volume used to dissolve residual material (section 3.2.4)

5.1.3 Analyte in sample. Calculate the analyte concentration, R, in the original sample as follows:

$$A = \frac{F \times R}{C}$$

Where

A = concentration of analyte in original sample (µg/g or ppm)

F = response factor, (µg/mL)/response unit (section 5.1.1)

R = average sample response unit from detector for sample

C = concentration of soil in final extract, g/mL (section 5.1.2)

5.2 Nonlinear Response Calculation Methods

For detector responses that significantly deviate from linearity, the following curve fit equations may be used to calculate extract concentrations (any valid curve fitting program may be used).

5.2.1 Calculation of extract concentration. Second-order. polynomial curve fit: $Y = AX^2 + BX + C$, or power curve fit: $Y = BX^m$

Where

Y = concentration of analyte in extract

X = detector response

A, B, C, M = constants

5.2.2 Calculation of analyte in sample. Calculate the analyte concentration, R, in the original sample as follows:

A (μg/g or ppm) = Y / C

Where

Y = calculated extract concentration from the curve fit equation (μg/mL)

C = soil concentration in extract, from section 5.1.2 (g/mL).

6 MATRIX EFFECT

The composition and characteristics of the soil extract matrix can affect several aspects of the analysis. These include, but are not limited to, the derivatization efficiency, and the gas chromatographic behavior of the analytes. In order to determine the magnitude of these effects, the following procedures can be employed.

6.1 Derivatized Matrix Standard

A derivatized matrix standard (DMS) is primarily used to determine whether there is a variation in derivatization efficiency caused by the matrix. The DMS is prepared by adding a known amount of analyte to a known amount of control extract (obtained in section 3.1.2). This standard is then derivatized as usual. This procedure subjects an exact amount of analyte to the derivatization process in the presence of the extract matrix. A response is obtained that is independent of the extraction process and is primarily a

function of the derivatization efficiency. Prepare this matrix standard at a level equivalent to 0.50 mg/kg (final analyte concentration = 0.01 µg/mL).

6.2 Fortified-Control Extracts

A fortified-control extract (FCE) can be prepared to determine the effect of the matrix on the chromatographic behavior of the analytes. In this case, a derivatized control extract (obtained in section 3.2.4) is fortified with a high-level derivatized standard (usually 10:1, vol/vol, control:standard) just prior to injection. The response from the FCE is compared to that obtained from a similarly prepared nonmatrix standard. The nonmatrix standard should be prepared by diluting the high-level standard with a derivatized reagent blank extract. The FCE is usually prepared and analyzed when differences are noted in the chromatographic behavior of analytes in standards versus sample extracts. Examples of the such behavior are shifts in retention time, changes in peak shape (broadening or tailing), or differing responses.

When small differences exist in peak shape that can be attributed to a matrix effect, calculation using peak areas may be appropriate.

6.3 Matrix Effects - Inlet System Reliability

The derivatized extract matrix has a number of effects on the inlet liner, and column. These effects are not well understood, but may relate to the residual absorptivity of the column and how the matrix and analytes can affect the column, or be affected by it. For example, in section 4.3, step 1 suggests the injection of a fortified control sample extract in order to equilibrate the column. This is an especially significant requirement when a new inlet liner has been installed. It has been noted that the response of standards is increased after several injections of sample extracts, controls, or fortified controls. Since it is not

known whether matrix components or the analytes themselves are binding to adsorptive sites, it is recommended that injections of high-level fortified controls are made prior to any other injections.

Depending on the nature of the extracts analyzed, the useful life of the inlet liner and column inlet (1 to 3 ft) can be limited. From 20 to 200 injections usually can be made before the chromatography deteriorates. The number of injections that can be made before these procedures are necessary may be directly related to the equivalent soil concentration of the soil extract. For best results., use extracts with a soil concentration of $\leq$0.02 g/mL.

It is recommended that each analytical set starts with a new inlet liner and the removal of 1 to 3 feet of the column. Initial equilibration, as described above and in section 4.3 is still required after inlet replacement.

7 INTERFERENCES

Extracts of control samples from untreated plots showed that no significant coextractive impurities interfered with the analyte peaks. However, extractives from soils and reagents can result in interferences with retention times coincident with or near that of the analytes.

Satisfactory selectivity can usually be achieved with the use of an alternate ion(s) (see section 8). If the use of alternate ions is unsuccessful, oven temperature manipulation may be used to separate the analytes from the interference.

7.1 Determination of Interference Sources

Extracts from untreated plots should always be analyzed to demonstrate the absence of significant interferences. If significant interferences are detected in the untreated control, two types of reagent blanks can be analyzed to determine the source of the interference or confirm its

origination in the untreated control. The first reagent blank consists of a 20-µL aliquot of deionized water added to the derivatization reagent mixture (see section 3.2.3). The second reagent blank consists of an aliquot of the extraction solution added to the reagent mixture. Comparison of the results from two reagent blanks may help locate the source of the interference. Refer also to section 11.5.4 for a discussion on the elimination of ghost peaks.

8 CONFIRMATORY TECHNIQUES

Unexpected positive results, as in untreated controls or preapplication samples, should be confirmed by other means. Confirmation can be achieved by quantitation using a different m/z ion, and comparing ratios of two or more ions. Ion ratios should be confirmed using a standard run on the same instrument. Alternate m/z ions for the derivatives of AMPA and PMG are given below.

AMPA Derivative	
Ion (m/z)	Abundance*
126	99
247	19
372	51
446	100
502	32

PMG Derivative	
Ion (m/z)	Abundance
213	100
338	31
584	25
611	34
612	37

* approximate percent relative abundance

In general, best results are obtained using the higher mass fragment, due to the improved signal-to-noise ratio. When using a manual tune program, use care to correctly identify alternate ions. The manual tune parameters may shift ion masses slightly. See Appendix A for a description of mass calibration procedures.

The use of a second, confirmatory column with a different stationary phase may also be used to confirm the presence of the analytes, or to distinguish them from interferences. The

fortification of treated samples with a known amount of derivatized standard may be helpful in distinguishing the analytes from interfering compounds.

9 DISCUSSION

9.1 Scope

This method is suitable for the determination of AMPA and PMG in a variety of soil types. However, due to the nature of some soils, variations in the method may be needed to maintain the accuracy and precision of the method. Recovery data given in Table I reflect the methodology described herein.

9.2 Precision and Accuracy

Fortified soil samples were prepared as described in section 3.3, and analyzed according to this method to establish recovery. Recoveries of PMG from soil fortified at 0.05 and 0.50 mg/kg ranged from 86 to 100%, with a mean recovery of 95% (n = 6), and coefficient of variation (CV) of 5.9%. Recoveries of AMPA from soil fortified at 0.05 and 0.50 mg/kg ranged from 92 to 107%, with a mean recovery of 98% (n = 6), and a CV of 6.3%. Table I lists the recoveries obtained from the soil type used for the fortifications. Table IV lists the properties of the soil.

The precision of the method depends on variations in extraction, derivatization and instrumental analysis. These variations can be evaluated from the data obtained during analysis of fortified samples. The coefficients of variation given in Table I are a measure of precision.

9.3 Detection Limit

The detection limit for a specific analyte in a specific soil is based on the minimum detectability of the analyte, and the soil concentration in the extract. The minimum detectable

concentration has been established as a response large enough that a 25% change can be distinguished. Also required is a signal-to-noise ratio of at least 10. The detection limit for a specific soil is obtained by dividing the minimum detectable concentration by the soil concentration in the extract.

9.4 Lower Limit of Quantitation (LOQ)

The lower limit of quantitation is defined as the lowest concentration at which a method has been verified. It may differ from the detection limit. Due to the variability in instrumental performance this value may vary and exhibit some inter-laboratory variation. LOQ values of 0.05 mg/kg for PMG and AMPA were obtained from work conducted for this report. Instrumental performance has allowed detection limit values of 0.01 mg/kg for both PMG and AMPA, but instrumental variability makes this performance level difficult to maintain.

9.5 Matrix Effects

The absence of chromatographic matrix effects was verified by the analysis of fortified control extracts, as described in section 5.2. Results are listed in Table II. Sample chromatograms are included in Figures 1 through 3.

9.6 Alternate Ion Analysis

Three major fragments (372, 446, and 502 m/z) can be used for the quantitation of AMPA, while two major fragments (584 and 611/612) exist for the quantitation of PMG. While 446 and 611 provide the greatest response for AMPA and PMG, respectively, the abundances of the alternate ions is high enough for accurate quantitation. In this work, soil was fortified at 0.05 mg/kg and analyzed while monitoring the 446, 372, and 502 m/z fragments for AMPA; and the 611 and 584 m/z fragments for PMG.

In all cases, the MSD response was sufficient to allow accurate quantitation at the limit of quantitation (0.05 mg/kg). See Figures 1 through 4 for chromatographic profiles.

9.7 HPLC Method Comparison

To compare method performance to another accepted method, aged soil samples previously analyzed using HPLC method RR 85-34R (reference 1) were reanalyzed using this method. Two soil samples previously analyzed in duplicate by HPLC were again analyzed in duplicate by GC-MSD. The amounts of PMG found by the GC/MSD method averaged 90% of the amount determined by the HPLC method (reference 2). The amount of AMPA found by the GC/MSD method averaged 106% of the amount determined by the HPLC method. See Table III for the method comparison data.

9.8 Dry-Weight Basis

This method determines the residues of AMPA and PMG in soil on an as-received basis. If it is desired to express the values on a dry-weight basis, compensation is necessary for water present in the sample.

9.9 Extraction Efficiency

The extraction efficiency for both analytes using the solvents given in this method was tested by analyzing fortified soils. The results are listed in Table 1.

9.10 Safety Precautions

Personnel untrained in the routine safe handling of chemicals and good laboratory practices must not attempt to use this procedure. Information on any specific chemical regarding physical properties, hazards, toxicity, and first-aid procedures can be found on the Material Safety Data Sheet accompanying the chemical, available from the supplier, or

from the GLP archive. In general, always wear safety glasses with side shields, work in a well ventilated area, avoid inhaling vapors, and avoid contact of the chemicals with skin and clothing. Flammable solvents should always be kept away from potential sources of ignition. Latex gloves are strongly recommended when handling trifluoracetic anhydride.

9.10.1 Flammable solvents. Ethyl acetate and trifluoroacetic anhydride are flammable.

9.10.2 Pressure buildup. Mixing trifluoracetic anhydride and heptafluorobutanol can generate heat and pressure in a closed container. When combining these reagents use care in mixing and venting of the container. Trifluoroacetic anhydride also reacts violently with water.

9.10.3 Chemical sensitivity. The use of the derivatization reagent mixture has been linked to an allergic reaction in some personnel. The response consists of an initial itching followed by a skin rash. The post-derivative evaporation step in section 3.2.3 appears to cause the most problems. It is essential to exercise all precautions to avoid contact with the derivatizing agents.

10 CONCLUSION

The method is specific for the analysis of AMPA and PMG residues in soil. Only commercially available laboratory equipment and reagents are required. The analysis can be completed by one person in an 8-hr period if an adequately homogenized sample is available. When possible, untreated and fortified samples should be extracted and analyzed with each set of samples to demonstrate absence of interferences and adequate recovery. If determination of AMPA and PMG residues at a concentration other than 0.05 and 0.50 ppm is required, suitably fortified samples must be analyzed to validate the method at that concentration.

This method may be extended to other soil types if a proper validation is conducted. Validation should include analysis of control and fortified samples to ensure the absence of interferences and adequate recovery. Samples should be fortified at the LOQ and 10 times the LOQ. The absence of significant matrix effects should be demonstrated by the analysis of fortified control extracts.

11 METHOD NOTES

11.1 Extraction Efficiency

The extraction solvent described in section 3.1.1 provides adequate recovery of PMG and AMPA for many soil types. Some soils may require a stronger solvent. Strong solute binding may be related to high clay content of the soil. Increasing the molarity of the ammonium hydroxide may increase recovery. Increasing the concentration of the phosphate ion is less effective. However, a minimum of 0.05 M phosphate is required when using ammonium hydroxide-based solutions. An ammonium hydroxide solution without added phosphate ion provides little or no recovery of PMG. Phosphate ion appears to compete with the phosphonic acid moiety of the analytes for the binding sites within the soil. However, to improve the chromatographic reliability of the method, avoid high molarity solutions whenever possible, as the minimization of coextractives can improve reliability. Higher concentrations of phosphate may produce insoluble residues upon evaporation of the derivatizing reagents. This material may be removed by filtration or centrifugation.

11.2 Derivatization

11.2.1 Direct derivatization of aqueous extracts. The standard practice for acetylation and/or esterification using trifluoroacetic anhydride and heptafluorobutanol is to evaporate aqueous samples to dryness and then dissolve the residuum in the derivatizing reagents. However, contact of aqueous solutions with glass can cause adsorption of PMG and

AMPA on the glass surface. When an evaporation step is employed this adsorption phenomenon can be more pronounced and result in low recoveries of the PMG and AMPA derivatives. Recoveries from dried soil extracts can be lower still. To prevent losses due to analyte adsorption, drying of the aqueous extracts is eliminated in this procedure. Instead, a small aliquot (20 µL) of extract is added directly to the derivatization reagent mixture.

The addition of water to trifluoroacetic anhydride at ambient temperature can cause a violent, exothermic reaction. Addition to a premixed solution of the heptafluorobutyl alcohol and trifluoroacetic anhydride reduces some of the danger. Addition to a chilled mixed reagent further reduces this risk. No more than 40 µL of aqueous sample or standard should be added to 1.0 mL of reagent. The described procedure (section 3.2.3) of sample addition and pipet tip rinsing ensures quantitative transfer of the sample aliquot. The procedure also ensures good initial mixing of the sample and reagent. Good mixing is essential for uniform heat dissipation and analyte derivatization.

