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.
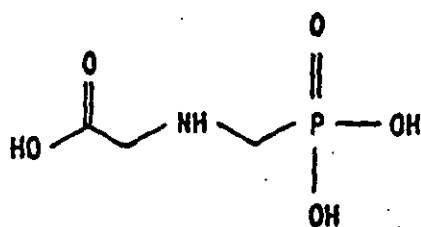
PMG
MW=169 daltons
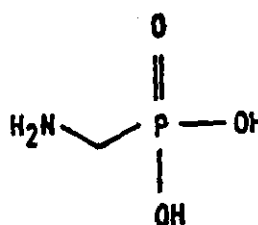
AMPA
MW=111 daltons

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

2.1.9   <u>Eppendorf automatic pipet</u>. 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 <u>Filters</u>. 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 <u>Glass pipets</u>. 2-, 5-, and 10-mL disposable, graduated glass
        pipets for general use.

2.1.12 <u>Glass bottles</u>. 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 <u>Auto-sampler vials</u>. 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 <u>Derivatization vials</u>. 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 <u>Evaporation manifold</u>. 12-unit evaporation manifold with
        aluminum heating/cooling block, 13-mm tube size (Chemical
        Research Supplies #201188).

2.1.16 <u>IEC Model K centrifuge</u>.

2.1.17 <u>Eberbach mechanical shaker (horizontal)</u>.

2.1.18 <u>Thermometer</u>. -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:

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

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 ($\leq$0.02 g soil/mL extract) when sensitivity requirements allow.

3.3   <u>Fortification</u>

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   <u>Calibration and Analysis</u>

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:

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

Where

F = response factor

C = concentration of calibration solution, $\mu g/mL$

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

5.1.2 <u>Soil in extract</u>. 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 <u>Analyte in sample</u>. 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 <u>Nonlinear Response Calculation Methods</u>

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 <u>Calculation of extract concentration</u>. 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 <u>Calculation of analyte in sample</u>. 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 <u>MATRIX EFFECT</u>

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 <u>Derivatized Matrix Standard</u>

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 ≤ 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   <u>CONFIRMATORY TECHNIQUES</u>

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		PMG Derivative	
Ion (m/z)	Abundance*	Ion (m/z)	Abundance
126	99	213	100
247	19	338	31
372	51	584	25
446	100	611	34
502	32	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.

9.6   <u>Alternate Ion Analysis</u>

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.

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 <u>Corrosivity of derivatizing reagent</u>. 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 <u>Solution Stability</u>

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 <u>Matrix Considerations</u>

11.4.1 <u>Matrix modification - citral addition</u>. 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 <u>Nonsoil matrix considerations</u>. 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.

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.

Table A.1   Comparison of AutoTune and Manual Tune Parameters

Acquisition Parameters		AutoTune	Manual Tune
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.

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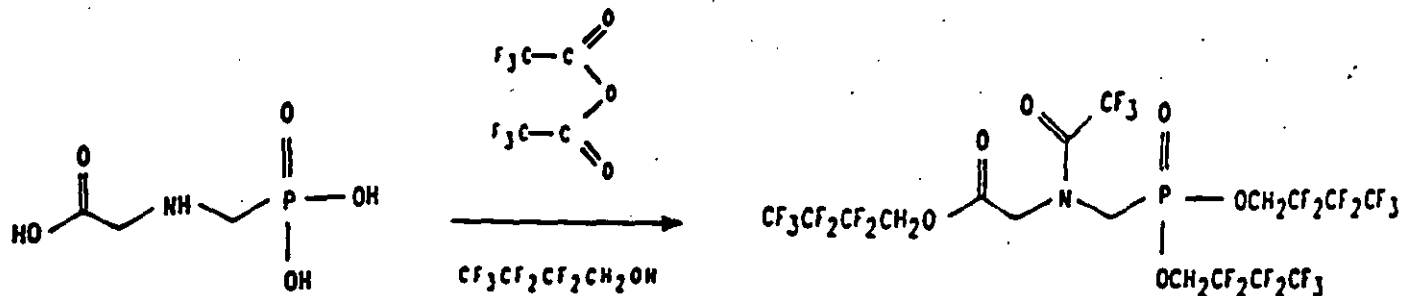
Chemical name of PMG derivative: N-[[Bis(2,2,3,3,4,4,4-heptafluorobutoxy)phosphinyl]methyl]-N-(trifluoroacetyl)glycine, 2,2,3,3,4,4,4-heptafluorobutyl ester
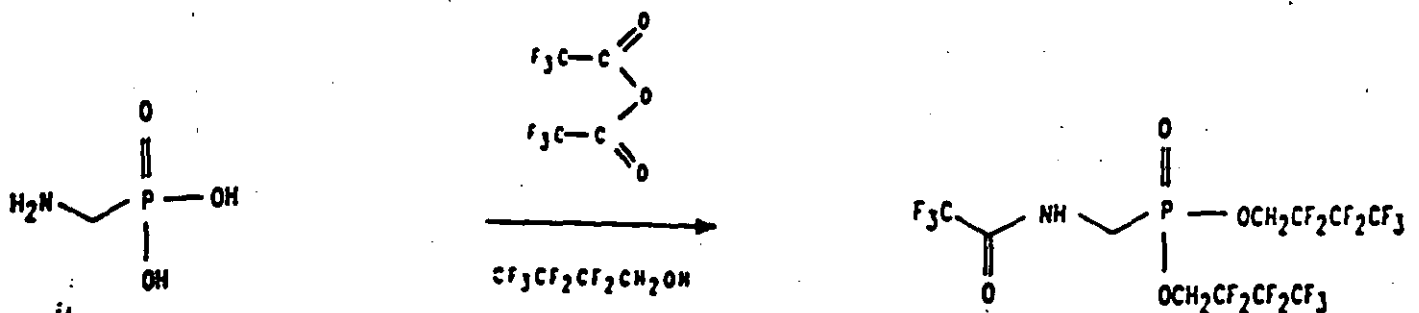
Chemical name of AMPA derivative:
(Trifluoroacetylamino)methylphosphonic acid, bis[2,2,3,3,4,4,4-heptafluorobutyl] ester

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

Calculation of representative soil concentration in final extract:

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

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

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:

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

Analyte Concentration:

Fortified Sample:

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

Control:

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

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. <u>Condition of MSD source unit (4.2.3).</u> 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. <u>Oven temperature profile (4.1.2).</u> 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.