11.2.2 Syringe carry-over. As discussed in section 11.2.1 contact of aqueous solutions with glass can result in adsorption of PMG and AMPA onto the glass surface. The effect on sample analysis is especially pronounced with dilute solutions involving high glass surface area to volume ratios. To avoid carry-over, eliminate the use of glass pipets and syringes. This is especially critical if using the same syringe for standard solutions of widely different concentrations, or in the preparation of controls. The use of an Eppendorf pipet, as described in section 3.2.3, eliminates the possibility of syringe carry-over. Derivatives of PMG and AMPA are not subject to adsorption and may be handled with glass syringes.

The use of volumetric pipets should be avoided when preparing fortification and calibration standards. It has been shown that pipets used for diluting high concentration standards cannot be adequately cleaned and then reused for diluting

daily-use standards. For this reason the preparation of standards using dilutions by weight, rather than volume is recommended.

11.2.3 Corrosivity of derivatizing reagent. The combination of the corrosive nature of the derivatizing reagent and the high temperature at which the reaction takes place requires that special attention be taken in the selection and use of the derivatization vial. Single-layer, PTFE-lined septa have been known to deteriorate and leak. Double-thickness PTFE septa should be used (see section 2.1.14). Open-top caps made with nonphenolic plastic should not be used since they are softer and have become loose during derivatization. Caps with punctured septa should not be used. Solid-top caps with PTFE liners may be used, but are not as cost effective as the vial/cap system described here.

11.3 Solution Stability

Aqueous extracts should be derivatized within four hours of extraction. Stock and intermediate standards have an expiration date of one year if stored at a temperature of less than 5 °C. Daily-use standards (underivatized) should be derivatized within 4 hours of dilution. Derivatives of AMPA and PMG are stable in ethyl acetate. Care should be taken to avoid evaporation of solvent. Although derivatized extracts can be stored indefinitely, they should only be quantitated with standards prepared in the same analytical set. Ethyl acetate/citral solutions should be given an expiration date of one month.

11.4 Matrix Considerations

11.4.1 Matrix modification - citral addition. Citral (3,7-dimethyl-2,6-octadienal) is added to the injection solvent at a concentration of 2.0 µL/mL of ethyl acetate. The addition of citral helps decrease losses due to analyte adsorption and increase peak symmetry. Because citral is a labile compound

susceptible to air oxidation, only fresh material should be used.

11.4.2 Nonsoil matrix considerations. Considerations should be given to the components of the final extract other than those contributed by the soil matrix. The amounts of water, ammonium hydroxide, and phosphate that are derivatized make significant, chromatographic-related contributions to the final extract. In order to keep the final concentration of these components constant for both the standards and the sample extracts, the following guidelines are offered.

1. Daily-use calibration standards must be prepared by diluting intermediate calibration standards with the extracting solution.

2. The concentration of the injected standards should be determined by varying the concentration of the daily-use standards, not by varying the aliquot volume of the daily-use standard added to the derivatization mixture. The volume of the standard or sample aliquot to be derivatized must be kept constant for the entire analysis sample set.

3. The amount of ethyl acetate (containing 2.0 µL citral/mL) used to dissolve the residuum in section 3.2.4 must be kept constant for all samples and standards within the sample set.

4. It is preferred that individual samples be diluted with derivatized control or reagent blank extract in order to bring any unexpectedly high residue results within the calibration range. Retention time to height/area ratios should be monitored to ensure consistency with standards.

11.5 Operational Parameters

This method relies on high sensitivity (i.e., low minimum detectability) and selectivity to offset the limitations imposed by the method's direct derivatization of small aliquots of aqueous soil extracts. Inherent to this method is the need for modifications of standard practices and inclusion of some nonstandard practices involving sample introduction, MSD tuning, and matrix modification.

11.5.1 Injection volume. An injection volume of 5 µL is used as a direct method for increasing sensitivity. Optimal chromatographic bandwidths can usually be maintained with this volume if other considerations are made, including inlet liner, column inlet, and oven temperature profile. In some cases the final matrix may contribute to excessive peak broadening or splitting. Diluting the extracts with the ethyl acetate/citral solution, or reducing the injection volume is sometimes helpful. If analyte detectability allows, use an injection volume less than 5 µL.

11.5.2 Inlet liner. A single-piece, double-restrictor inlet liner is used (Restek #20784). This liner has the advantage of an increased internal volume, when compared to two-piece types. The greater volume allows for retention of more solvent/sample vapor within the liner and subsequent introduction onto the column. Liner volume can be a limiting factor when the injected volume is greater than 2 µL. Serious chromatographic difficulties have been traced to the use of single-restrictor or straight-tube inlet liners.

Fused-silica wool is used to pack the liner. Packing of the single piece liner can be accomplished using the pulling end of a puller/inserter tool (Restek #20114). Fused-silica wool is recommended over glass wool due to it's inherent inertness. Pack the wool to a moderate density in a 15-mm plug at the center of the liner. Install the inlet end of the retention gap column so that the column end approaches the wool packing. This can be achieved by extending the

column end about 23-25 mm from the tip of the column inlet connection ferrule.

11.5.3 Oven-temperature profile. The use of 5-µL injection volumes requires special considerations be given the oven-temperature profile and injection solvent. In general, the initial oven temperature at which the solvent is allowed to recondense within the retention gap should be at least 100 °C less than the analyte elution temperature. At an initial temperature between 60 and 85 °C, the analytes are trapped at the head on the column. The analytes will continue to remain focused at the inlet of the analytical column while the solvent passes through. After the solvent has completely evaporated the oven temperature can be increased and the analytes eluted at 180 to 200 °C.

The oven-temperature profile described in section 4.1.2 outlines a program that yielded the chromatograms given in this report. At other times however, the described profile gave poor results and a second intermediate temperature ramp was required. The exact mechanism of the solute band refocusing is not fully understood. Since solute band refocusing can be achieved using initial temperatures both below and above the boiling point of ethyl acetate, it is not clear whether cold trapping or solvent trapping is occurring. Some experimentation may be required to obtain optimum chromatography and the level of sensitivity required. A typical alternative to the profile given in section 4.1.2 is:

Initial Temp.:	60 °C
Initial Time:	1.5 min
1st Rate:	70 °C/min (or ballistic) to 90 °C
Hold Time:	2.0 min at 90 °C
2nd Rate:	30 °C/min to 280 °C
Hold Time:	3.0 min

It should be noted that the oven temperature profile can have a great effect on the overall analyte response. Evaluation

of the chromatographic performance should not be based solely on peak shape. Minor adjustments in the temperature profile can result in major changes in analyte response.

Adequate sensitivity may rely on maintaining narrow chromatographic bandwidths. Conditions given in this report routinely resulted in final peak widths (PW at 1/2 height) of 0.80 to 1.2 seconds.

11.5.4 Injection port temperature. Increasing the injection port temperature to above 200 °C may increase the deleterious effect of the sample matrix on the column inlet. The higher temperature may also increase the degradation of these coextractive compounds within the inlet liner to more polar, adsorptive compounds. However, lower injection port temperatures may produce "ghost peaks" in subsequent injections. In general, select the lowest temperature at which "ghost peaks" do not occur. Test for "ghost peaks" by making two injections of a high level (0.50 mg/kg) fortified control extract, followed by two injections of an unfortified control extract. Lower injection port temperatures may also have the effect of minimizing solvent vapor expansion within the liner, and may slightly increase the amount of sample transferred to the column. (Note: injection port temperatures in excess of 320 °C have been used with no apparent analyte degradation.)

11.5.5 Post-study method modifications. In work conducted after this method validation study, minor modifications were developed which were found to improve the ruggedness and reliability of the method. These modifications are incorporated in the method given in this report. However, the data presented in Table I was obtained using the following parameters:

1. A retention gap column (2 to 5 m, 0.25 mm i.d.) was placed between the inlet and the analytical column. The carrier gas head pressure was 15 psi. The inlet temperature was 230 °C. The initial oven temperature

of 85 °C was held for 3 minutes. The final oven temperature was 280 ° was held for 3 minutes.

2. The pre-chilling of the derivatization reagents was conducted at a temperature of < -10 °C.

3. The derivatization vials were left in a stream of nitrogen for an additional 10 minutes after apparent dryness was reached.

By changing these parameters to those given in the method, the method is made more rugged. The method as presented in this report has been used in other GLP studies. As an example, the procedural recoveries obtained during the analysis of samples from study GLYP-91-SD-02 (report RR 92-074B) are given in Appendix D.

12 TABLES AND FIGURES

Table I. Recovery of PMG and AMPA from Soil

Trial No.	Sample No.	Amount Added, Each Analyte [mg/kg]	Amount PMG Found [%]	Amount AMPA Found [%]
99-CA-92-4017	H4017-04	0.05	91	92
	H4017-05	0.05	100	94
	H4017-06	0.05	94	97
	H4017-07	0.50	98	107
	H4017-08	0.50	100	105
	H4017-09	0.50	86	95
		Average	95	98
		CV	5.9	6.3

Table II. Recovery of PMG and AMPA from Fortified Control Extracts (Determination of Chromatographic Matrix Effect)

Trial No.	Sample No.	Amount Added, Each Analyte [mg/kg]	Amount PMG Found [%]	Amount AMPA Found [%]
99-CA-92-4017	H4017-10	0.05	98	98
	H4017-11	0.05	103	96
		Average	101	97

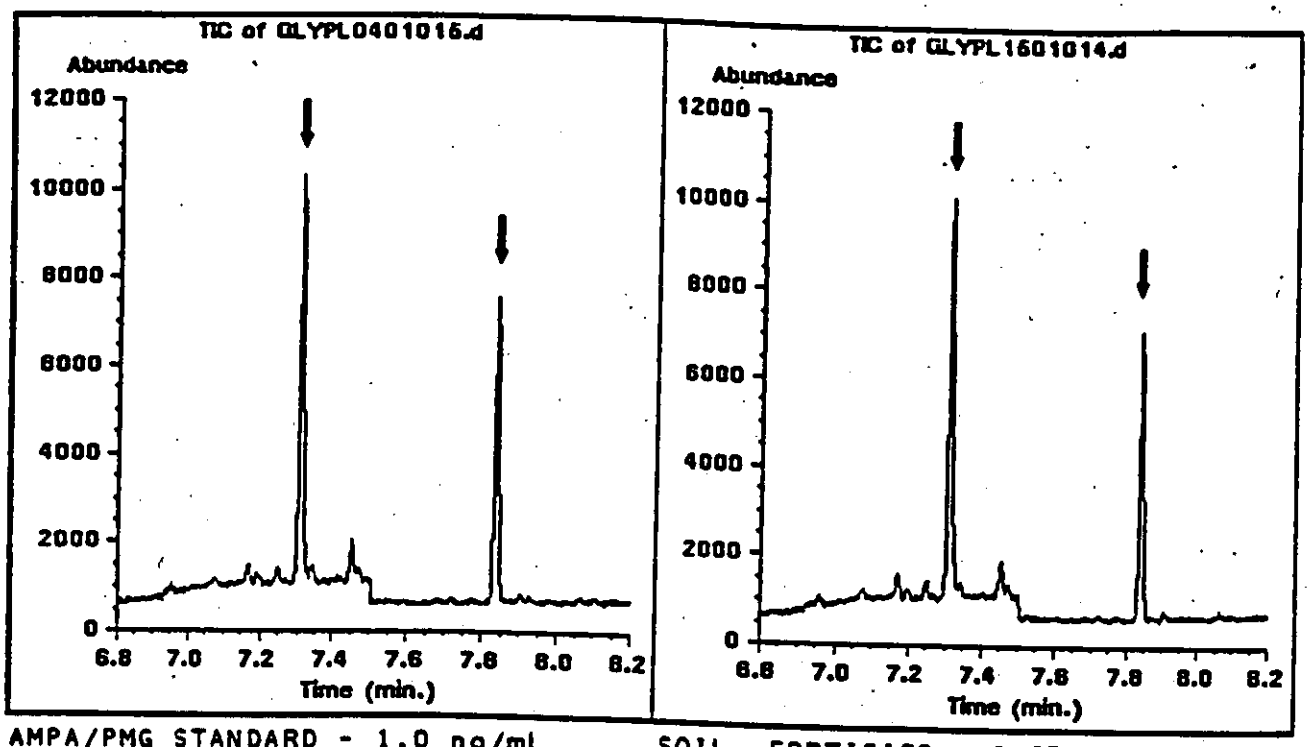
Table III. Method Comparison: GC-MSD versus HPLC

	Amount PMG [mg/kg]			Amount AMPA [mg/kg]		
Sample No.	HPLC	GC-MSD	% Difference	HPLC	GC-MSD	% Difference
D147-61	1.2	1.21	0.8	0.50	0.54	8.0
D147-61D	1.4	1.26	-10.0	0.59	0.60	1.7
D147-73	0.76	0.65	-14.5	0.80	0.82	2.5
D147-7D	0.80	0.68	-15.0	0.80	0.89	11.2
Average			-9.7			5.9

Table IV. Soil Characteristics

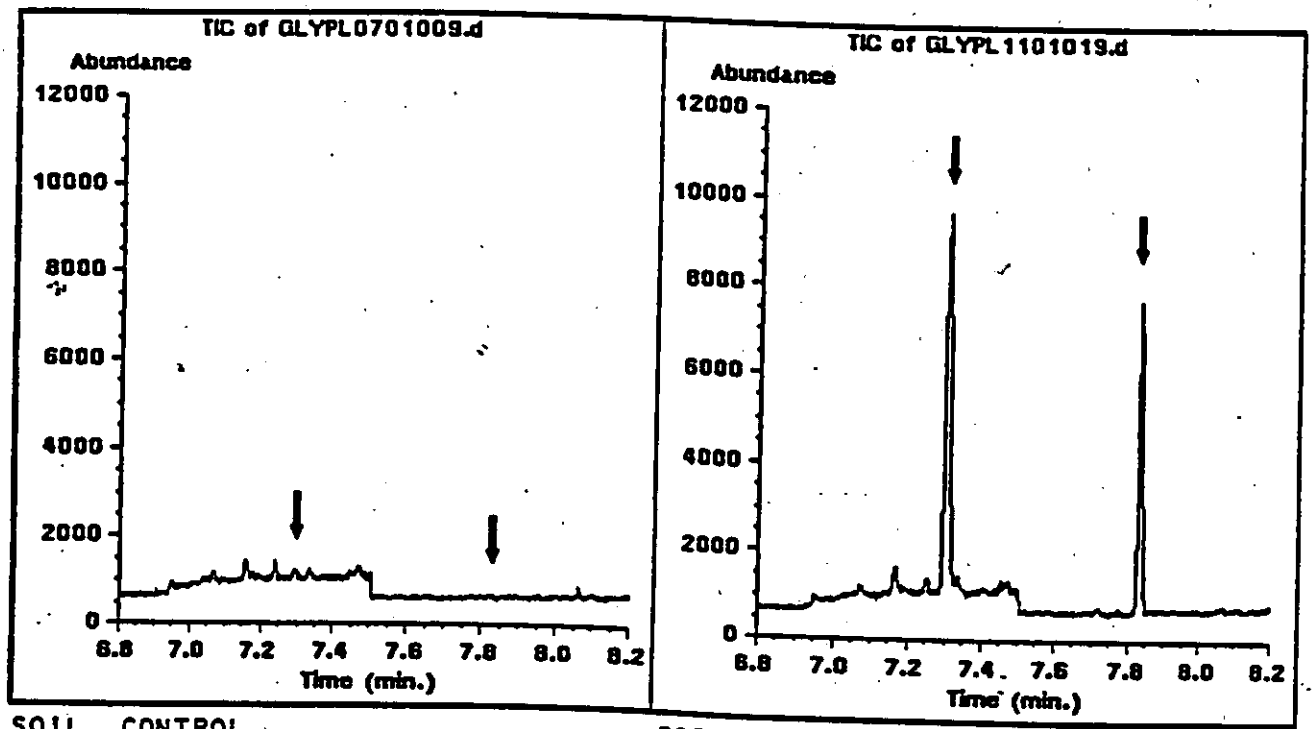
Textural Class:	sandy loam
Soil Origin:	Othello, WA
Soil Depth:	4 - 7 inches
Organic Matter:	0.6%
Cation Exchange:	7.8 meq/100 g
pH:	8.0
Sand:	66%
Silt:	29%
Clay:	6%

Figure 1. Sample chromatograms - soil fortified at 0.05 mg/kg (masses monitored: 445.5 and 611.25 m/z)



AMPA/PMG STANDARD - 1.0 ng/mL
Rt: AMPA-7.30, PMG-7.83 min.

SOIL, FORTIFIED - 0.05 ug/g
H4017-05 0.02 g/mL

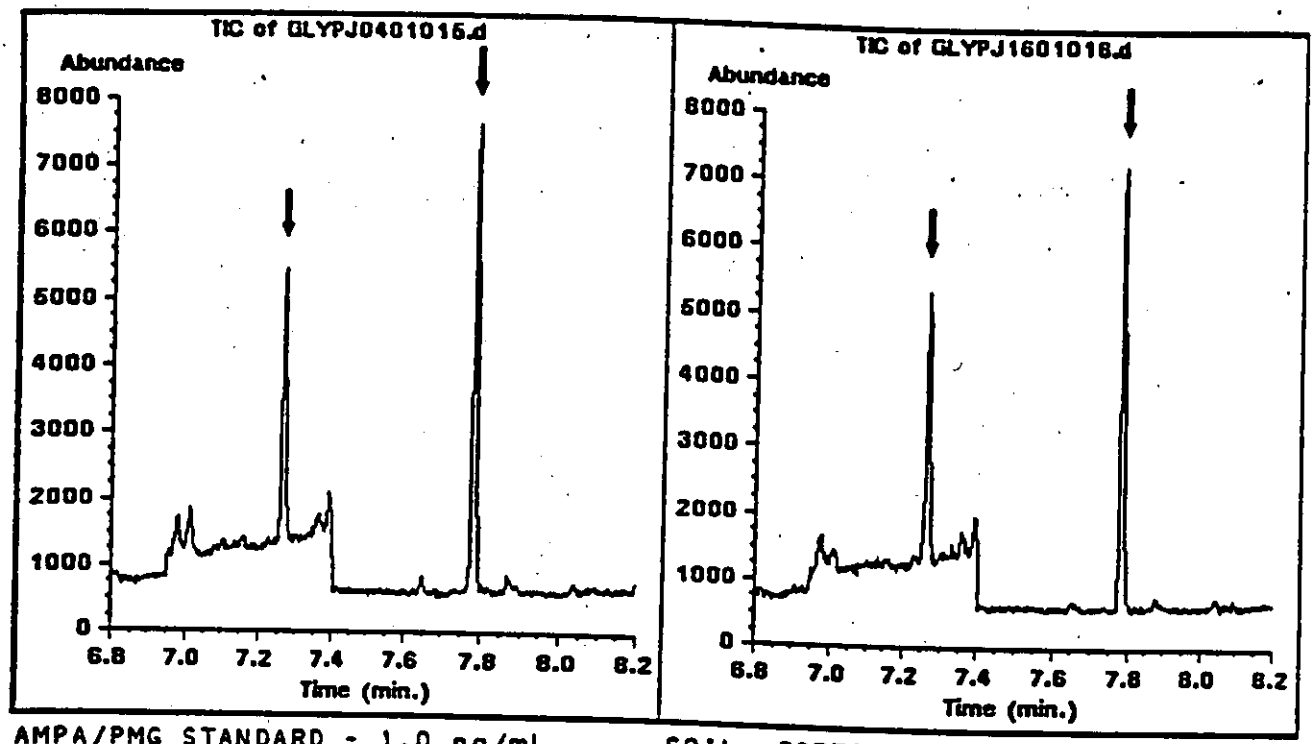


SOIL, CONTROL
H4017-02 0.02 g/mL

FORTIFIED CONTROL EXTRACT-0.05 ug/g
H4017-11 0.02 g/mL

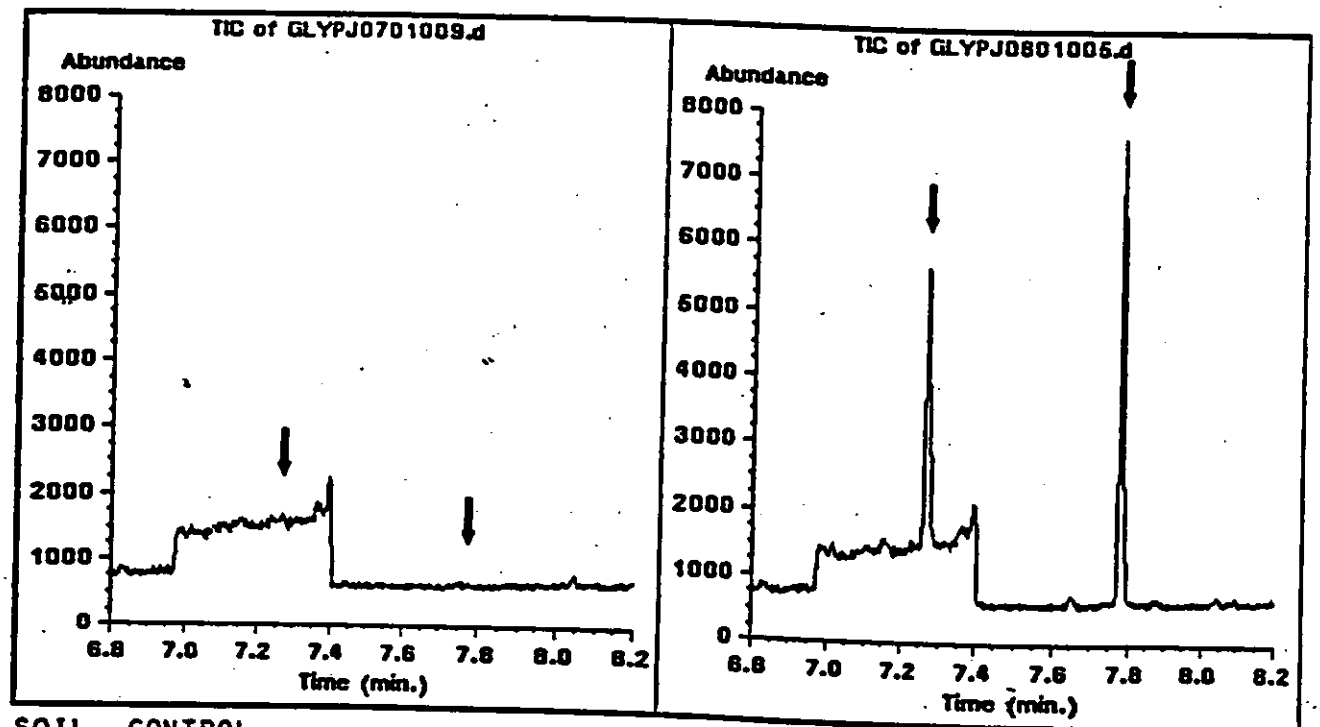
Note: AMPA monitored at 445.5 m/z, PMG monitored at 611.25 m/z.

Figure 2. Sample chromatograms - soil fortified at 0.05 mg/kg (masses monitored: 372 and 611.25 m/z)



AMPA/PMG STANDARD - 1.0 ng/mL
Rt: AMPA-7.27, PMG-7.77 min.

SOIL, FORTIFIED - 0.05 ug/g
H4017-06 0.02 g/mL

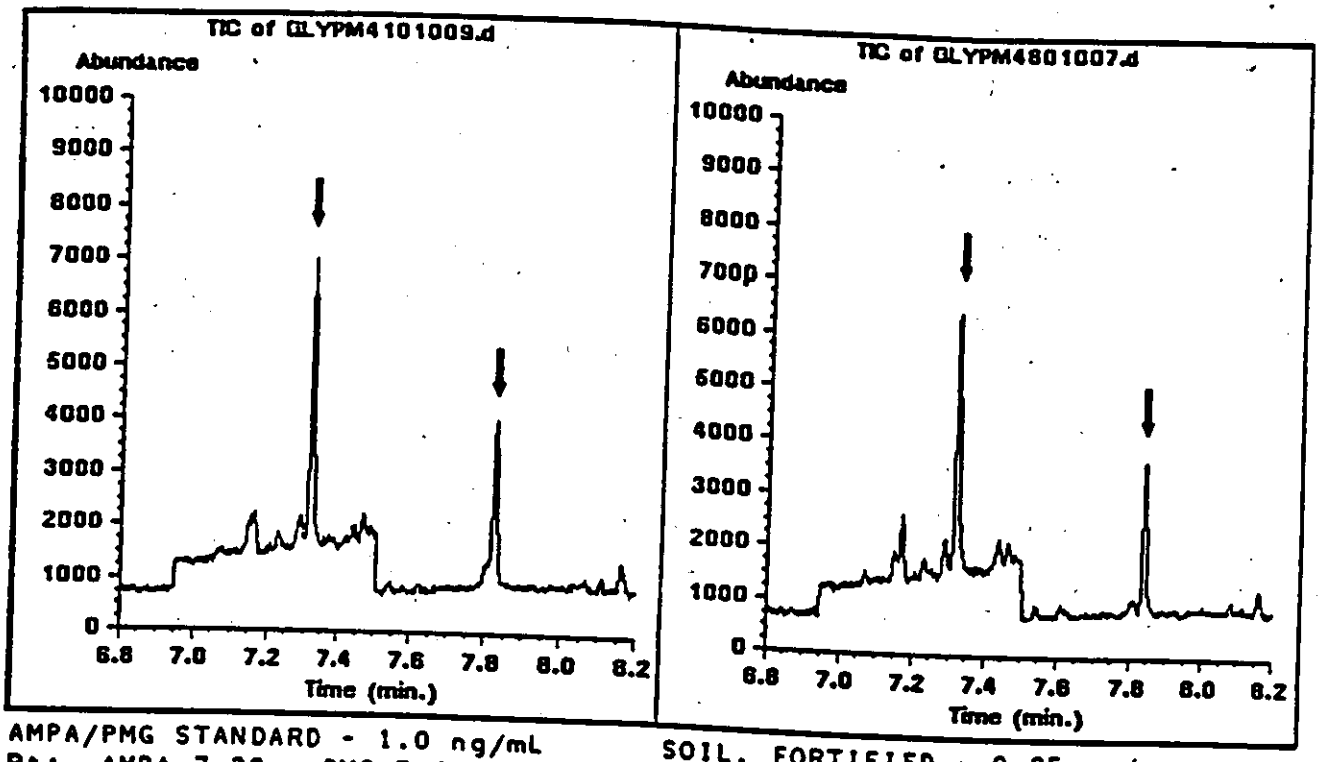


SOIL, CONTROL
H4017-03 0.02 g/mL

FORTIFIED CONTROL EXTRACT-0.05 ug/g
H4017-10 0.02 g/mL

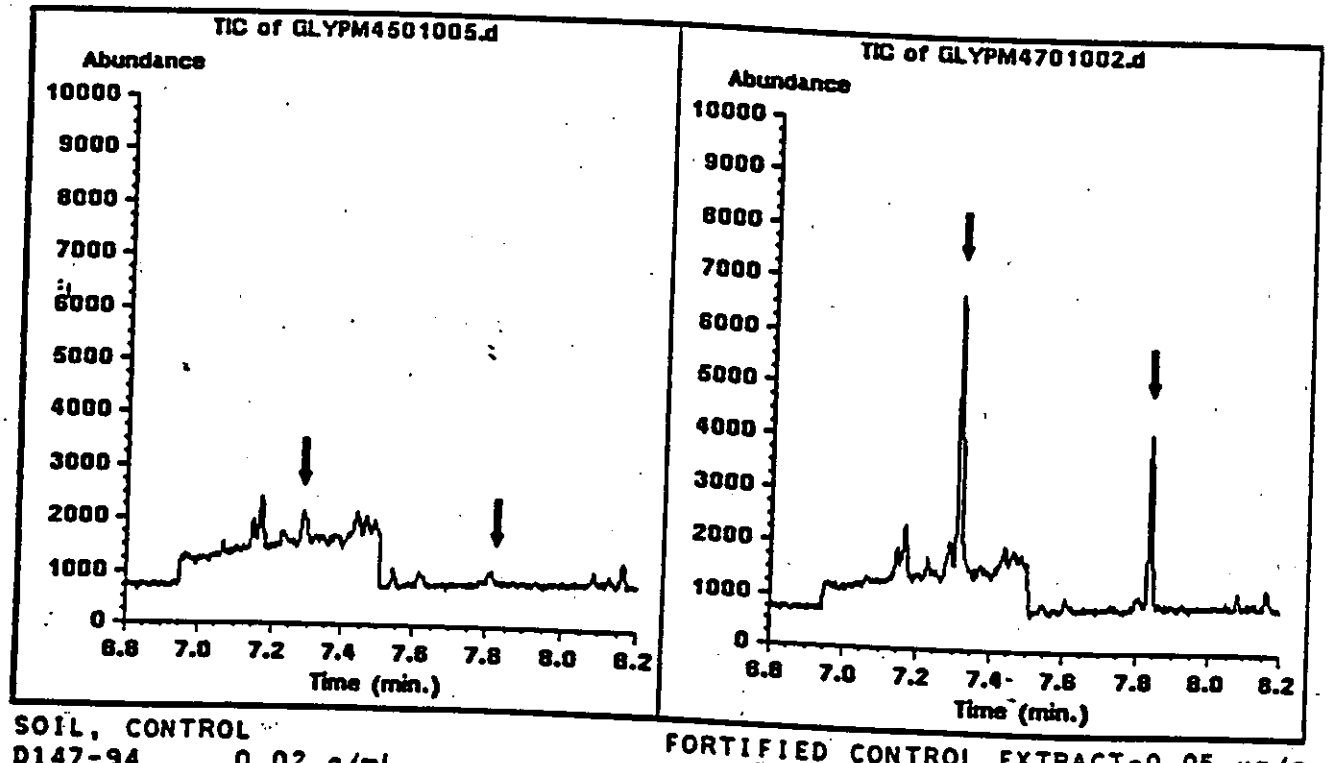
Note: AMPA monitored at 372 m/z, PMG monitored at 611.25 m/z.

Figure 3. Sample chromatograms - soil fortified at 0.05 mg/kg (masses monitored: 502 and 584 m/z)



AMPA/PMG STANDARD - 1.0 ng/mL
Rt: AMPA-7.30, PMG-7.83 min.

SOIL, FORTIFIED - 0.05 ug/g
D147-94 0.02 g/mL

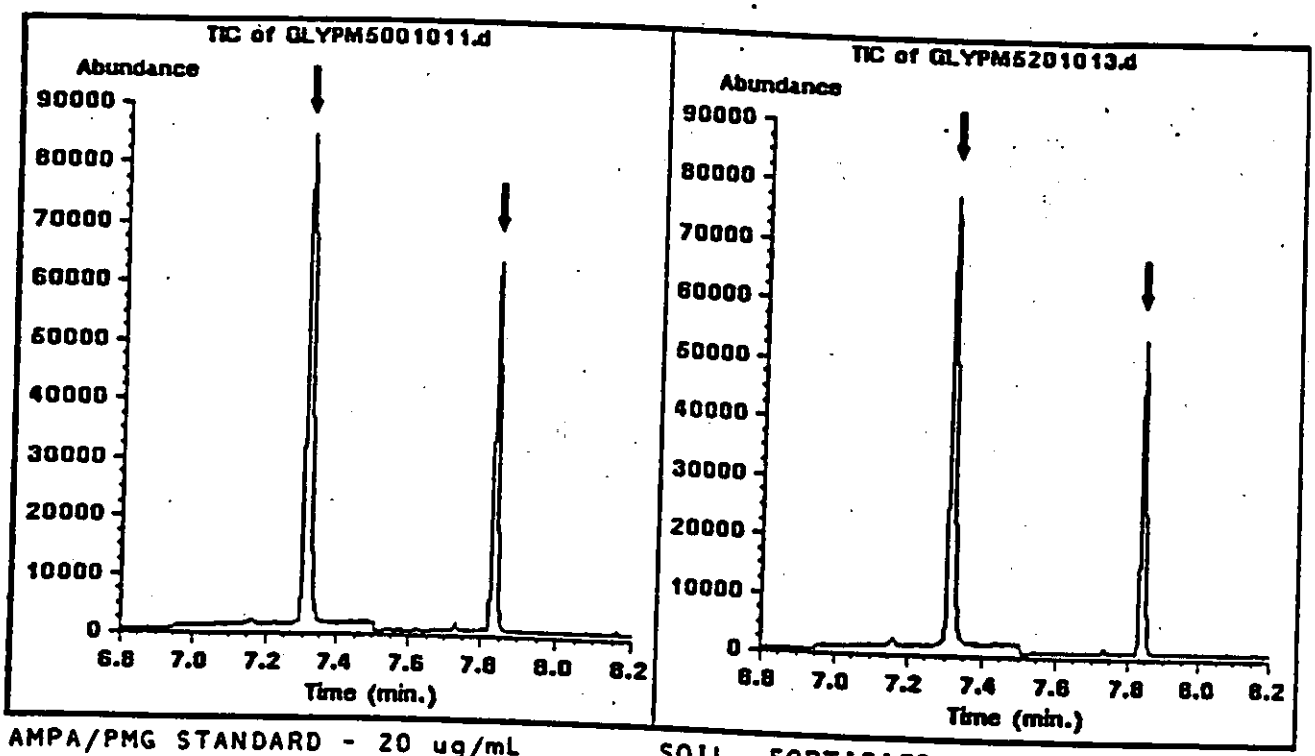


SOIL, CONTROL
D147-94 0.02 g/mL

FORTIFIED CONTROL EXTRACT-0.05 ug/g
D147-94 0.02 g/mL

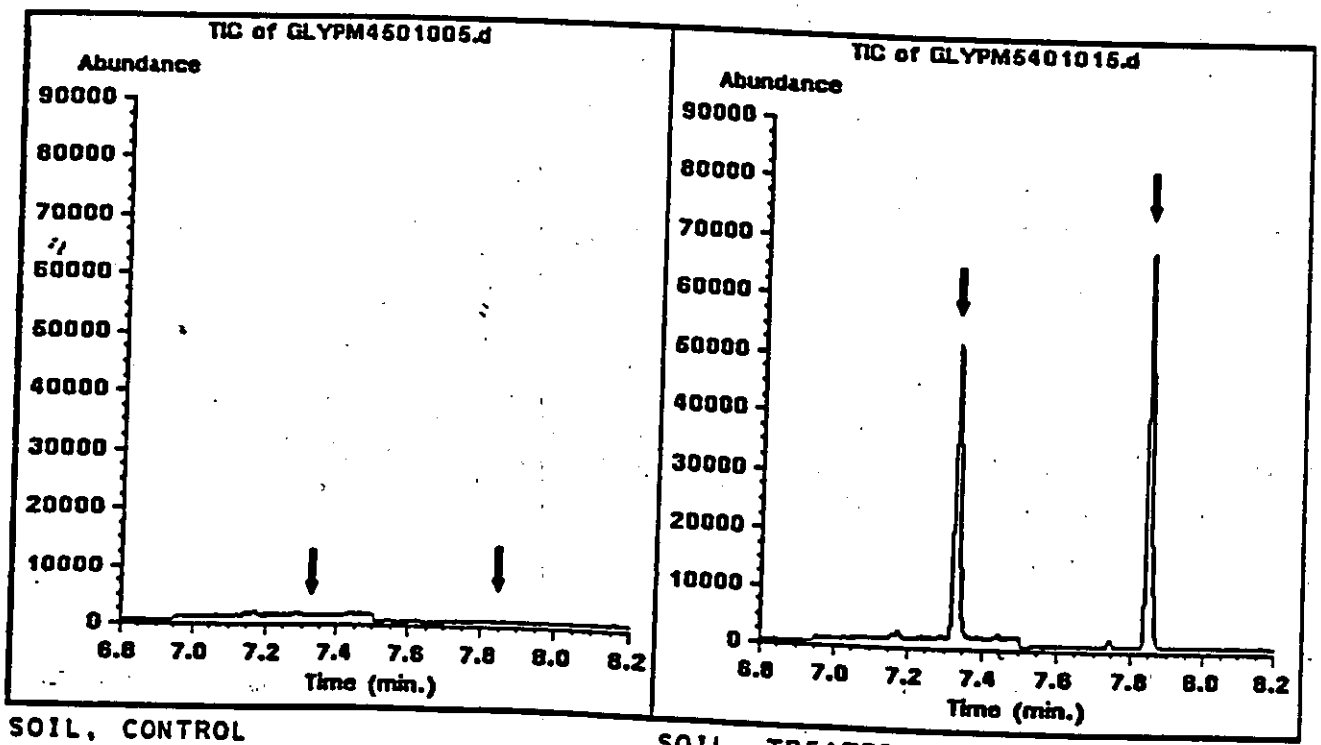
AMPA-monitored at 502 m/z, PMG monitored at 584 m/z.

Figure 4. Sample chromatograms - soil treated at 8 lb ai glyphosate-trimesium / acre (masses monitored: 502 and 584 m/z)



AMPA/PMG STANDARD - 20 ug/mL
Rt: AMPA-7.33, PMG-7.85 min.

SOIL, FORTIFIED - 1.0 ug/g
D147-94 0.02 g/mL

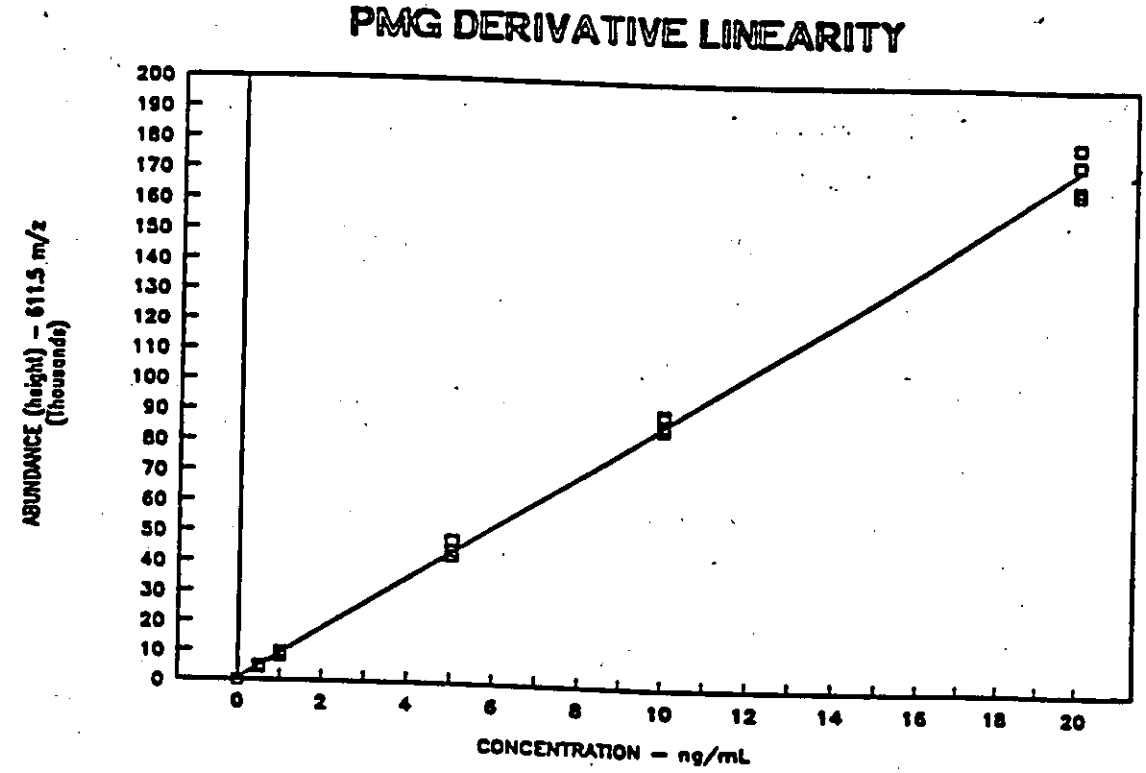


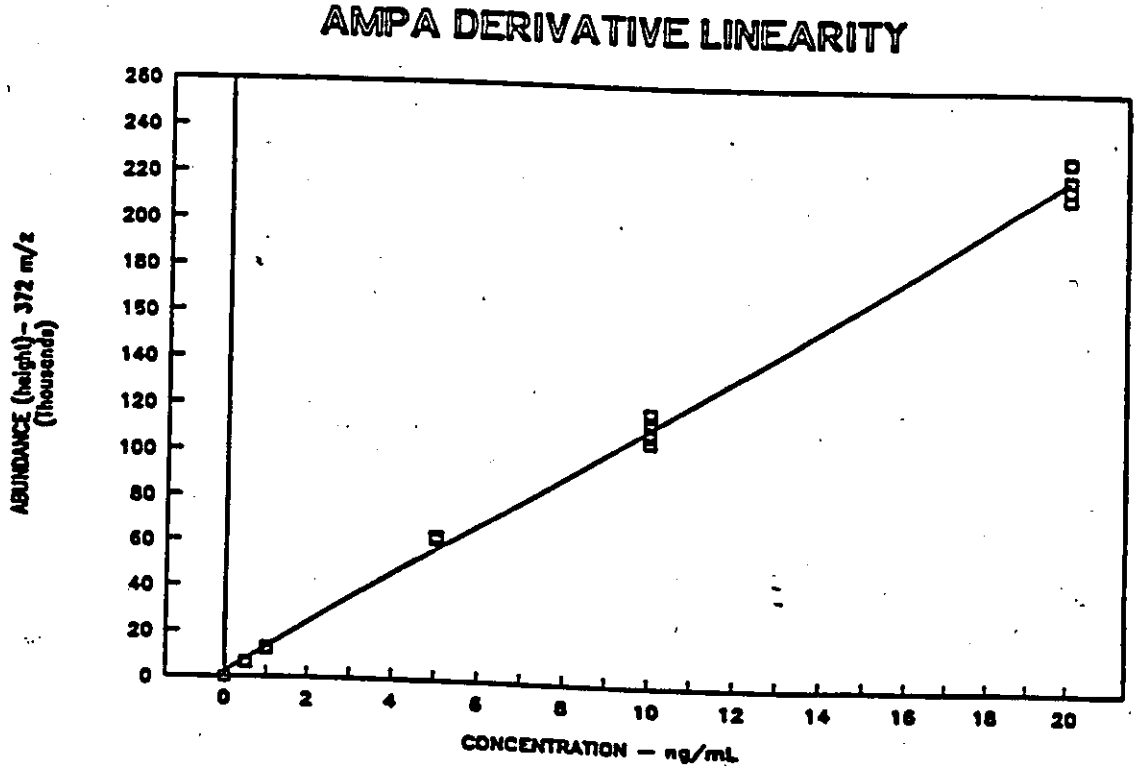
SOIL, CONTROL
D147-94 0.02 g/mL

SOIL, TREATED - 8 lb A.I./acre
D147-61 0.02 g/mL

AMPA monitored at 502 m/z, PMG monitored at 584 m/z.

Figure 5. Calibration curves for PMG and AMPA derivatives based on injections of 0.5-, 1.0-, 5.0-, 10.0-, and 20.0-ng analyte/mL solution





13 RETENTION OF RECORDS

All of the raw data, the protocol, and final report are located in the GLP Archive at the Western Research Center of Zeneca Ag Products, 1200 South 47th Street, Box 4023, Richmond, California 94804-0023.

14 REFERENCES

1. Patchett, G.G.; Katague, D.B. (1989) *Determination of SC-0224 Anion Residues in Crops, Soil, and Water by Liquid Chromatography*; Western Research Center report number WRC 85-34R.

2. Roper, E.M. (1992) *Touchdown: Field Dissipation Study for Terrestrial Use, Othello, Washington, 1988-1989*; ICI Americas Western Research Center report number RR 92-059B [DRAFT].

3. WRC Laboratory Notebook 13506 (unpublished).

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15 APPENDICES

Appendix A. Mass-selective detector manual tune procedures

Appendix B. Analyte derivatization: structures and mass spectral data

Appendix C. Sample calculations; recommended fortification procedures; recommended dilutions of calibration solutions, critical elements

Appendix D. Recovery of PMG and AMPA from soil analyzed for study GLYP-91-SD-02

Appendix A. Mass-selective detector manual tune procedures

I. INTRODUCTION

The derivatives of AMPA and PMG provide the mass spectra shown in Figure A.1. The AMPA derivative, with a parent ion of 571 AMU, provides 3 fragments (m/z 372, 446, and 502) that are suitable for quantitation purposes in the selective-ion monitoring (SIM) mode. Of the three, m/z 446 provides the best signal-to-noise (S/N) ratio.

The derivative of PMG has a parent ion of m/z 811 AMU. This ion is above the range of the mass-selective detector, but its existence has been confirmed on other instruments. Fragments at m/z 584, 611 and 612 are best for quantitation. Using the manual tune procedure described below, both the m/z 611 and 612 fragments can be detected simultaneously in the SIM mode.

The manual tune procedure is based on increasing the AMU bandwidth as a direct method for increasing sensitivity. In addition, the tuning range is limited to the range of 300 to 650 m/z. The loss of resolution caused by increasing the bandwidth can cause a decrease in selectivity, resulting in minor increases in chromatographic interferences. However, the manual tune procedure will provide a 2- to 4-fold increase in the minimum detectable amount of AMPA, and a 4- to 8-fold increase for PMG, when compared to the AutoTune procedure, as measured by comparison of the chromatographic signal-to-noise ratio.

II. PROCEDURES (Unix ChemStation)

A. Manual Tune Procedure

1. Run an "AutoTune" tuning procedure using the standard acquisition parameters (see Table A.1).

2. Enter the MANUAL TUNE screen.

a. Under the Data Acquisition menu item, change the data acquisition parameters to those found under the manual tune parameter heading in Table A.1

b. Run an "Extended Tune" tuning procedure. Save parameters under a new file name. Obtain spectrum scan by typing "SCAN" on the command line of the MANUAL TUNE screen.

c. Under the "Change Parameters" menu item, turn on the MSD, and slowly reduce the AMU gain to between 65 and 75. Observe the increase in the peak width of the calibration ions. Decrease AMU gain until a peak width of 2.4 to 3.0 AMU is obtained. AMU offset may also have to be adjusted. Increase the Multiplier voltage to obtain an abundance of 2,000,000 for the m/z 414 ion. Obtain spectrum scan.

Compare scans obtained in b and c above. The increase in peak width should result in reduced resolution. Compare scans obtained in b and c, above to those in Figures A.2. Note any shift in the m/z assignments when compared to the AutoTune scan obtained in a, above. Also note the absence of isotopes m/z 515, 503 and 615. This is normal due to the decrease in resolution.

B. AMPA and PMG Mass Calibration - SIM Mode

The manual tune procedure has a tendency, to shift the detected masses of the fragments from their actual masses. For example, under AutoTune parameters the AMPA m/z 446 fragment will give maximum response when the m/z 446 ion is selected. Under manual tune parameters, however, the maximum response may be found by selecting m/z 445.0 or 445.5 when in the SIM mode.

There are two cases when the actual masses should be calibrated. The first, described above, is when maximum response is needed. The second, is when alternate ions are monitored for residue confirmation, or when questioned peaks must be differentiated from interferences.

1. Modify the normal SIM-mode method to monitor 6 to 8 ions at 0.5 AMU intervals for the fragment in question. For example, if calibrating the m/z 611 fragment of PMG, select m/z 609.5, 610.0, 610.5, 611.0, 611.5, 612.0, 612.5 and 613.0. Change the dwell time for each ion to 40 msec. Calibrate one PMG and one AMPA fragment ion per run.

2. Make an injection using the tune file resulting from the AutoTune procedure, above. Make another injection using the manual tune file resulting from the manual tune, above.

Figure A.3 shows the expected results from this procedure. The AutoTune spectrum shows narrower bandwidths for both m/z 446 and 611. The abundance maximum for m/z 446 has shifted slightly to 445.5 for the manual tune spectrum, although 445.0, 445.5, or 446 would give similar results. The higher resolution obtained for the AutoTune spectrum clearly shows both m/z 611 and 612 fragments for PMG. In the case of the manual tune, m/z 611.0 or 611.5 would give equal results in the SIM mode.

C. AMPA AND PMG Mass Calibration - SCAN Mode

If the m/z assignments of AMPA and PMG ions remain in doubt, an alternative method is to produce full mass spectra of AMPA and PMG derivatives using the SCAN mode. A derivatized standard solution (1.0 to 5.0 µg/mL) is prepared and analyzed in the SCAN mode. First with the AutoTune parameters, then analyzed again using the manual tune parameters. Fragment mass assignments can then be made by comparing the two spectra. See Figure A.4.

III. RESULTS AND DISCUSSION

Figure A.5 shows improvement in the signal-to-noise ratio that is possible by conducting the manual tune procedure described above. The results shown here were obtained after the replacement of the MSD source. Over time, the response obtained with either the manual tune or the AutoTune procedure will degrade. The rate of degradation will depend on the number of injections, the amount of coextractives in the extracts, and the integrity of the vacuum system. The sensitivity of the detector is due primarily to the condition of the source unit. However, the response of the analytes should not be the sole parameter used to judge the condition of the source, as chromatographic parameters may also play a role. As a result, spectrum scans should be obtained on a regular basis using the current manual tune file with PFTBA. When the 502 m/z abundance drops below 500,000, the operator should consider source replacement. The manual tune procedure and mass calibration described above should be performed after source replacement. Performing the manual tune and mass calibration is not usually required on a regular basis.

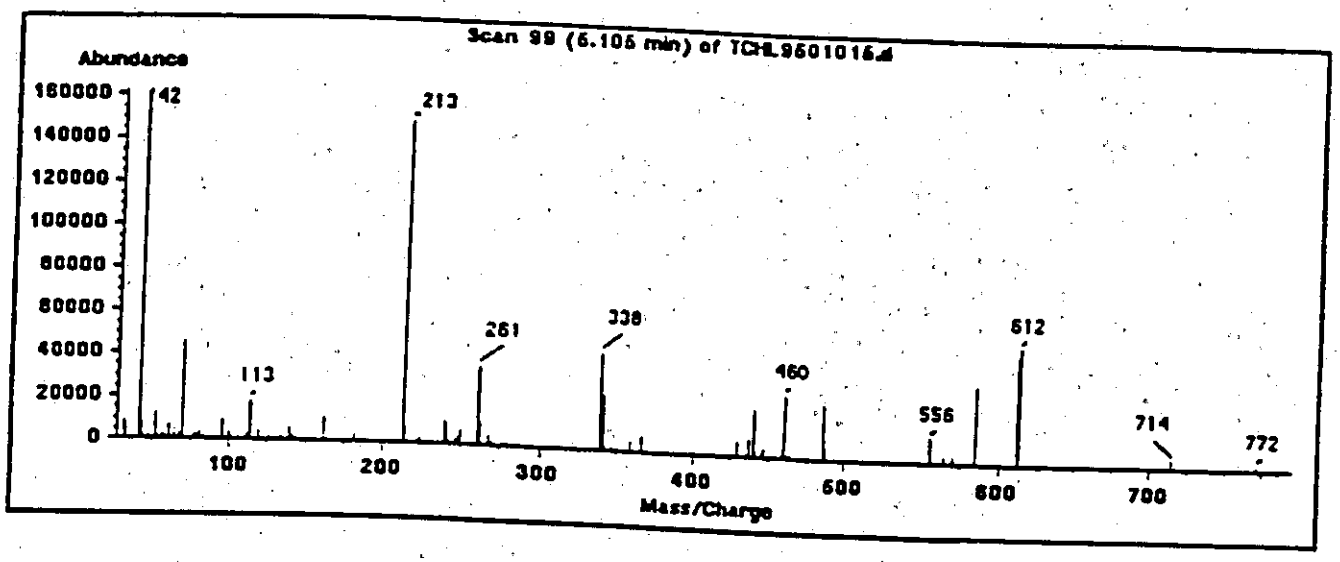
IV. CONCLUSIONS

The purpose of this procedure is to manually tune the mass-selective detector in order to increase the minimum detectable amount of the PMG and AMPA derivatives. This is accomplished by, first, narrowing the tuning range to focus on the ions of interest. Second, the bandwidths are manually increased from 0.5 to 2.6 AMU in order that both the m/z 611 and 612 fragment of the PMG derivative are simultaneously detected using a single ion in the SIM mode. Performing this procedure can increase the response to the PMG derivative by a factor of eight.

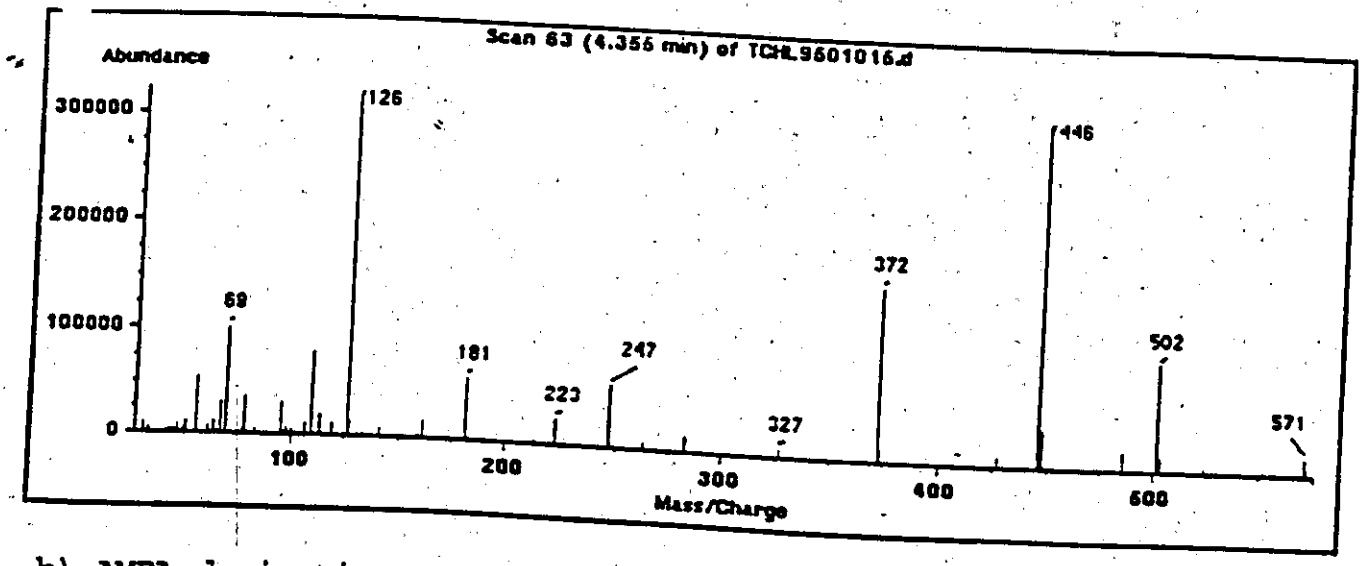
In order to maintain the integrity of the mass calibration (a main goal of the "AutoTune" procedure), additional procedures can be conducted. First, the instrument is tuned using the "AutoTune" procedure to correctly calibrate the ions resulting

from the calibration compound, PFTBA. Second, spectrum scans are obtained after the AutoTune procedure, the manual tune procedure, and the AMU gain adjustment to ensure that no significant m/z shifts have occurred. Third, m/z assignments of the derivative ions are checked by comparing spectrum scans using AutoTune and manual tune parameters in the normal SCAN mode and by producing a modified "spectrum" in the SIM mode.

Figure A.1 Mass spectra of PMG and AMPA derivatives - mass-selective detector (positive electron impact mode)



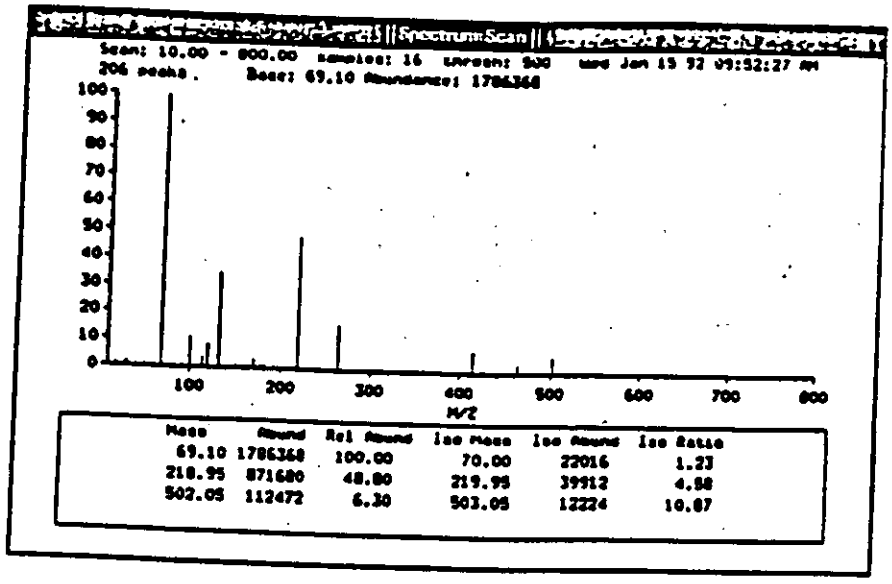
a) PMG derivative



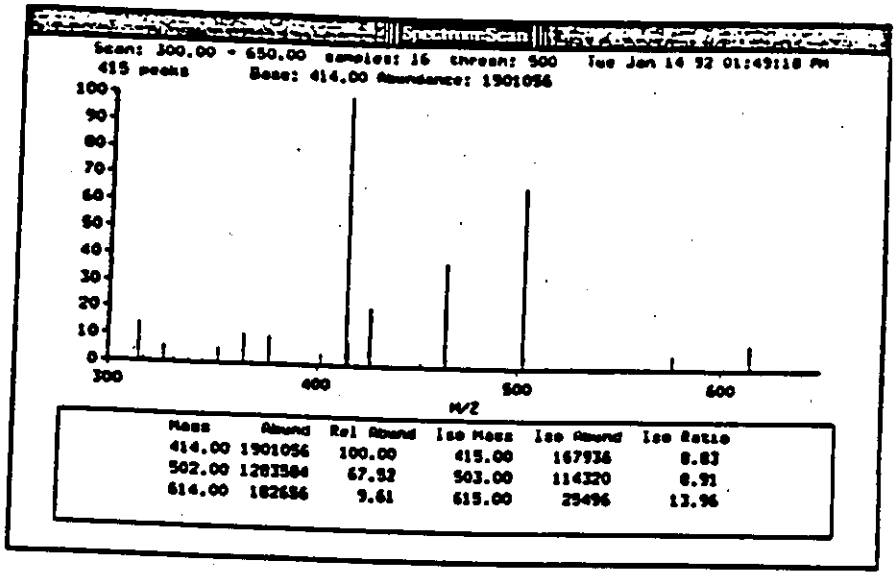
b) AMPA derivative

Figure A.2 Manual tune procedure - spectrum scan (PFTBA)

a) After AutoTune



b) After manual tune procedure (extended tune)



c) After manual tune and AMU gain adjustment

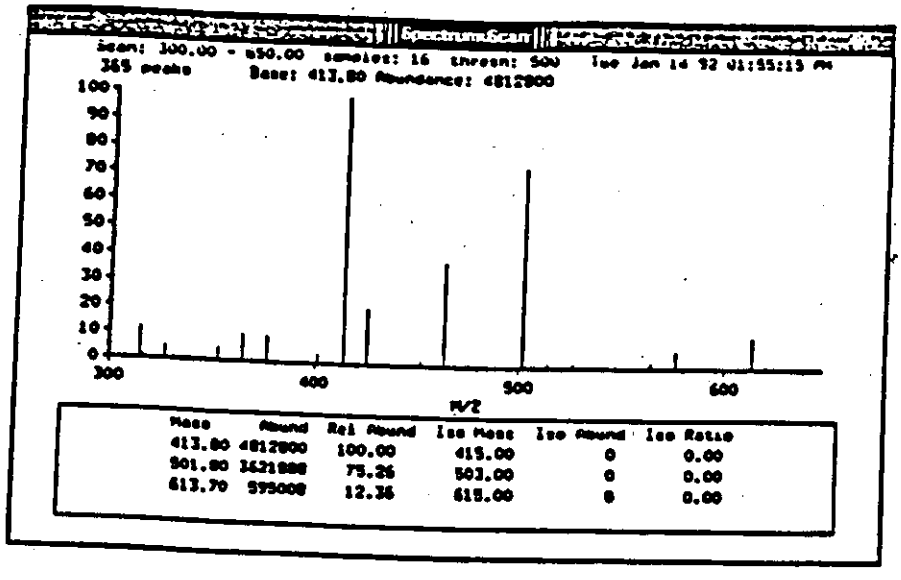
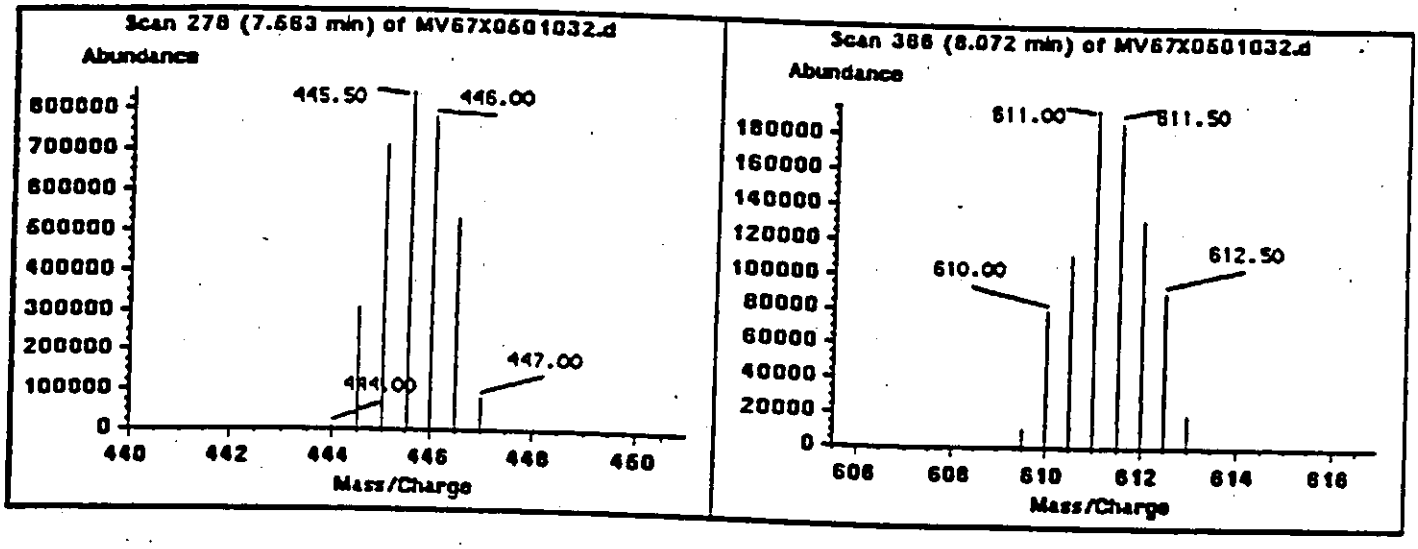


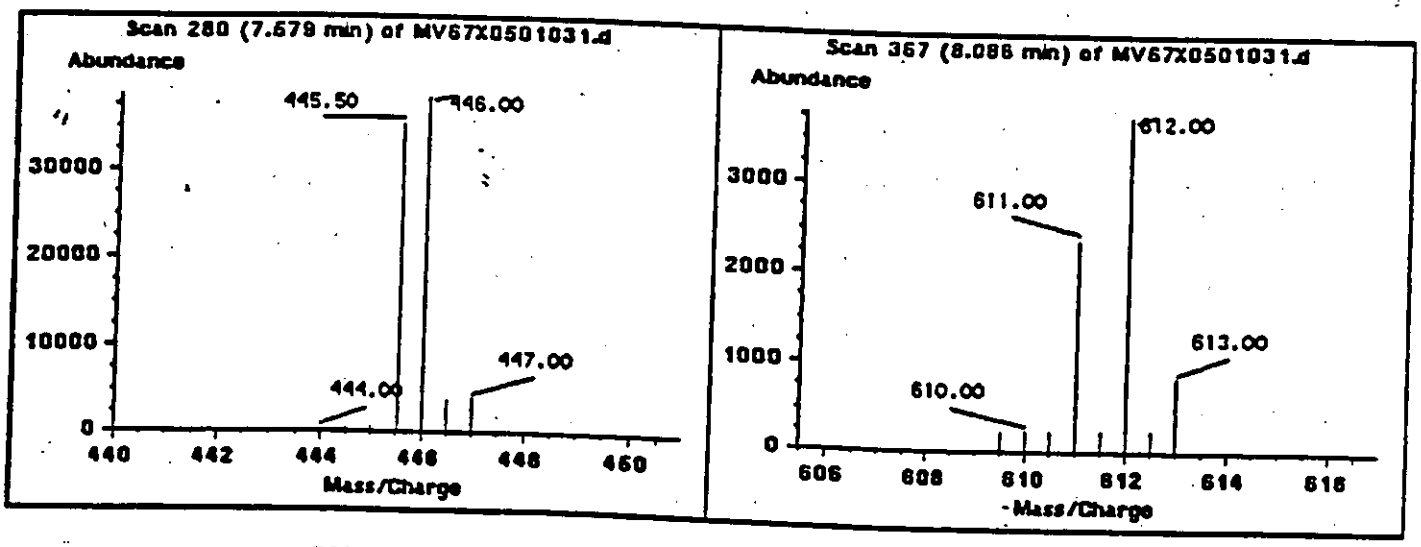
Figure A.3 SIM mode mass calibration

a) Calibration scan using manual tune parameters.



AMPA PMG

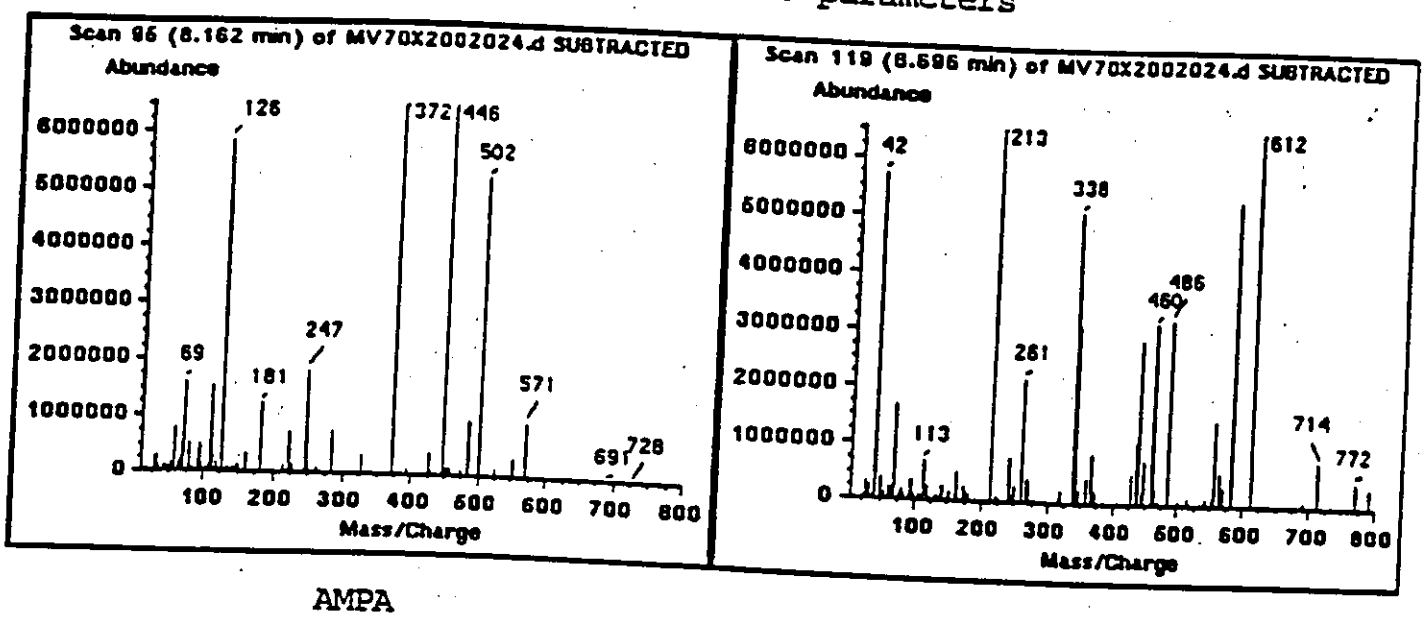
b) Calibration scan using AutoTune parameters.



AMPA PMG

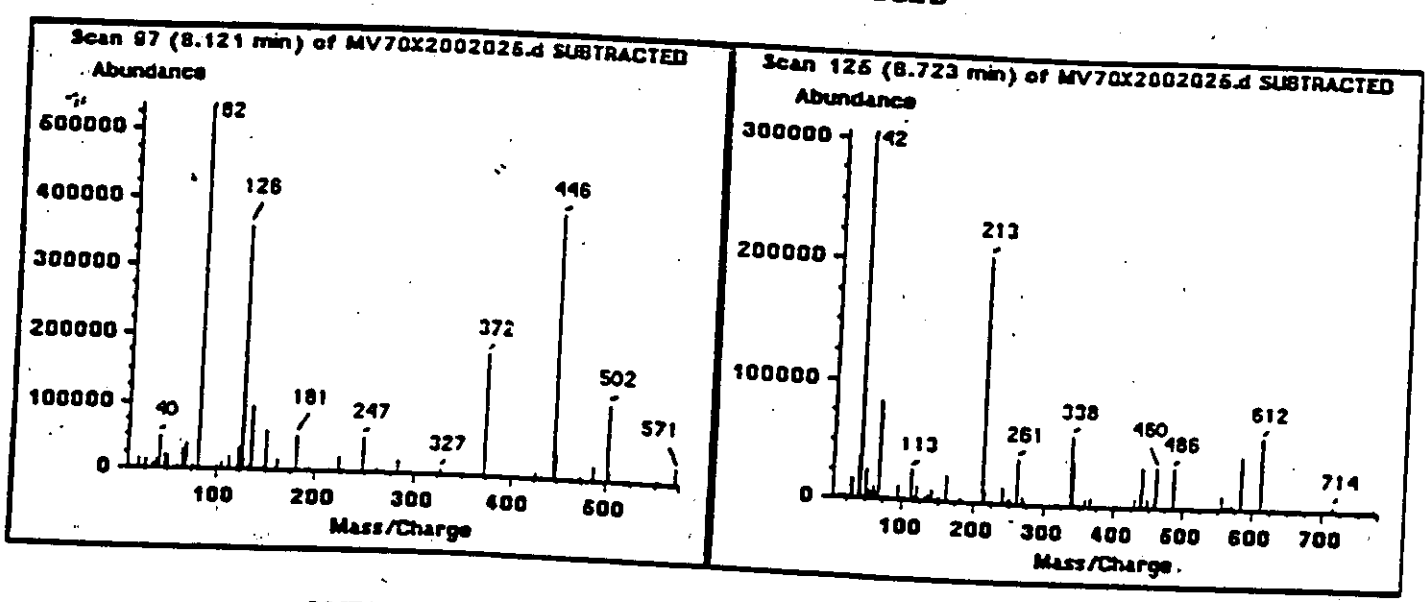
Figure A.4 SCAN mode mass calibration

a) Calibration scan using manual tune parameters



AMPA PMG

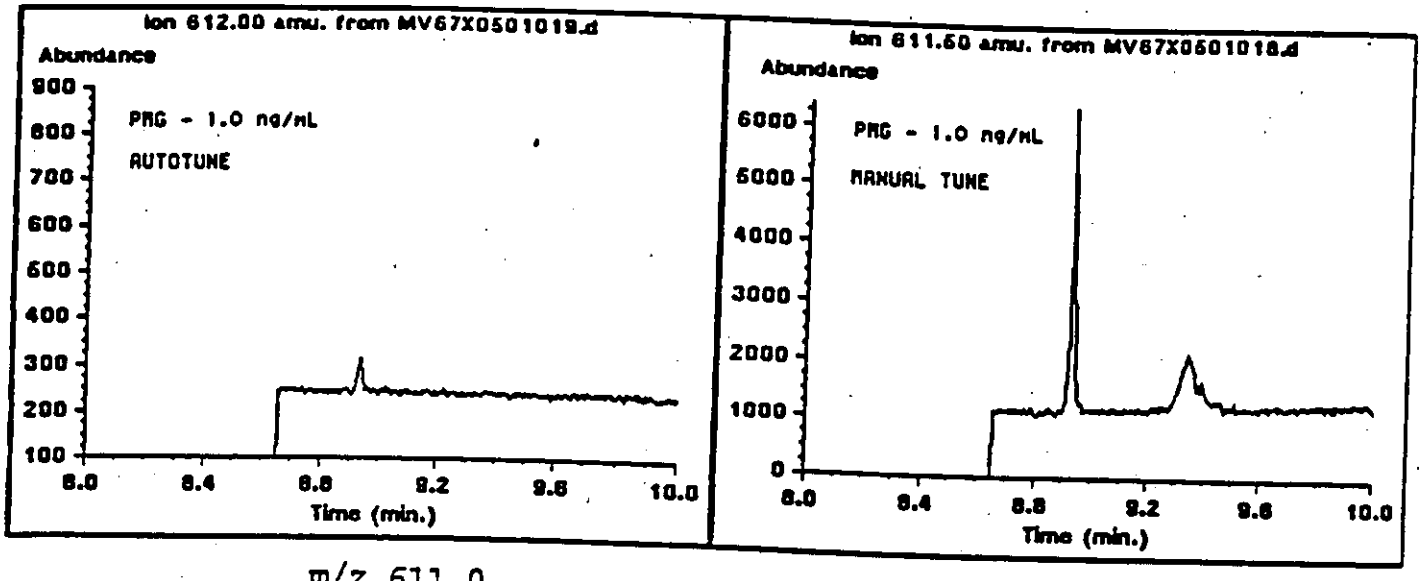
b) Calibration scan using AutoTune parameters



AMPA PMG

Figure A.5 Chromatographic signal-to-noise comparison

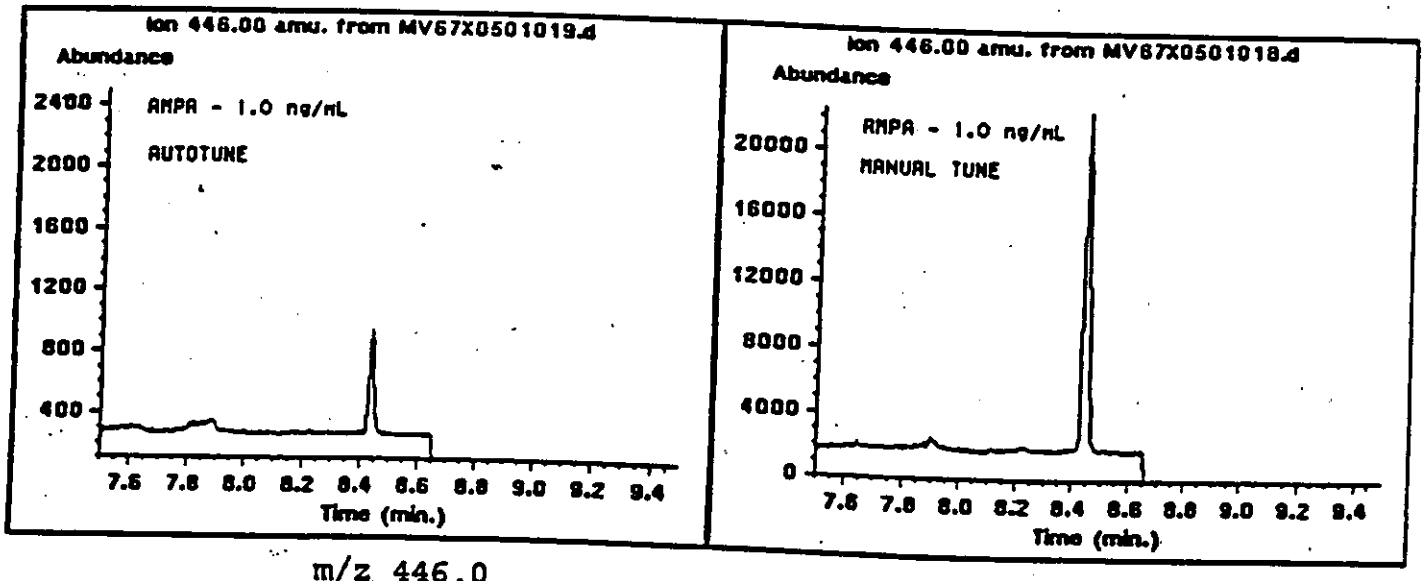
a) PMG derivative



m/z 611.0

m/z 611.5

b) AMPA derivative



m/z 446.0

m/z 445.5

Table A.1 Comparison of AutoTune and Manual Tune Parameters

		AutoTune	Manual Tune
Acquisition Parameters			
Tuning Masses (m/z)	#1	69	414
	#2	219	502
	#3	502	614
Range (m/z)		10 to 800	300 to 650
Samples		4 (2^N)	4 (2^N)
Threshold		500	500
Averages		1	1
Mass Spectrometer Parameters			
Multiplier Voltage		1800	2400
Entrance Lens		46	54
Repeller		10.2	9.36
Ion Focus		0.0	5.6
X-Ray		56.0	61.6
AMU Gain		126	73
AMU Offset		58	61 *
Mass Gain		-9	4 *
Mass Offset		16	17 *

Highlighted parameters require manual adjustment

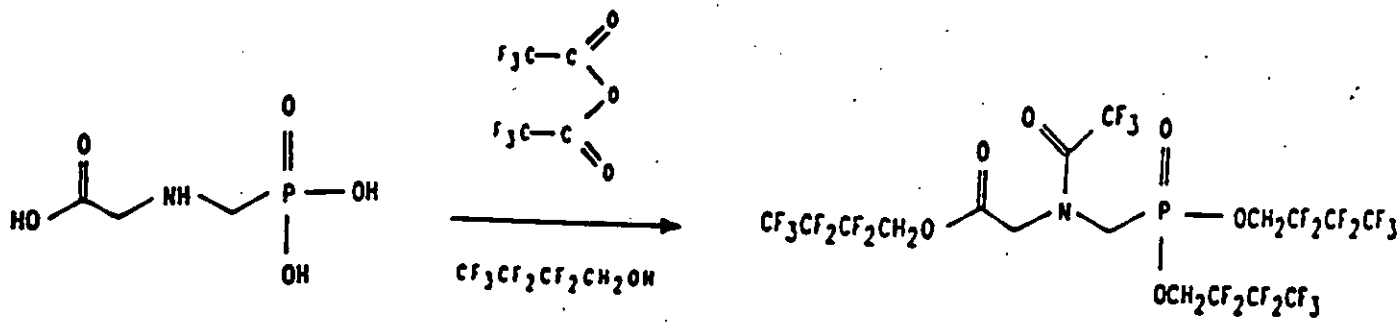
* Minor adjustment needed in some cases.

Appendix B. Analyte derivatization: structure and mass spectral data

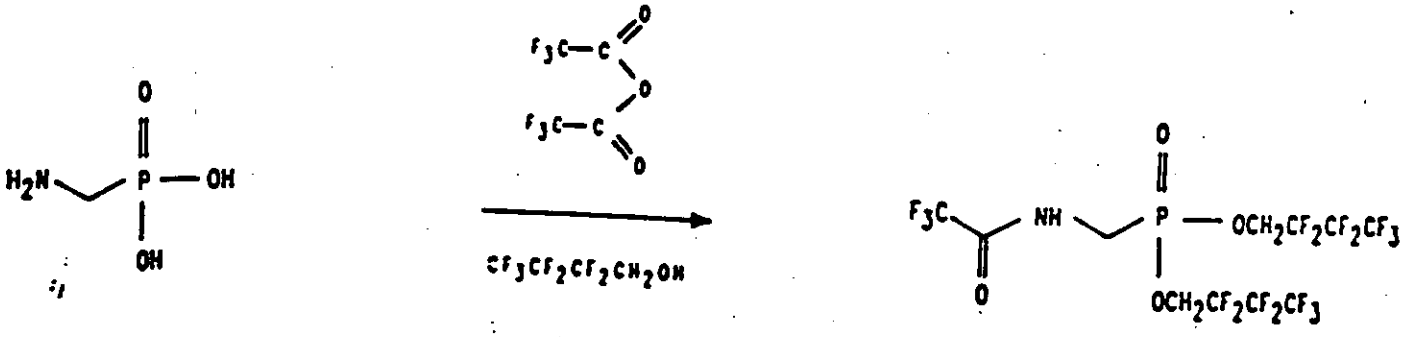
The mixture of trifluoroacetic anhydride and heptafluorobutanol provide a single-step derivatization for all the chromatographically active, functional groups found in PMG and AMPA. The carboxylic and phosphoric acid functional groups are derivatized to form the corresponding heptafluorobutyl esters. The amine functional groups are derivatized to form the corresponding trifluoroacetyl derivatives. Figure B.1 shows the structures of PMG and AMPA and the proposed structures of the PMG and AMPA derivatives.

The molecular weights of the derivatives of PMG and AMPA are 811 and 571 AMU, respectively. Although the molecular weight of the parent ion of the PMG derivative exceeds the range of the mass-selective detector, the presence of the molecular ion was confirmed on an alternative mass spectrometer. Figure B.2 shows the full mass spectra (positive electron impact) of the PMG and AMPA derivatives. Note also the presence of both the 611 and 612 m/z ions obtained from the PMG derivative. Figure B.2 also shows the proposed fragmentation and m/z assignments of the two derivatives.

Figure B.1 Derivatization of PMG and AMPA



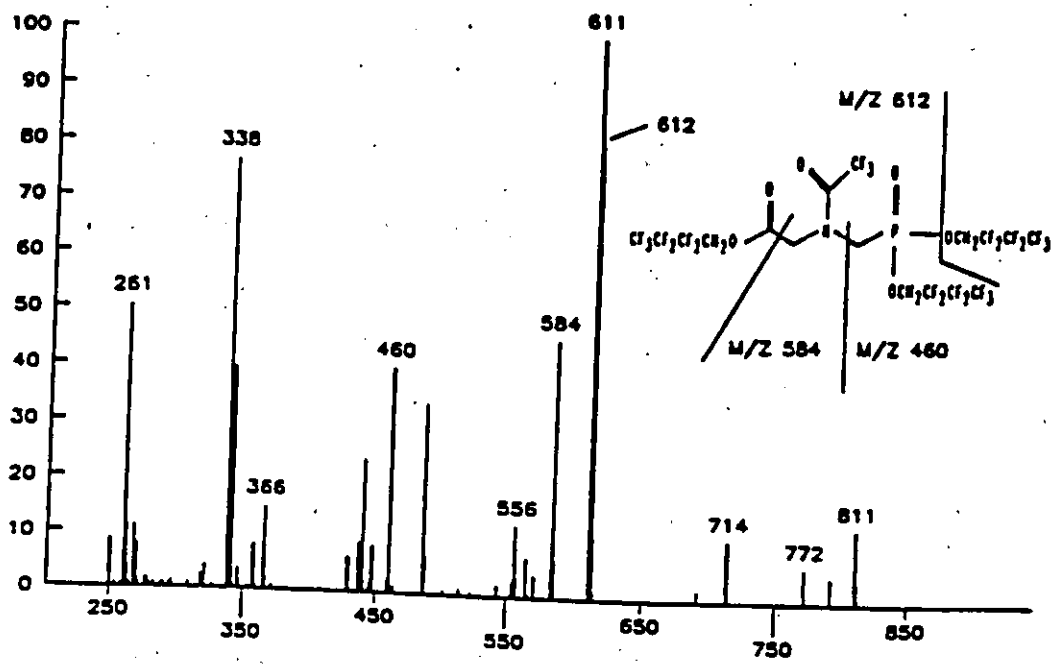
Chemical name of PMG derivative: N-[[Bis(2,2,3,3,4,4,4-heptafluorobutoxy)phosphinyl]methyl]-N-(trifluoroacetyl)glycine, 2,2,3,3,4,4,4-heptafluorobutyl ester



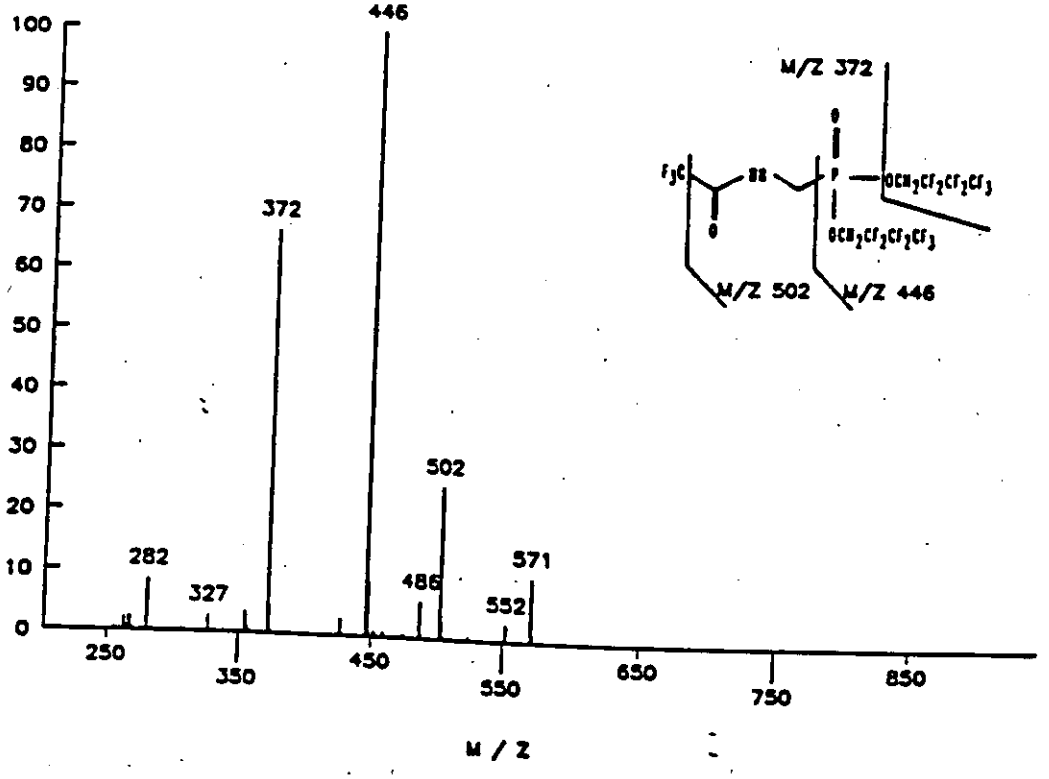
Chemical name of AMPA derivative: (Trifluoroacetylamino)methylphosphonic acid, bis[2,2,3,3,4,4,4-heptafluorobutyl] ester

Figure B.2 Mass spectra (positive electron impact) of PMG and AMPA derivatives

PMG derivative



AMPA derivative



Appendix C. Sample calculations
Recommended fortification procedures
Recommended dilutions of calibration solutions
Critical elements

EXAMPLE OF CALCULATIONS USED IN THE PREPARATION AND ANALYSIS OF A SOIL SAMPLE CONTAINING 0.05 µg/g OF PMG AND AMPA

STANDARD SOLUTIONS

2.3.4 Dilute stock solution (containing PMG and AMPA at 100 µg/mL) to an intermediate standard with a concentration of 0.50 µg/mL.

$$100\ \mu g/mL \times \frac{0.25\ g}{50\ g} = 0.50\ \mu g/mL$$

2.3.5 Dilute intermediate standard (0.50 µg/mL) to a daily-use standard at 0.0125 µg/mL, using the extracting solution as the diluent.

$$0.50\ \mu g/mL \times \frac{0.100\ mL}{4.0\ mL} = 0.0125\ \mu g/mL$$

3.2.3 Derivatize the daily-use standard by adding 20 µL standard to 1.0 mL derivatization solution. After evaporation, dilute to a final volume of 250 µL.

$$0.0125\ \mu g/mL \times \frac{0.020\ mL}{0.250\ mL} = 0.001\ \mu g/mL$$

Result: An injection standard at a concentration of 0.001 µg/mL.

SAMPLE PREPARATION

3.1.1 Extract 20 g soil (with an analyte concentration of 0.05 µg/g) with 80 mL extraction solution.

Calculation of representative soil concentration in final extract:

$$\frac{20\ g}{80\ mL} = 0.25\ g/mL$$

3.2.3 Derivatize the crude extract by adding 20 µL extract to 1.0 mL derivatizing solution. After evaporation, dilute to a final volume of 250 µL.

$$0.25\ g/mL \times \frac{0.020\ mL}{0.250\ mL} = 0.02\ g/mL$$

CALCULATION CHECK

Calculation of analyte concentration in final extract (compare result to standard concentration in 3.2.3 above):

$$0.05\ \mu g/g \times \frac{20\ g}{80\ mL} \times \frac{0.020\ mL}{0.250\ mL} = 0.001\ \mu g/mL$$

Result: A derivatized extract with a soil "concentration" of 0.02 g/mL, and an analyte concentration of 0.001 µg/mL

Reference numbers in the left-hand column refer to the appropriate method section.

ANALYTE CONCENTRATION

Assuming:

Control fortification level:	0.05	µg/g
Soil concentration in extract:	0.02	g/mL
Standard concentration:	0.001	µg/mL
Average standard response:	5000	units
Average sample response	4500	units
Average control response:	500	units

Response Factor Calculation:

$$\text{Response Factor} = \frac{0.001\ \mu g/mL}{5000\ \text{units}} = 2.0E\text{-}7\ \mu g/mL/\text{unit}$$

Analyte Concentration:

Fortified Sample:

$$\text{Concentration} = \frac{2.0E\text{-}7\ \mu g/mL/\text{unit} \times 4500\ \text{units}}{0.02\ g/mL} = 0.045\ \mu g/g$$

Control:

$$\text{Concentration} = \frac{2.0E\text{-}7\ \mu g/mL/\text{unit} \times 500\ \text{units}}{0.02\ g/mL} = 0.005\ \mu g/g$$

Percent Recovery:

$$\%\ \text{Recovery} = \frac{(0.045\ \mu g/g - 0.005\ \mu g/g)}{0.05\ \mu g/g} \times 100 = 80\%$$

RECOMMENDED FORTIFICATION PROCEDURES

Matrix	Fortification Level (mg/kg)	Fortification Solution Concentration (µg/mL)	Sample Weight (g)	Fortification Volume (µL)
Soil	0.05	10	20	100
	0.50	100	20	100

RECOMMENDED DILUTIONS OF CALIBRATION SOLUTIONS

Matrix	Fortification Levels (mg/kg)	Final Soil Concentration (g/mL)	Intermediate Standards (µg/mL)	Dilution (mL)	Daily-Use Standard (µg/mL)	Dilution (mL)	Derivatized Standard (ng/mL)
Soil	0.05, 0.50	0.02	0.50	0.10/4.0	0.0125	0.020/0.25	1.0
			0.50	0.25/4.0	0.03125	0.020/0.25	2.5
			5.0	0.10/4.0	0.125	0.020/0.25	10.0

CRITICAL ELEMENTS

The following procedural elements should be reviewed. They indicate elements of the method that have been shown, or are suspected of being of critical importance to the success of the method. Report sections are referenced in parentheses.

1. Double-restrictor inlet liner (2.1.4). The use of single-restrictor liners has resulted in chromatographic failure.

2. PTFE double-thickness septa (2.1.14). Standard single-thickness septa may not withstand the derivatization conditions required.

3. Use of glass syringes or pipets (various). Underivatized PMG and AMPA may bind onto glass surfaces. Eppendorf pipets should be used whenever possible. This is especially critical when handling standard solutions. Glass pipets should not be reused after contacting standard solutions.

4. Derivatization aliquot size (11.4.2). Maintain the same aliquot volume for all additions of standards and extracts to the derivatization reagent. This allows the final injected solutions to contain the same amounts of nonmatrix components.

5. Complete evaporation of excess derivatizing reagents (3.2.3). Maintain vials under a stream of nitrogen for 30-40 minutes after liquid has evaporated. Small amounts of volatile, acidic residues remaining after too short of an evaporation period can cause interfering peaks or degrade chromatography of analyte peaks.

6. Expiration of ethyl acetate/citral solution (3.2.4, 11.3). This solution should be prepared monthly and kept in sealed bottle. Older solutions have been linked to poor chromatography, including peak broadening, and shifting retention times (especially AMPA). Neat citral turns a darker yellow with age. It is best to purchase citral in 5-g containers.

7. Condition of MSD source unit (4.2.3). Analyte response is directly related to the condition of the source unit. It is advantageous to begin work with this method with a recently cleaned source. In addition to higher analyte response, a baseline response can be established which can help in troubleshooting and other diagnostic procedures.

8. Oven temperature profile (4.1.2). The profile given in this section is a recommended starting point. Small changes may be necessary. Significant changes in peak area:height ratio may occur with changes in the initial oven temperature as small as 3 to 5 degrees.

Appendix D. Recovery of PMG and AMPA from soil analyzed for study GLYP-91-SD-02

Recovery of PMG and AMPA From Soil Analyzed for Study GLYP-91-SD-02, Using GC Parameters as Described in Text[1].

Sample Number	Fortification Level, mg/kg	Recovery, %	
		PMG	AMPA
G403-1A	1.0	84	91
G403-1A	1.0	90	98
G403-1A	1.0	92	100
G403-1A	2.0	79	89
G403-1A	2.0	86	92
G403-1A	2.0	88	94
G403-1A	5.0	78	90
G403-1A	5.0	82	96
G403-1A	5.0	84	98
G403-1A	0.1	96	86
G403-1A	0.1	122	105
G403-1A	0.05	85	79
G403-1A	0.05	90	96
G403-1A	0.05	94	97
G403-1A	0.05	96	98
G403-1A	0.05	100	101
G403-1A	0.05	101	105
G403-1A	0.05	102	106
G403-1A	0.05	104	108
G403-1A	0.05	105	112
G403-1A	0.05	106	115
G403-1B	0.1	109	116
G403-1B	0.05	96	102
G403-13B	0.1	102	104
G403-13B	0.05	79	96
G403-23B	0.1	95	108
G403-23B	0.05	87	94
G403-2C	0.1	97	112
G403-2C	0.05	85	88
G403-2D	0.1	86	93
G403-2D	0.05	87	98
G403-2E	0.1	94	102
G403-2E	0.05	92	100
G403-59B	0.1	92	102
G403-59B	0.05	81	90
		97	102
Mean:		93	99
Range:		78-122	79-116
CV:		10.4	8.4
N:		36	36

[1] Values obtained in course of analyses for GLP study GLYP-91-SD-02, using GC parameters found in Sections 3 and 4. Recovery values in Table I were obtained using parameters found in Section 11.5.5.